Ghrelin Levels Increase After Pictures Showing Food

Petra Schüssler[1], Michael Kluge[1], Alexander Yassouridis[1], Martin Dresler[1], Manfred Uhr[1] and Axel Steiger[1]

The neuropeptide ghrelin is a major signal for food intake in various species including humans. After exogenous ghrelin administration, food intake and body weight increase in rodents. In normal human subjects, ghrelin administration increases self-rated appetite and calorie intake and prompts the imagination of favorite meals. It is unclear so far whether ghrelin levels are affected by external cues such as sight of food. We investigated the influence of pictures showing food compared to neutral pictures on ghrelin levels in young normal male subjects (n = 8). The study consisted of two consecutive sessions with a one-week interval. During each session, blood for later analysis of plasma concentrations of ghrelin was collected between 08:15 and 13:00 every 15 min (between 10:30 and 11:30 every 10 min). Breakfast and lunch was provided at 08:30 and 12:00, respectively. Fifty pictures were presented from 10:30 to 10:45 showing neutral images during the first session and food contents during the second session. As expected, ghrelin levels increased before each meal independent of the picture contents. In addition, ghrelin levels during the 30-min interval following the presentation of pictures with food increased significantly compared to the 30-min interval before this presentation (area under the curve (AUC): 188 % vs. 158 %, $P < 0.05$). The difference in the increases between the two picture conditions was also significant ($P < 0.05$). Our findings suggest that sight of food elevates ghrelin levels in healthy volunteers.

Obesity (2012) doi:10.1038/oby.2011.385

INTRODUCTION

Environmental factors such as the visual exposure to food are claimed to influence eating behavior in humans (1). Sight of hedonic food enhanced the desire to eat and calorie intake, even when subjects were satiated (2,3). The specific physiological mechanisms, which trigger motivation for food consumption independent of metabolic energy status, are so far unclear.

The neuropeptide ghrelin, mainly secreted by the stomach and proximal small intestine, is the major stimulus for food intake in humans and rodents. Ghrelin acts via the ghrelin receptor (GHS-R1a), which is particularly found in the neuropeptide Y and growth hormone-releasing hormone neurons in the hypothalamus-pituitary unit (4,5). After exogenous ghrelin administration, feeding and body weight increase in rodents (6,7). In normal human subjects, ghrelin administration increases self-rated appetite and calorie intake (8) and prompts the imagination of favorite meals (9). Ghrelin levels increase in humans before meals and decrease after meals regardless whether meal times were fixed (10) or meals were initiated voluntarily (11). Peripheral ghrelin secretion is supposed to be controlled by cephalic mechanisms, likewise the sympathetic neural system stimulates directly peripheral ghrelin secretion (12,13).

In fMRI studies, it was demonstrated that the amygdala, orbitofrontal cortex, insula, anterior cingulated, and fusiform gyrus contain neurons, who respond specifically to visual food stimuli (14,15). These areas participate in a complex neural circuit that represents food identification and estimation. The intravenous administration of ghrelin increases the response to images with food content in most of these above-mentioned areas (16).

A reciprocal interaction exists in the energy balance between ghrelin and leptin and between ghrelin and insulin (11,17). Leptin is produced in the adipose tissue, and leptin receptors are found in the arcuate nucleus located within the hypothalamus, the same region where ghrelin is engaged. Leptin decreases food intake and increases energy expenditure (18). Ghrelin inhibits insulin secretion, and ghrelin secretion is decreased by insulin (19,20). Data are conflicting, if leptin declines before meal initiation (11,21).

As far as we know, it is unclear whether ghrelin plasma levels are affected by external cues such as sight of food. Since such cues are omnipresent in nowadays society it appears important to clarify this issue. Therefore, we examined the effect of visual presentation of images of food on ghrelin plasma levels in healthy, normal weight subjects. In addition, plasma concentrations of insulin and leptin were evaluated because they are postulated to influence the course of ghrelin secretion. In

[1]Max Planck Institute of Psychiatry, Munich, Germany. Correspondence: Axel Steiger (steiger@mpipsykl.mpg.de)

Received 3 August 2011; accepted 1 December 2011; advance online publication 12 January 2012. doi:10.1038/oby.2011.385

more detail, we wanted to test the hypothesis that ghrelin levels increase in healthy volunteers after presentation of pictures showing food. The courses of leptin and insulin in this experiment are difficult to predict. Since ghrelin and these hormones may interact, in addition the course of their plasma concentrations was assessed.

METHODS AND PROCEDURES

Subjects

The sample consisted of eight healthy male volunteers of normal weight (mean age 22.3 ± 5.5 years, range 21–28, mean BMI 22.9 ± 0.9 kg/m^2). Detailed characteristics are given in **Supplementary Table S1** online. All subjects were drug-free for at least 3 months and entered the study after passing rigid psychiatric, physical, and laboratory examinations. All were emmetropic or corrected to normal vision. Reasons for exclusion from the study were as follows: major or chronic diseases (e.g., eating disorders, diabetes, heart failure, hepatitis), acute or previous psychiatric or chronic neurological disorder (e.g., schizophrenia, epilepsy) in the own or family history, stressful life events, shift work, aberrancies in the blood chemistry or electrocardiogram. Other exclusion factors were excessive physical exercise; food allergies; specific diet (e.g., vegan, vegetarian); eating habits differing from the local culture, abuse of drugs, nicotine (more than 5 cigarettes per day), loss or gain of weight in the last 3 months more than 3 kg.

The experiment was approved by the Ethics Committee for Human Experiments of the University of Munich. After the purpose of the study had been explained to the subjects, all of them gave their informed consent according to the tenets of the declaration of Helsinki.

Materials

Endocrine measurements. Plasma total ghrelin and leptin were measured using commercial radio-immunoassays (ghrelin: Phoenix, Belmont, CA; intra- and interassay coefficients of variation <13%; leptin: Linco Research, Saint Charles, Mo; intra- and interassay coefficients were below 7% and 9%, respectively). For the quantitative measurement of insulin, a chemiluminescent immunoassay was used (Immulite 2000; Siemens, Eschborn, Germany, intra- and interassay coefficients of variation were below 5.5% and 7.35%).

Random samples for each hormone were analyzed in duplicate. According to standard procedures for time series, the remaining specimens were analyzed only once.

Visual presentation. Fifty color-photographs of food and 50 nonedible objects were selected for visual presentation on a computer screen (23 × 37.5 cm). The taste intensity of the 50 food photographs was scored by 21 male volunteers, who were asked to assign to them a score between 1 (very tasty) and 7 (not tasty).

The food photographs demonstrated savory and sweet meals which are typical for lunch in Germany (e.g., steak, Viennese Schnitzel, pizza, ice cream, chocolate cake). The nonfood images consisted of various objects and sceneries without any context to eating behavior (bicycle, piano, pair of shoes, clouded sky etc.).

During the experiment, images were presented to each of the eight subjects in randomized order for a total time of 15 min. Each picture was shown on average three times for 6 s.

At the end of the second session, subjects were committed to evaluate the picture's taste intensity using the same scale as the 21 volunteers. This task was necessary in order to examine the objectivity in scoring the contents of the used pictures.

Experimental paradigm

The study consisted of two consecutive sessions with a 3- to 8-day interval. During each session, 5 ml blood was drawn from the adjacent room, using an intravenous cannula and a tubic extension for later analysis of plasma concentrations of ghrelin, leptin, and insulin every 15 min from 08:15 to 10:30, from 11:30 to 13:00, and every 10 min between 10:30 and 11:30. We choose a frequency of blood sampling which is higher than in most other studies on the course of ghrelin levels in humans (11,22), but in accordance to a recent study by Spiegel *et al*. (23). Similar to their study, we elevated the sampling frequency during the interval of major interest (10:30 to 11:30). Breakfast (1 cup of coffee, 2 buns, butter, jam, 2 slices cheese or sausage) and lunch (meat, pasta or potatoes, and vegetables and 2 glasses of water) were provided at 08:30 and 12:00, respectively. Such meals are typical for Germany.

Fifty pictures in 6-s intervals were presented from 10:30 to 10:45 showing neutral images during the first session and food pictures during the second session. This order was chosen to avoid anticipation in the subjects during the second session. A flow chart of the experiment is given in **Figure 1**.

Subjects were not informed before of the food-related content of the photographs presented on a computer screen from a distance around 1 m. They were informed in advance to receive breakfast and lunch during the experiment.

All subjects reported regular eating patterns including breakfast, lunch, and dinner. Study participants were advised to have dinner as usually and not to be sleep deprived before the experiment.

The experiment was carried out in a quiet room, and subjects were placed in a comfortable armchair in sitting position. Subjects were watched by video monitoring not to fall asleep. Beside breakfast, lunch, and the time for presentation on the computer screen, subjects were allowed to read magazines or newspapers, which were free of any eating-related matters.

At the end of each session at 13:00, subjects completed a recognition test, presenting five of the previously seen pictures with food or neutral content and five new pictures with food or neutral content randomly intermixed. Subjects rated whether each photograph had been seen previously (yes) or had not been seen (no) during the experiment. The number of correct answers was counted for each subject.

Statistical analysis

Metrical variables are expressed as mean ± s.d. Differences of mean ghrelin, leptin, and insulin plasma levels subsequent to presentation of neutral or food pictures at single time points were investigated only on the exploratory level. For inferential statistics, the area under the curve (AUC) determined according to the trapezoid rule and the mean location (ML) was taken into account for each hormone and each of two intervals (phases) namely the preintervention (10:00–10:30) and postintervention period (10:50–11:20). The phase

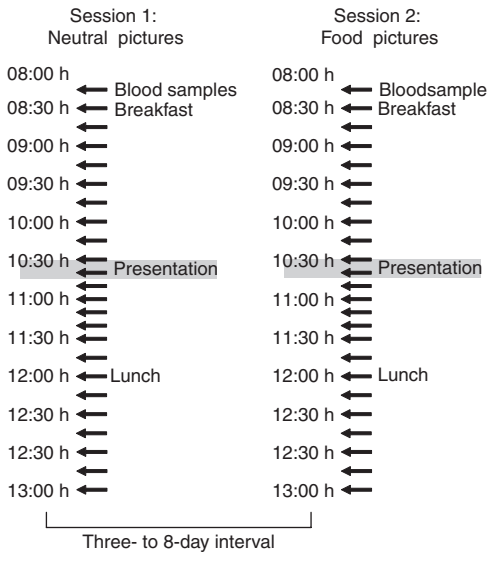

Figure 1 Flow chart of the experiment.

Table 1 Mean ± s.e.m. of the area under the curve (AUC) of the hormone concentration levels during intervals before (interval 1, 10:00–10:30) and after (interval 2, 10:50–11:20) picture presentation as well as of their increments (quotients of AUC after to AUC before presentation)

	Ghrelin			Insulin			Leptin		
Variables	Mean	s.e.m.	*N*	Mean	s.e.m.	*N*	Mean	s.e.m.	*N*
AUC_neut pict interval 1	368.19	37.58	8	42.82	8.14	8	7.08	1.72	8
AUC_neut pict interval 2	584.00	56.94	8	47.01	8.50	8	10.92	2.20	8
	$F(1,7) = 82.75, P < 0.0001$			$F(1,7) = 0.039, P = 0.551$			$F(1,7) = 46.19, P < 0.0001$		
AUC_food pict interval 1	327.93	30.97	8	49.47	9.04	8	6.77	1.69	8
AUC_food pict interval 2	619.42	63.60	8	42.54	7.67	8	10.50	2.31	8
	$F(1,7) = 72.08, P < 0.0001$			$F(1,7) = 0.030, P = 0.601$			$F(1,7) = 29.49, P = 0.001$		
Quot_neut (interval 2 to interval 1)	1.58	0.05	8	1.44	0.48	8	1.64	0.09	8
Quot_food (interval 2 to interval 1)	1.88	0.06	8	1.15	0.34	8	1.65	0.12	8
	$F(1,7) = 28.43, P = 0.001$			$F(1,7) = 0.025, P = 0.630$			$F(1,7) = 0.02, P = 0.887$		

Ghrelin and leptin but not insulin pointed to significant differences between the two intervals in both presentation modules. But only for ghrelin the increments showed significant differences between neutral and food pictures (*P* values < 0.05, by univariate F-tests in MANOVA).

and picture-stimulus effects on the AUCs and MLs of each of the hormones were then tested about significance by two-factorial multivariate analyses of variance with repeated measures designs. Thereby both influential factors "phase" and "picture stimulus" were two within-subjects-factors with two levels. To assess better the picture-stimulus effect on changes of the hormone levels before and after the stimulus, we calculated in addition for each hormone the quotients of the AUC (ML) in the postintervention phase to the AUC (ML) in the preintervention phase and then applied a multivariate analysis of variance on all of these quotients. Possible associations between the three hormones at each of the three intervals were tested about significance by means of the Pearson correlation coefficients. The Pearson correlation coefficient was also used to test agreement between subjects and raters in the scoring of the pictures' intensity of taste.

Differences in the nonendocrine data (recognition test) were tested by the nonparametric Wilcoxon matched pairs' tests. As nominal level of significance, $\alpha = 0.05$ was accepted and corrected by all post hoc tests according to Bonferroni procedure.

RESULTS

Ghrelin, insulin, and leptin

Table 1 shows some descriptive and inferential statistics concerning the AUC and ML values of the hormones. **Supplementary Figure S1** online shows ghrelin, insulin, and leptin levels of each subject from 08:15 to 13:00. ANOVA revealed for ghrelin significant interaction effects of the factors (Wilks multivariate test of significance; effect of phase × picture-stimulus: $F(2,6) = 5.87$, sig of $F = 0.039$), which were significantly remarkable on both the ML and AUC values as well (univariate F-tests, $P < 0.05$). By scrutinizing the simple effects of phase and picture stimulus on ghrelin, i.e., by investigating the phase differences in its ML and AUC values within each picture stimulus separately and vice versa, we identified that both ghrelin ML and AUC values increased significantly during the 30-min interval following the presentation of food pictures (intervention) compared to the preintervention interval. When neutral pictures were presented, then only ghrelin AUC values showed significant differences between the two presentation periods (tests with contrasts in multivariate analysis of variance (MANOVA), $P < 0.05$).

Leptin showed only a significant phase effect (Wilks multivariate test of significance; effect of phase: $F(2,6) = 19.26$, sig of $F = 0.002$) attributed exclusively to the AUC values (univariate F-tests, $P < 0.05$), whereas insulin seemed not to be affected neither from the picture stimulus nor from the intervention period, although their ML and AUC values before are stably greater than after intervention.

Additionally we compared the increments, i.e., the quotients of the ML or AUC values after to before presentation between the two types of pictures. We found a significant picture stimulus effect on the ghrelin increments (Wilks multivariate test of significance; effect of picture stimulus: $F(2,6) = 13.68$, sig of $F = 0.006$), attributed to both indicators ML and AUC (univariate F-tests, $P < 0.05$). Figure 2 gives an impression about the increments of ghrelin after the neutral and food pictures. Neither for leptin nor for insulin significant picture stimulus effects on the quotients of the ML or AUC values were found.

As expected, ghrelin decreased after breakfast (mean maximum ghrelin: neutral picture session at 08:15: 210.0 ± 36.2 pg/ml; food picture session at 08:30: 234.0 ± 30.9 pg/ml) to the lowest ghrelin values between 09:30 and 10:30 (mean minimum ghrelin: neutral picture session at 09:30: 166.0 ± 24.8 pg/ml, food picture session at 09:30: 152.0 ± 21.7 pg/ml). Shortly after lunch at 12:15, ghrelin reached its maximum in both sessions (neutral picture session: 261.0 ± 44.9 pg/ml, food picture session: 247.0 ± 35 pg/ml).

Insulin increased to peak after breakfast between 09:15 and 09:45 (mean maximum insulin: neutral picture session at 09:30: 52.9 ± 24.9 pg/ml, food picture session at 09:15: 56.6 ± 49.1 g/ml) and fell steadily before lunchtime to its nadir (mean minimum insulin: neutral picture session at 12:00: 7.7 ± 4.5 pg/ml, food picture session at 11:45: 8.4 ± 28.1 pg/ml). After lunch at 12:45, insulin increased again to a postprandial surge

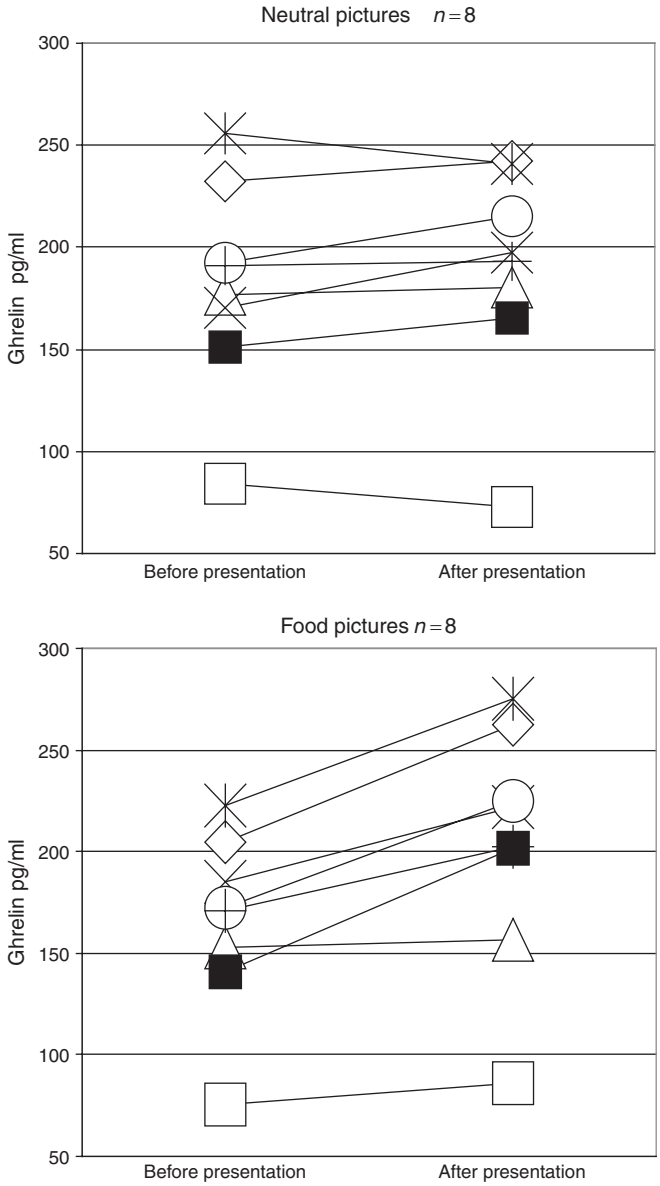

Figure 2 Ghrelin concentration levels of the single individuals before (interval 1, 10:00–10:30) and after (interval 2, 10:50–11:20) presentation of neutral and food pictures ($n = 8$).

(neutral picture session: 42.4 ± 24.5 pg/ml, food picture session: 36.7 ± 28.1 pg/ml).

Plasma leptin decreased mildly from 08:15 until 09:30. Apart from that, leptin did not display an interpretable variation in the course of time.

Association between ghrelin, insulin, and leptin

Not any significant correlation was found between the AUC values of the hormones ghrelin, insulin, and leptin, in each of the two experimental phases, neither for the neutral nor for the food picture presentation (Pearson correlation coefficients. *P* values n.s., see **Table 2**). However, when taking into account the increments of the AUC values after to before picture presentation, we found a significantly positive association ($r = 0.767$, $P < 0.05$) between insulin and leptin by the presentation of neutral pictures and a significantly negative association ($r = -0.736$, $P < 0.05$) between ghrelin and insulin by the presentation of food pictures. That means that increases of ghrelin AUC after pictures showing food are related with decreases of insulin AUC.

Furthermore, we analyzed by contrast tests the single effects, i.e., interval differences within each picture presentation-modus and vice versa differences in picture presentation-modus within each interval and identified that ghrelin levels during the 30-min interval following the presentation of food pictures (intervention) increased significantly (more than 18%), compared to the preintervention interval (tests with contrasts in ANOVA, $P < 0.05$). In contrast, after presentation of the neutral pictures, ghrelin levels remained unchanged (+3%). The difference in the increases between the two conditions was also significant for ghrelin ($P < 0.05$, Wilcoxon tests for matched samples).

Insulin and leptin showed no significant differences, neither between the picture presentation conditions nor between the intervals.

Recognition test

Subject 1 did not recognize four of 10 pictures in both sessions. Subject 5 and 8 made a mistake in one experimental session.

There is no significant correlation between the results of the recognition test and ghrelin increase.

Subjective taste rating

For the set of the 50 food pictures, we found a high correlation ($r = 0.7516$, $P < 0.0001$) between the averaged scoring of the 21 raters and the eight subjects of the study. That indicates a relative high objectivity in scoring the contents of the pictures.

We did not find a significant correlation between the mean taste scores and the increase of ghrelin ($r = -0.53$).

DISCUSSION

Our major findings are visual presentation of food resulted in a significant increase of ghrelin (AUC: 30%) compared to the time interval before the visual presentation. This change differed significantly from the presentation of neutral pictures, which did not prompt a significant increase in ghrelin levels. The course of leptin and insulin concentration was not affected by the visual presentation. As expected, ghrelin decreased between 09:30 and 10:30 to its lowest levels and increased at lunchtime. Insulin raised to peak after meal times. A negative association between the AUCs of ghrelin and insulin after presentation of food pictures was found. The taste rating of the presented food pictures at the end of the experiment was not correlated to plasma ghrelin levels.

As far as we know, this is the first study demonstrating an effect on peripheral ghrelin plasma levels, mediated by an external cue such as visual presentation of hedonic food. This supports the view that peripheral ghrelin secretion is regulated by the central nervous system (12). In subjects fasting for 24 h, ghrelin levels increased and spontaneously

Table 2 Pearson correlation coefficients of the hormone AUC values and their increments (quotients) in the two intervals (before and after picture presentation)

	Association intensity between the hormones					
	Interval 1		Interval 2		Quotients of AUCs (interval 2 to interval 1)	
	Insulin	Leptin	Insulin	Leptin	Insulin	Leptin
Presentation of neutral pictures						
Ghrelin	0.1781	0.5853	–0.3786	0.3054	0.2054	0.5286
	$P = 0.673$	$P = 0.127$	$P = 0.355$	$P = 0.462$	$P = 0.626$	$P = 0.178$
Insulin		0.4908		0.5222		0.7672
		$P = 0.217$		$P = 0.184$		$P = 0.026$[a]
Presentation of food pictures						
Ghrelin	0.2319	0.4312	–0.6175	0.3209	–0.7363	0.5329
	$P = 0.581$	$P = 0.286$	$P = 0.103$	$P = 0.438$	$P = 0.037$[a]	$P = 0.174$
Insulin		–0.2232		–0.0442		–0.0873
		$P = 0.595$		$P = 0.917$		$P = 0.837$

By the presentation of neutral pictures the increments of insulin were significantly positive correlated with the increments of leptin, whereas by the presentation of food pictures the increments of insulin were significantly negative correlated with those of ghrelin (P values < 0.05).
AUC, area under the curve.
[a]Significant values.

decreased at customary mealtimes (22), which points to a cephalic mechanism in terms of a circadian rhythm or a cognitive process such as a conditioned reflex. In our study, food pictures were presented at a time (at 10:30) where food intake usually is not expected and ghrelin levels reach their lowest point between breakfast and lunch. Viewing food pictures at that time resulted in a significant increase in the ghrelin plasma level compared to the interval before the presentation of food images, which is a weaker effect than the increase in ghrelin at mealtimes in our experiment and in previous studies (10,11). We did not assess the feeling of hunger in our subjects, because we did not want to call their cognitive attention on appetite-related contents beside the visual presentation. We suggest that our subjects did not feel very hungry before the presentation of pictures, because there is evidence that the temporal course of ghrelin is closely positively correlated with subjective hunger scores. Low ghrelin plasma levels and low hunger scores were reported 60–150 min after a meal (11), the time period where our subjects received the visual presentation. So we assume that the increase of ghrelin is related to the visual stimulus and not induced by an internal cue such as a conditioned time schedule for food intake or a (preexisting) feeling of hunger. Otherwise, the relative moderate rise of ghrelin after the visual food stimuli indicates that there are other, maybe additional mechanisms causing the stronger ghrelin surge at mealtimes.

There is a strong evidence that ghrelin mainly produced in the stomach induces its orexigenic effects via the vagus nerve and the brainstem to the hypothalamus (24). In subjects, who had surgery involving vagotomy, ghrelin did not stimulate food intake (25). On the other hand, there is an active transport of ghrelin through the blood–brain interface (26). Perception of hedonic food by the central nervous system appears to stimulate ghrelin secretion in the stomach. The elevated ghrelin levels may then induce appetite via the vagus nerve or an active transport through the blood–brain barrier to the central nervous system.

The pictures with food content, which were used as a visual stimulus for the experiment, were chosen based on rating in healthy male volunteers, around 2–3 h after food intake, and illustrated highly preferred savory or sweet food. Subjects participating in the ghrelin–visual stimuli experiment rated the appetizing appearance of the food images shortly after lunch in a satiated condition. These different states of hunger did not influence the valuation of the food pictures. Furthermore, we also did not find a significant correlation between the taste scores and the increase in ghrelin, though this evidence is statistically limited by the small number of subjects in the present study.

Leptin is mainly produced in the adipose tissue, and peripheral leptin levels are approximately proportional to body fat content. A small amount of leptin is secreted by the stomach (27). Therefore, it could play a role in the short-term regulation of food intake or food digestion/absorption (28). In our study, we did not find a correlation between leptin and ghrelin or leptin and insulin. Leptin plasma levels did not show any variation after the visual presentation. The mild decrease in leptin after breakfast is consistent with the observation that leptin displays a diurnal rhythm with a nadir around 09:00 (10,29). Consistent with previous reports (11,30), the insulin level increased after breakfast within an average of 60 min and decreased steadily toward their basal value shortly before lunch. Insulin is thought to have a modulating effect on ghrelin (31), likewise hyperinsulinemia suppressed peripheral ghrelin secretion (32) and in turn ghrelin inhibited insulin secretion (19,33). The negative association between ghrelin and insulin in our study supports the hypothesis of an insulin suppressing effect of physiologic doses of ghrelin in humans (34).

However, the increase in ghrelin after visual food stimuli in our study did not modulate insulin secretion. The rising of insulin levels known to be closely linked with carbohydrate ingestion (30) was not affected by sight of food per se.

Our finding suggests that an external cue such as sight of hedonic food elevates ghrelin levels in young, healthy subjects of normal weight. The exposure to the food stimuli was carried out at a time point, where subjects were near to the intermediate state on the continuum hungry to sated. Leptin and insulin were not affected by the visual presentation. It is possible that ghrelin is directly controlled by the central nervous system and that as reported before from other research groups sympathetic nerves act as a signaling pathway of the gut–brain axis.

In our study, secretion of ghrelin, the strongest orexigenic hormone known so far, was induced by perception of pictures with food. This finding supports the hypothesis that environmental factors contribute to eating behavior in modern society, where the visual presentation of food products is common.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Rolls BJ, Rowe EA, Rolls ET. How flavour and appearance affect human feeding. *Proc Nutr Soc* 1982;41:109–117.
2. Cornell CE, Rodin J, Weingarten H. Stimulus-induced eating when satiated. *Physiol Behav* 1989;45:695–704.
3. Marcelino AS, Adam AS, Couronne T, Köster EP, Sieffermann JM. Internal and external determinants of eating initiation in humans. *Appetite* 2001;36:9–14.
4. Valassi E, Scacchi M, Cavagnini F. Neuroendocrine control of food intake. *Nutr Metab Cardiovasc Dis* 2008;18:158–168.
5. van der Lely AJ, Tschöp M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* 2004;25:426–457.
6. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908–913.
7. Wren AM, Small CJ, Ward HL *et al*. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000;141:4325–4328.
8. Wren AM, Seal LJ, Cohen MA *et al*. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001;86:5992–5995.
9. Schmid DA, Held K, Ising M *et al*. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. *Neuropsychopharmacology* 2005;30:1187–1192.
10. Cummings DE, Purnell JQ, Frayo RS *et al*. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;50:1714–1719.
11. Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 2004;287:E297–E304.
12. Mundinger TO, Cummings DE, Taborsky GJ Jr. Direct stimulation of ghrelin secretion by sympathetic nerves. *Endocrinology* 2006;147:2893–2901.
13. Cummings DE, Overduin J. Gastrointestinal regulation of food intake. *J Clin Invest* 2007;117:13–23.
14. Killgore WD, Young AD, Femia LA *et al*. Cortical and limbic activation during viewing of high- versus low-calorie foods. *Neuroimage* 2003;19:1381–1394.
15. Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science* 2003;301:1104–1107.
16. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metab* 2008;7:400–409.
17. Sun Y, Asnicar M, Smith RG. Central and peripheral roles of ghrelin on glucose homeostasis. *Neuroendocrinology* 2007;86:215–228.
18. Nogueiras R, Tschöp MH, Zigman JM. Central nervous system regulation of energy metabolism: ghrelin versus leptin. *Ann N Y Acad Sci* 2008;1126:14–19.
19. Broglio F, Arvat E, Benso A *et al.* Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 2001;86:5083–5086.
20. Broglio F, Gottero C, Prodam F *et al*. Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycaemia but unaffected by glucagon and arginine in humans. *Clin Endocrinol (Oxf)* 2004;61:503–509.
21. Chapelot D, Aubert R, Marmonier C, Chabert M, Louis-Sylvestre J. An endocrine and metabolic definition of the intermeal interval in humans: evidence for a role of leptin on the prandial pattern through fatty acid disposal. *Am J Clin Nutr* 2000;72:421–431.
22. Natalucci G, Riedl S, Gleiss A, Zidek T, Frisch H. Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *Eur J Endocrinol* 2005;152:845–850.
23. Spiegel K, Tasali E, Leproult R, Scherberg N, Van Cauter E. Twenty-four-hour profiles of acylated and total ghrelin: relationship with glucose levels and impact of time of day and sleep. *J Clin Endocrinol Metab* 2011;96:486–493.
24. Murphy KG, Dhillo WS, Bloom SR. Gut peptides in the regulation of food intake and energy homeostasis. *Endocr Rev* 2006;27:719–727.
25. le Roux CW, Neary NM, Halsey TJ *et al*. Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. *J Clin Endocrinol Metab* 2005;90:4521–4524.
26. Banks WA, Tschöp M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* 2002;302:822–827.
27. Bado A, Levasseur S, Attoub S *et al*. The stomach is a source of leptin. *Nature* 1998;394:790–793.
28. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev* 2007;8:21–34.
29. Weigle DS, Cummings DE, Newby PD *et al*. Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. *J Clin Endocrinol Metab* 2003;88:1577–1586.
30. Erdmann J, Töpsch R, Lippl F, Gussmann P, Schusdziarra V. Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. *J Clin Endocrinol Metab* 2004;89:3048–3054.
31. Broglio F, Prodam F, Riganti F, Muccioli G, Ghigo E. Ghrelin: from somatotrope secretion to new perspectives in the regulation of peripheral metabolic functions. *Front Horm Res* 2006;35:102–114.
32. Flanagan DE, Evans ML, Monsod TP *et al*. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab* 2003;284:E313–E316.
33. Akamizu T, Takaya K, Irako T *et al*. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J Endocrinol* 2004;150:447–455.
34. Castañeda TR, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010;31:44–60.