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ORIGINAL RESEARCH

The Effect of Potato Protease Inhibitor II on Gastrointestinal Hormones and Satiety in Humans During Weight Reduction

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Context: It is questioned whether the potato protein protease inhibitor II (PI2) reduces appetite and exerts effects on the satiety hormone cholecystokinin (CCK).

Objective: To investigate PI2 impact on gastrointestinal hormones and appetite measures during weight reduction.

Design: In a randomized, placebo-controlled trial over 20 weeks, fifty-two overweight/obese participants (BMI 25.2–38.0 kg/m^2) received a protein-rich diet (30%) adjusted to 500 kcal below their individual daily needs. Subjects ingested a capsule containing either PI2 (150 mg) or placebo twice daily 1 hr before lunch and dinner. At week 0 and week 10 participants joined breakfast test meals to determine CCK, GLP-1, ghrelin, leptin, glucose and insulin concentrations in a time course experimental manner. Appetite sensations were measured on test meal days and in week 4, 9, 14 and 19 using visual analogue scales.

Results: Weight loss at week 10 and 20 in the PI2 group was 4.3±3.1 kg and 5.6±4.1 kg, in the control group: 4.7±4.0 kg and 6.8±3.7 kg. A significant effect of PI2 on circulating CCK levels was observed at week 10. The other hormones were unaffected by PI2. At week 10, PI2 group subjects showed higher satiety and decreased desire to eat compared to placebo. During study duration, PI2 showed a significant impact on appetite ratings prior to lunch, one hour before dinner and just before dinner.

Conclusion: PI2 increased circulating CCK plasma levels during the diet intervention. Likewise, PI2 modulated appetite sensation from week 4 to 20. The study demonstrated that the PI2 can modulate a key satiety signal.

Keywords: dietary supplement, appetite, satiety modification, obesity

Introduction

Gastrointestinal peptide hormones such as leptin, ghrelin, cholecystokinin (CCK) or glucagon-like peptide 1 (GLP-1) influence the biochemical processes controlling hunger and satiety and therefore have a therapeutic value in conditions such as obesity.[1,2] However, when orally delivered, the peptides are not effective, because they are degraded by enzymes in the gastro-intestinal tract. This enzymatic degradation of the hormones may be slowed down by protease inhibitors (PIs) from plants.[3,4] The naturally occurring protein in potatoes, the protease inhibitor II (PI2), has been shown to inhibit serine proteases such as trypsin and chymotrypsin, thereby enhancing enzymatic activity.[5,6]

In the gastrointestinal tract CCK is synthesized in endocrine and neuronal cells in response to intraluminal stimuli associated with ingestion of a meal.[7] High levels of circulating CCK reduce food intake in humans.[8] It has been reported that by the use

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of PI2 as a supplement in food, the levels of CCK increased and the caloric intake at the next meal was reduced.[4]

During a meal, the CCK-releasing peptide (CCK-FP) is secreted into the intestinal lumen. A proteinase inhibitor now causes the CCK-FP to degrade with delay. CCK-FP stimulates the release of CCK from endocrine cells into the small intestine and thus increases the concentration of CCK.[9] Luminal trypsin activity inhibition and direct stimulation of CCK secretion diminished the food consumption.[10–12]

Although, further clinical studies in humans with PI2 as a putative functional agent for appetite control showed different results. In response to PI2 intake, reduced energy intake,[3,4] lowered subjective appetite rates[9,13,14] and increased CCK levels[3,13,15,16] were reported. On the contrary, Peters et al found no efficacy on subjective appetite measures, ad libitum energy intake or CCK concentrations.[17]

In obesity and after weight reduction there are altered gut and adipose tissue hormones and changes are associated with disturbances in the gut-brain axis.[18,19] Regarding appetite sensations, previous studies show different results from the effects of weight loss, with self-reported perceptions of hunger and appetite increasing or generally decreasing.[20,21]

For a deeper insight into the potential of PI2 (150 mg), the study investigated the effect of PI2 on appetite measures in overweight and obese subjects during a 20-week diet. In order to investigate the influence of PI2 on the gastrointestinal hormones CCK, GLP-1, ghrelin, insulin and leptin, test meals were taken at the beginning and after 10 weeks.

Subjects and Methods

Subjects

Fifty-two healthy overweight and obese women (n=41) and men (n=11), all non-smokers, aged between 24 and 60 years and a body mass index of 25.2–38.0 kg/m^2, were recruited from the University Obesity Outpatient Clinic and from advertisements on bulletin boards at the University of Ulm (Table 1). All subjects underwent a medical examination and were evaluated in good health. None of them reported a history of diabetes, high blood pressure, cardiovascular or endocrine diseases, chronic illnesses or cancer. The weight of each volunteer had been stable in the previous 3 months (<2 kg change).

Study Protocol

The study was a prospective, randomized, double-blind, placebo-controlled parallel comparison trial over 20 weeks, which was approved by the Ethics Committee of the University of Ulm. All participants gave their written informed consent before the start of the study. This trial was conducted in accordance with the Declaration of Helsinki. The ratio of test compound to placebo control was 3:1 with a total number of 52 inclusions, whereby study subjects were randomized by computer-generated code. The weight reduction diet included three main meals and each subject was instructed to take one capsule with either 150 mg PI2 or placebo (microcrystalline cellulose) one hour before lunch and one hour before dinner every day with a glass of water.

At a screening visit subjects' energy requirements were measured by bioelectrical impedance analysis (BIA, Body Cell Analyzer, Biodynamics, BIA 450, Seattle, USA). A dietitian instructed each patient how to prepare meals according to individual needs, 500 kcal less daily for the weight loss period of 20 weeks. The diet was protein-rich with 30 E% protein, 40–45 E% carbohydrate and 25–30 E% fat. Breakfast was a commercially available formula shake (F1, Herbalife Nutrition, Germany), lunch and dinner consisted of conventional food. No adverse effects for diet or study compound PI2 were reported from any of the subjects.

Table 1 Baseline Characteristics of Study Participants

Group	PI2 Group			Control Group		
	All	Men	Women	All	Men	Women
n	39	9	30	13	2	11
Age (yrs)	46.9±8.1	45.6±9.2	47.3±7.9	42.8±11.2	46.0±9.8	42.2±11.8
Height (cm)	166.0±8.1	176.1±4.3	163.0±6.4	166.0±5.8	170.5±2.1	165.2±6.0
BW (kg)	85.8±13.8	98.5±11.5	82.1±12.3	87.4±9.7	94.8±13.0	86.0±9.1
BMI (kg/m^2)	30.9±3.5	31.7±2.8	30.8±3.7	31.6±2.4	32.6±3.7	31.4±2.3
WC (cm)	100.4±8.9	108.5±8.9	98.2±7.5	102.1±8.8	111.5±12.0	104.4±7.6
HipC (cm)	109.9±8.2	109.2±7.0	110.1±8.6	112.0±7.6	105.0±4.2	113.2±7.5
Sagittal⦸(cm)	23.8±2.9	24.1±1.8	23.7±3.1	24.3±2.9	26.4±7.0	23.8±1.9

Notes: Values are means ± SD, statistically there were no differences between the PI2 group and the placebo group.

The subjects filled three-day diet diaries (two weekdays, one weekend day) in weeks 0, 4, 9, 14, and 19. The self-reported food documents were checked and analyzed by a registered nutritionist blinded for the study.

Anthropometry

The subjects arrived at the research facility at 08.00 a.m. after an overnight fast. The body weight in underwear was measured at the beginning, after 5, 10 and 20 weeks on a calibrated scale with an accuracy of 0.1 kg. At the start of the study, the standing height of each participant was measured with a precision of 0.5 cm. Waist circumference was measured monthly with a non-stretchable tape, with the participants in an upright position and measurements were taken midway between the lower ribcage and iliac crest with an accuracy of 0.5 cm. To measure the sagittal diameter, the subjects were supine and a caliper was used midway between the lower ribcage and iliac crest with an accuracy of 0.1 cm. After 10 mins of rest, blood pressure on the left arm was measured with an automated blood pressure monitor (Colin Corp, 2007–1 Hayashi, Komaki-city, Japan).

Biochemical Determinations

In order to monitor health status and physiological shape of study participants' blood samples were drawn at baseline, after 10 and after 20 weeks by puncture of a superficial arm vein, and measurements of insulin, blood glucose, glycated hemoglobin A1c (HbA1c), triglycerides (TG), cholesterol, HDL-cholesterol, aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (ALP), gamma-glutamyltransferase (GGT), creatinine, and blood urea nitrogen were performed with routine methods (Roche Cobas automatic analyzers) at the Institute for Clinical Chemistry of the University Hospital Ulm.

Insulin resistance (IR) was defined on homeostasis model assessment (HOMA-IR), which was calculated according to the following formula: fasting insulin (μU/mL) x fasting glucose (mmol/L)/405 (25). IR was defined as being in the highest quantile of HOMA score (≥2.0), according to the previous studies.[22]

Plasma Hormone Measurements and Test Meal Procedure

For the time course analysis of hormonal changes, which are subject to food uptake, the participants stayed 5 hrs in the nutrition research facility at baseline of the study and after 10 weeks for measurements of the hormone levels after a test meal. After fasting overnight for ≥12 h, an intravenous cannula was inserted and blood was drawn at −60, 0 (followed by food intake) and 5, 10, 15, 30, 60, 90, 120 and 180 min after ingestion of the test meal. Venous blood (7 mL) was collected in Lavendar Vacutainer EDTA-tubes pre-coded with aprotinin (500 KIU/mL blood; Sigma-Aldrich Chemistry, Steinheim, Germany). Each sample was carefully inverted and kept on ice. Within 1 h post-collection, the samples were centrifuged for 15 mins at 3000 g. Plasma was collected and stored at −80°C until further analysis. All samples of each individual were measured in the same assay.

Plasma ghrelin was measured using a commercially available radioimmunoassay with an analytical sensitivity of 40 pg/mL and an inter- and intra-assay coefficient of variation (CV) <10% (Mediagnost, Reutlingen, Germany). Leptin was measured using a commercially available RIA-Kit from Biotrend Chemikalien, Cologne, Germany. The intra-assay and inter-assay CVs were <5% and <7.6%, respectively, with a detection limit of 0.44 ng/mL. GLP-1 ELISA-kit from Biotrend (Biotrend Chemikalien GmbH, Köln, Germany) had a detection rate of 12.35–1000 pg/mL with a sensitivity of 5.44 pg/mL. The radioimmunoassay for CCK was purchased from Phoenix (Phoenix Pharmaceutical, Inc., Germany), the intra- and inter-assay CVs were 5–7% and 12–15%, respectively, and detection limit ranging from 10–1280 pg/mL. Before CCK measurement the peptide was extracted from plasma according to the manufacturer's recommendation. The extracted samples were dried, re-suspended in elution buffer, and stored on ice until assayed.

One hour before test meals, a capsule with verum or placebo was taken according to the randomization data. The standard test meal consisted of a commercially available formula shake combining 26 g basic powder (Herbalife Nutrition, Formula 1, healthy meal nutritional shake mix) with 10 g soy-protein (Herbalife Nutrition, Formula 3, protein powder) and 125 g mixed berries in 250 mL of semi-skimmed milk (1.5 % fat), all stirred with a hand blender. The meal provided 390 kcal, 28 g protein (30 energy %), 12 g fat (28 energy %), 38 g carbohydrate (40 energy %) and 10 g fiber. Nothing else was eaten until the end of the experiments, but subjects were allowed to drink bottled water.

Appetite Rating

Measures of satiety, hunger, desire to eat, and prospective consumption (amount of food that could be eaten) were

assessed using 100-mm visual analogue scales (VASs) inquiring questions such as "How hungry do you feel?", "How full do you feel?", "How strong is your desire to eat?", "How much food do you think you could eat?" VASs are widely used to measure subjective appetite perceptions in dietary studies.[23–25] Participants filled out home-based visual analogue scales during 5 consecutive days in week 4, 9, 14, and 19 one hour before lunch, as well as 1, 2, and 3 hrs thereafter plus one hour before dinner and just before dinner.

At test meal days, the VAS were filled out one hour prior to the test meal, just before eating, and 1, 2 and 3 hrs thereafter.

Statistics

Data are presented as mean changes from baseline ± standard error of the mean (SEM) unless otherwise stated. All subjects who completed the study were included in the data analysis, independent of reported dietary compliance as indicated by food records, or lack of weight loss according to intention-to-treat analysis. Hormone and appetite data were expressed as absolute values or as changes from baseline, during weight reduction. Serial measurements were analyzed by repeated measures ANOVA, with time and treatment as factors. Areas under the curve (AUCs) for hormone concentrations and appetite ratings were determined using the trapezoid rule. For the assessment of the appetite profile during the four weeks combined data of all reports were used. Clinical characteristics, biochemical markers, analysis of food diaries and individual time points with normal distributed data of both groups were compared using Student's *t*-test. Non-normal dispersed data were analyzed with the Mann–Whitney *U*-test. For paired comparison of not normal distributed values the Wilcoxon's paired test was applied. Relationships between hormones and subjective appetite were analyzed using bivariate correlations. A p-value <0.05 was regarded as statistically significant. Statistical procedures were performed using SPSS 21 (SPSS Inc., Chicago, Illinois, USA).

Results

Compliance and Baseline Clinical Parameters

Fifty-two subjects entered the study, 39 in the PI2 group and 13 in the placebo group. At termination of the study, 36 subjects finished successfully in the PI2 group and 11 in the placebo group. Baseline characteristics of subjects are summarized in Table 1 with no differences found between the groups.

Anthropometric Measures and Biochemical Data After Dietary Intervention

Ten weeks after start of the study, weight loss was 4.3 ±3.1 kg in the PI2 group and 4.7±4.0 kg in the control group ($p<0.001$, each within group). After 20 weeks of study duration, the weight loss was 5.6±4.1 kg in the PI2 group and 6.8±3.7 kg in the CG ($p<0.001$, each within group) (Table 2).

Waist circumference and the sagittal diameter decreased significantly in all study subjects ($p<0.05$, both groups each) (Table 2). As for blood pressure, the dietary intervention showed only a significant lower effect on systolic blood pressure in the PI2 group ($p<0.001$).

Basal blood glucose concentration of the study participants remained similar throughout the study, whereas insulin level showed a slight decrease. This had an effect on the HOMA-IR, which showed lower values in both groups in the course of the study ($p<0.05$). In the PI2 group, cholesterol, triglycerides and the liver enzymes AST, ALT, ALP and GGT declined significantly ($p<0.05$, each). HDL-cholesterol, hemoglobin A1c, creatinine and blood urea nitrogen levels were not affected in either group (data not shown) (Table 2).

Dietary Intake

On average, 78% of all subjects delivered evaluable food records. At screening visits, only 60% of dietary records were analyzable, because they contained partly unreliable data (obviously underreporting of food intake), the portion information was incorrect or the diary was not returned.

The proportion of records that were suitable for analysis increased gradually up to 83% until the end of the study (Table 3).

At baseline, energy intake was similar in all subjects (PI2 group: 1675±641 kcal/day vs CG: 1743±623 kcal/day). According to the recommendations for weight reduction, total calorie consumption decreased, but the lower caloric intake was not being maintained continuously. Over time the unintended trend went back towards higher energy intake. However, it should be noted that study participants adhered to the protein-rich and low-carbohydrate diet in both groups equally.

Table 2 Changes in Anthropometric Measures, Body Composition and Biochemical Characteristics According to PI2 Group (PI2) or Control Group (CG) After 20 Weeks of Dietary Intervention

Week		0	10	20	p
Body weight (kg)	PI2 CG	84.8±13.6 87.4±10.6	80.2±12.7 82.2±8.4	79.2±12.4 80.8±8.7	<0.001 <0.01
Waist circumference (cm)	PI2 CG	99.8±8.6 102.1±9.4	95.3±9.0 96.6±8.5	92.0±10.1 93.8±9.4	<0.001 <0.05
Sagittal diameter (cm)	PI2 CG	23.1±2.9 23.8±3.3	20.8±3.1 21.4±2.6	21.1±2.7 21.3±2.4	<0.001 <0.01
SBP (mmHg)	PI2 CG	140.9±18.2 135.1±12.5	132.6±15.8 132.8±9.5	132.7±13.6 131.0±12.7	<0.001 Ns
DBP (mmHg)	PI2 CG	80.1±9.2 75.6±8.3	77.4±9.3 74.6±10.1	77.5±9.5 75.3±8.6	Ns Ns
Blood glucose (mg/dL)	PI2 CG	94.3±10.5 98.6±12.8	90.7±13.4 93.2±12.6	93.7±11.2 96.7±12.0	Ns Ns
Insulin (µU/mL)	PI2 CG	10.5±5.4 10.0±5.2	8.6±4.1 7.8±4.5	8.1±3.9 7.4±4.2	<0.01 Ns
HOMA	PI2 CG	2.5±1.4 2.3±1.2	1.9±1.0 1.8±1.1	1.8±1.0 1.7±1.1	<0.01 0.05
TG (mmol/L)	PI2 CG	1.6±1.0 1.3±0.6	1.1±0.4 1.0±0.2	1.0±0.5 1.0±0.2	<0.05 Ns
AST (U/L)	PI2 CG	24.1±6.9 20.7±6.1	21.4±6.4 24.6±5.2	21.7±5.3 21.9±4.8	<0.05 Ns
ALT (U/L)	PI2 CG	33.8±20.2 25.7±16.5	26.9±13.6 25.2±9.6	23.3±12.0 25.6±4.5	<0.05 Ns
ALP (U/L)	PI2 CG	68.0±20.5 66.2±17.4	61.9±19.3 54.5±22.5	63.4±20.3 63.4±19.1	<0.001 Ns
GGT (U/L)	PI2 CG	31.9±28.9 26.6±28.4	22.4±15.8 22.6±11.1	24.3±2.7 19.6±13.5	<0.05 Ns

Notes: Values are mean values ±SD; p = after 20 weeks compared to baseline.
Abbreviations: PI2 group, intake of 150 mg potato protease inhibitor twice daily; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate-aminotransferase; ALT, alanine-aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl-transferase.

PI2 and Plasma CCK

Test Meal 1

CCK changes were not observed after test meal 1 in both groups (Figure 1Aa).

Test Meal 2

After 10 weeks of weight loss, a greater CCK release was observed in response to the test meal in the PI2 group during the study period. Postprandial AUC showed a higher CCK-concentration when PI2 was previously taken ($p<0.05$) (Figure 1Ba).

PI2 and Plasma GLP-1, Ghrelin, Leptin, Blood Glucose and Insulin

PI2 did not induce any effects on GLP-1, ghrelin, leptin, blood glucose and insulin at either test meal.

Test Meal 1

The GLP-1 concentration increased significantly immediately after food intake in all subjects with a plateau between 60 and 90 mins, and was still elevated at the end of the study ($p<0.0001$, each course) (Figure 1Ab).

Table 3 Energy (kcal) and Macronutrient Intake (%) of Available Dietary Diaries from PI2 and Control Group (CG) During the Study Course

Week	0	4	9	14	19
Number of evaluable diaries					
PI2	23	25	33	27	29
CG	8	10	11	10	11
Kcal/day (total energy intake)					
PI2	1675±641	1207±301**	1287±492**	1264±366**	1432±483*
CG	1743±623	1256±208	1436±428	1431±480	1515±608
Protein (E%)					
PI2	20.0±5.9	27.8±4.7**	27.7±6.3**	26.5±4.0**	28.4±11.9*
CG	17.7±5.3	26.6±5.8**	25.0±5.8**	27.2±5.6*	26.9± 5.7**
Carbohydrate (E%)					
PI2	43.7±8.1	41.1±7.3	39.4±7.1	38.2±6.9**	38.4±5.6
CG	46.7±5.6	40.0±8.9	41.3±5.5	42.9±5.0	38.6±5.1
Fat (E%)					
PI2	33.5±8.2	30.0±5.9*	30.8±7.2	31.1±6.2	32.1±6.3
CG	31.8±7.5	30.7±7.0	31.8±6.4	28.4±4.9	31.0±6.7
Alcohol (E%)					
PI2	3.6±1.6	2.0±4.2	2.2±4.3	4.0±5.0	1.5±2.8
CG	3.5±0.7	2.4±2.1	2.0±2.8	2.1±1.0	3.5±3.1

Notes: Values are means ±SD. **p<0.01, *p<0.05, compared to baseline, no significant difference between the groups.
Abbreviations: PI2 group, intake of 150 mg potato protease inhibitor twice daily.

Ghrelin concentrations increased significantly in both groups within one hour before the test meal (p<0.05). After eating the hormone concentration declined rapidly within the next 60 min, remained at low level for further 60 mins, reaching baseline levels after 180 min (p<0.0001, each course) (Figure 1Ac).

Leptin levels decreased significantly in all subjects without difference between the groups, and independent of any effect of PI2 intake (p<0.0001, each course) (Figure 1Ad).

Blood glucose and insulin (Figure 2A) showed a corresponding significant increase after the baseline test meal, with insulin remaining elevated longer than glucose levels. The patterns of hormonal changes were the same in all subjects (p<0.0001, each course).

Test Meal 2

The GLP-1 concentration increased similarly in both groups, although the overall increase was lower than after test meal 1 (Figure 1Bb).

Ghrelin (Figure 1Bc), leptin (Figure 1Bd), glucose and insulin (Figure 2B) showed similar courses in all subjects like in the first test meal.

Changes of Hormone and Glucose Concentration After Weight Loss

Figure 3 shows results for subjects of the PI2 group. Basal hormone concentration of CCK and ghrelin were significantly higher after the weight reduction (p<0.05 for CCK, p<0.01 for ghrelin), whereas leptin and insulin levels were significantly lower (p<0.001 for leptin, p<0.01 for insulin). Post-prandial concentrations were altered, higher levels were determined for CCK and ghrelin, lower levels were observed for leptin and insulin (AUC, p<0.01 each for these hormones). For GLP-1 and glucose concentrations no significant changes occurred.

Appetite Rating at Day of Test Meals

As reported by all subjects, on test meal days (week 0 and 10) appetite ratings one hour before eating were not

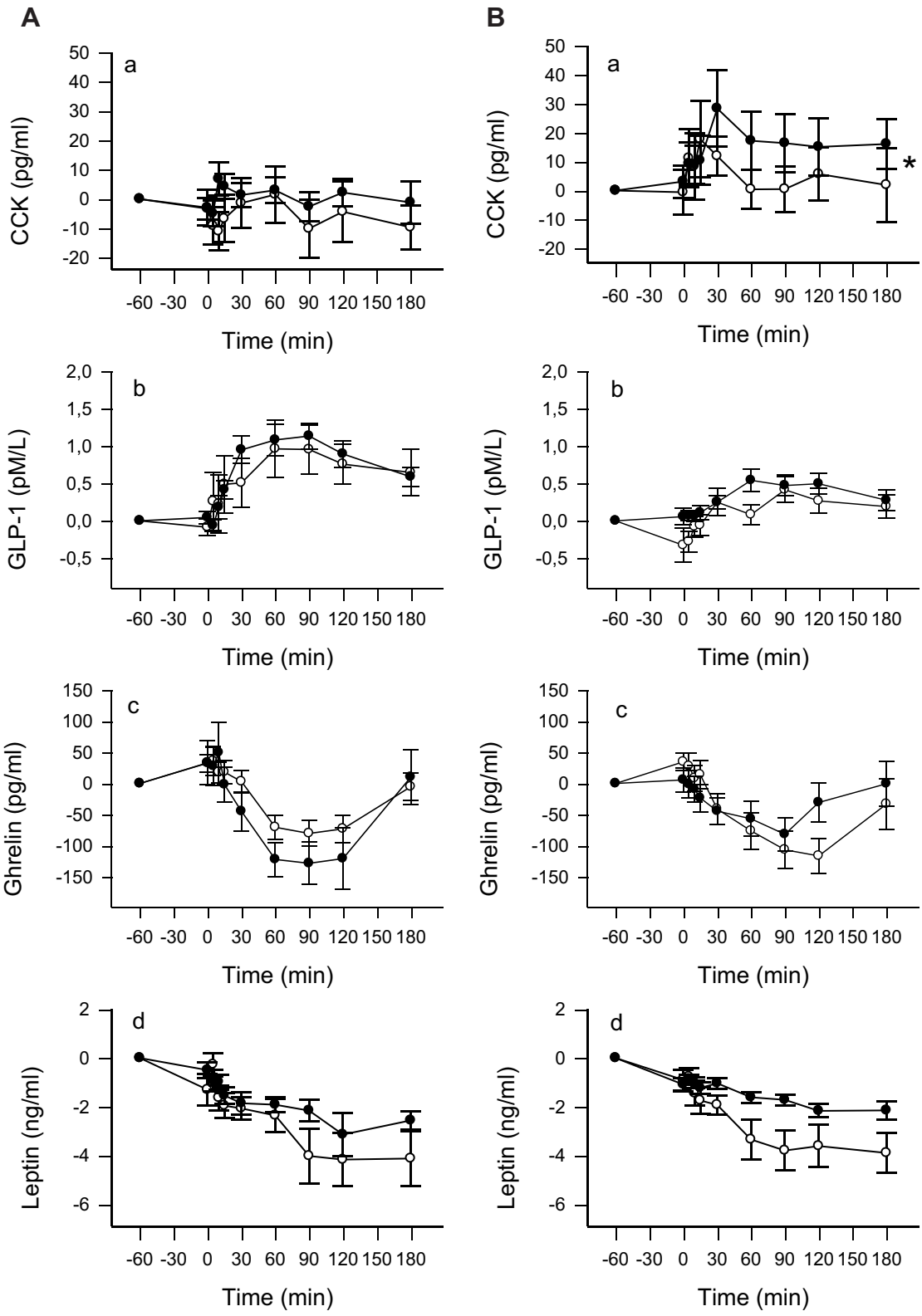

Figure 1 Change of CCK (a), GLP-1 (b), ghrelin (c) and leptin (d) concentrations for a fasting and meal-stimulated time course experiment in patients who ingested PI2 (●) or placebo (○) one hour before the test meal (taken at time 0 min), at the beginning of the study (column **A**) and after 10 weeks of weight reduction (column **B**). *AUC $p<0.05$ postprandial hormone release.

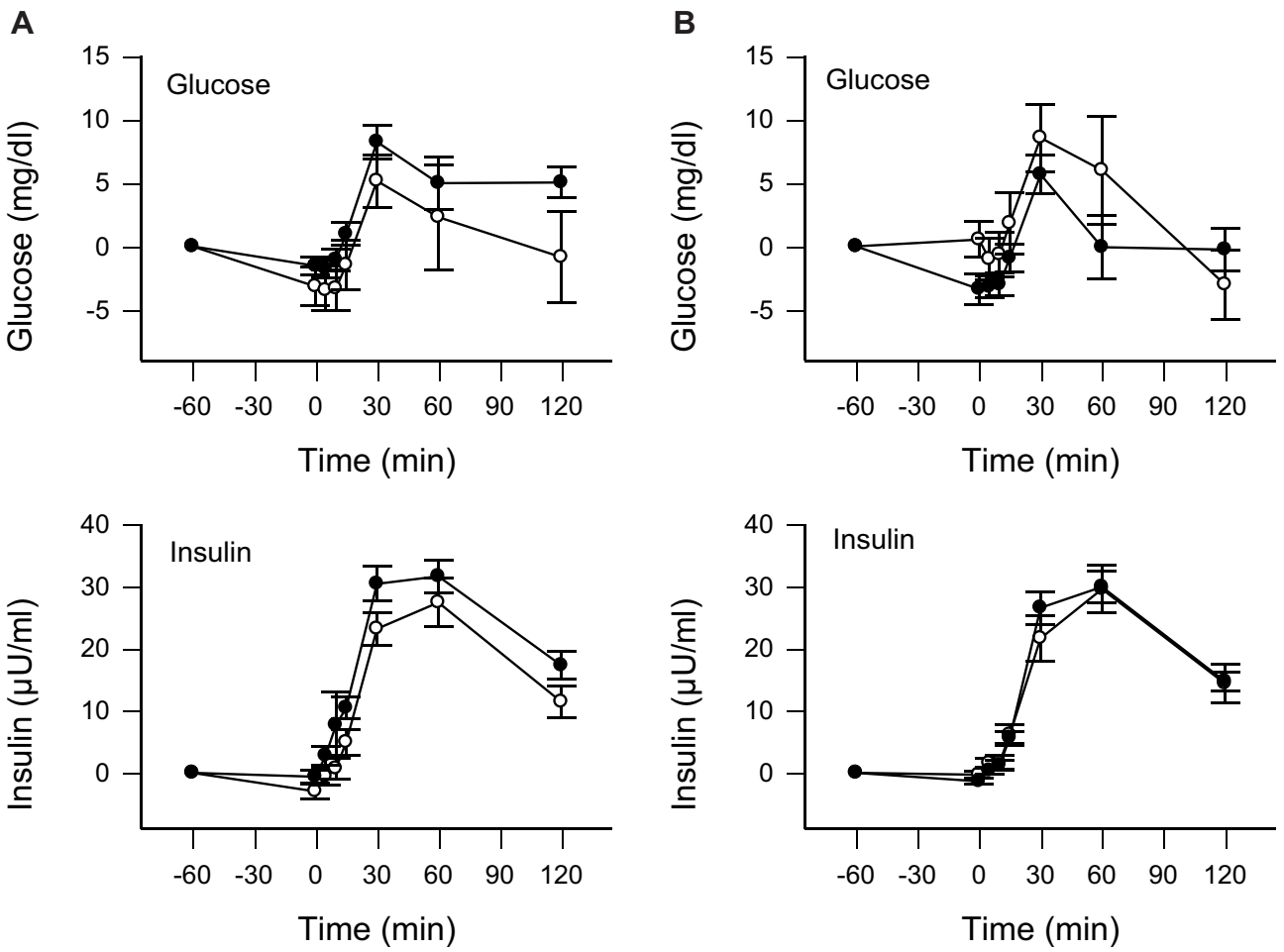

Figure 2 Change of glucose and insulin concentrations for a fasting and meal-stimulated time course experiment of in patients who ingested PI2 (●) or placebo (○) one hour before the test meal (taken at time 0 min) at the beginning of the study (column **A**) and after 10 weeks of weight reduction (column **B**).

different at any query. The results for change in VAS assessments are shown in Figure 4A for test meal at week 0 for the queries satiety (4a), hunger (4b), desire to eat (4c) and prospective consumption (4d), and in Figure 4B for test meal at week 10 for the queries satiety (4a), hunger (4b), desire to eat (4c) and prospective consumption (4d). PI2 or placebo was taken by the study participants one hour before the respective test meal after appetite rating (time −60 min).

At test meal week 0 after intake of PI2, no effect on the appetite sensations could be determined. After ten weeks, subjects who had taken PI2 showed similar results to test meal week 0. However, subjects who had taken placebo felt less saturated during the hour before the test meal ($p<0.05$) and had a greater desire to eat ($p<0.05$). No effect on the other appetite measures was observed.

Relationship Between Appetite Ratings and Gut Hormones

There was no association between CCK, GLP-1 and leptin levels and intensity of appetite measures. A significant correlation was determined between ghrelin and satiety, hunger and desire to eat ratings at week 0 ($p<0.05$) and week 10 ($p<0.01$). Insulin was significantly related to all subjective appetite ratings at both test meals ($p<0.01$, each).

Appetite Rating at Lunch and Dinner in Week 4, 9, 14 and 19

VAS data for the assessment of satiety, hunger, desire to eat and prospective consumption were each combined from the four weeks, since similar results were reported in each single week. For all appetite ratings, a significant favorable effect of PI2 compared to placebo was reported

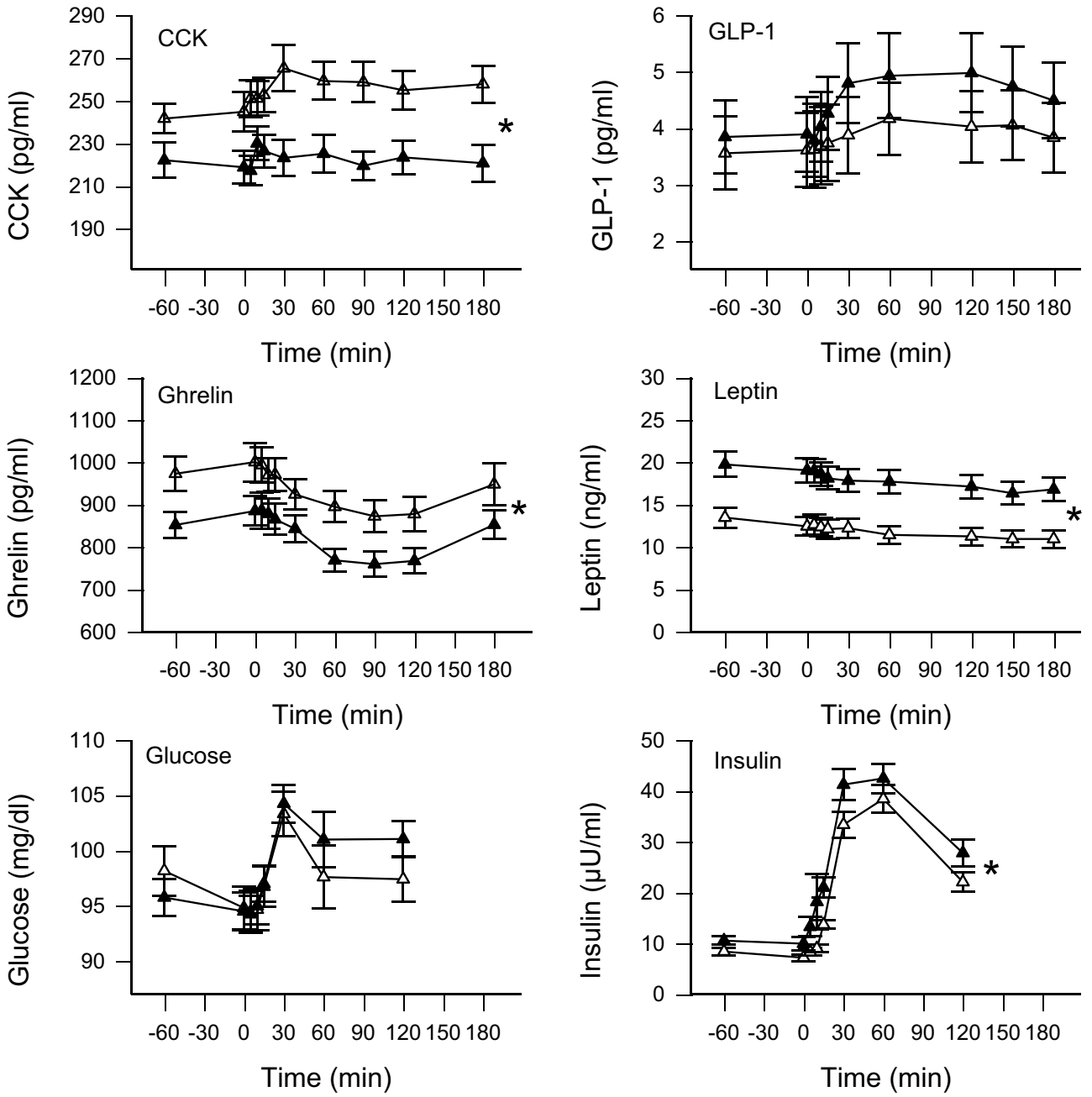

Figure 3 Absolute values of a fasting and meal-stimulated time course experiment analyzing CCK, GLP-1, ghrelin, leptin, glucose and insulin in patients who ingested PI2 one hour before the test meal (taken at time 0 min) at the beginning of the study (▲) and after 10 weeks of study duration (Δ). *AUC $p<0.01$ hormone concentration after 10 weeks.

by the study participants just before lunch, one hour before dinner and just before dinner ($p<0.05$) (Figure 5A–D).

Discussion

The study was conducted to investigate the effect of protein protease inhibitor II on gastrointestinal hormones and satiety sensation in obese subjects during a 20-week weight loss intervention. We report a significant effect of PI2 on circulating CCK plasma levels in response to standardized test meal in study participants, but only after a weight loss phase of 10 weeks. An effect of PI2 intake on subjective appetite was determined on the second test meal day and during the course of the study before lunch and at dinner.

The results of our study demonstrate that PI2 exerts an effect on the gastrointestinal hormone CCK in humans, but individual conditioning appears to be critical. Hormones from the gastrointestinal tract and from adipose tissue are

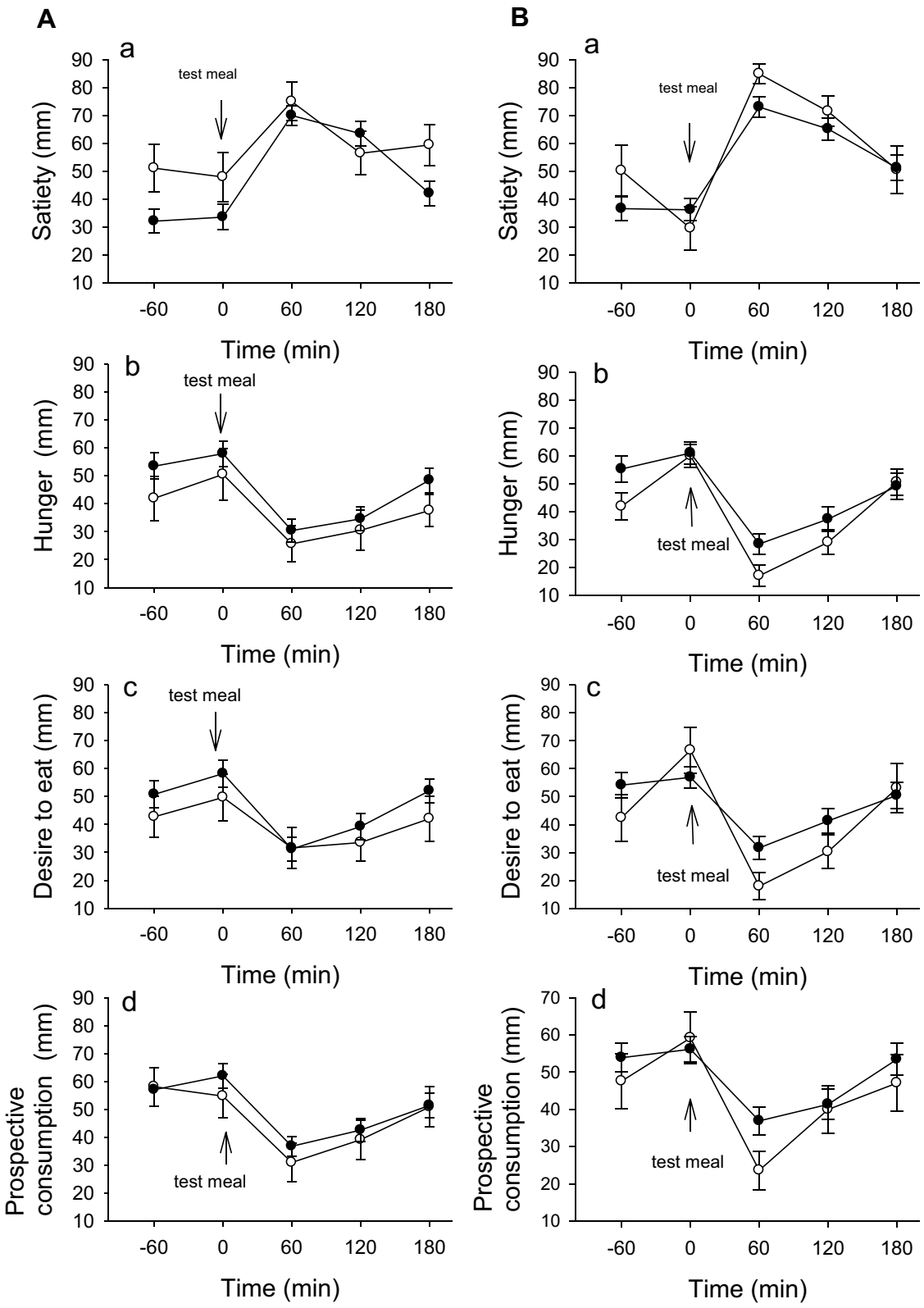

Figure 4 Subjective appetite ratings for satiety (a), hunger (b), desire to eat (c), and prospective consumption (d) in response to PI2 before and after a test meal (week 0 chart **A**, week 10 chart **B**). One hour before the test meal PI2 or placebo was taken; PI2 group (●), placebo group (○). Values are mean values ± SEM.

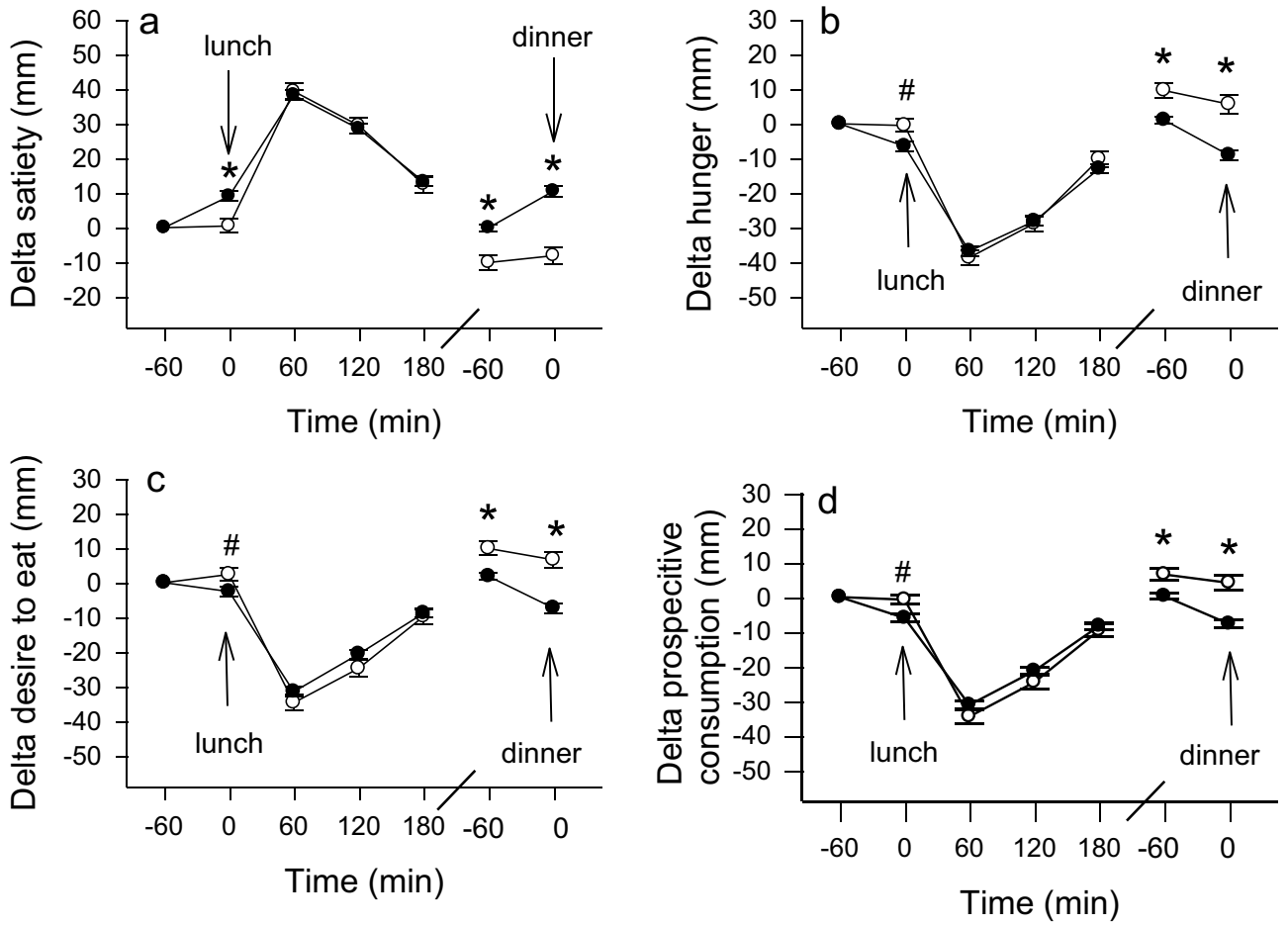

Figure 5 Changes in appetite ratings in response to PI2 for satiety (**A**), hunger (**B**), desire to eat (**C**) and prospective consumption (**D**). One hour before lunch (−60 min) and one hour before dinner (−60 min) PI2 or placebo was taken. VASs were completed in week 4, 9, 14, 19 and combined data of all weeks are shown; PI2 group (•), placebo group (○). Values are mean values ± SEM. *p < 0.0001, #p<0.05 PI2 vs GC at individual time points.

altered in overweight and obese subjects and change when obese individuals lose weight.[18] In our study basal, CCK hormone levels were higher in response to weight reduction and the stimulating influence of PI2 only became apparent in the weight loss phase. In previous studies the extent to which obesity and weight loss influences CCK secretion was controversial, it was unaltered or has been reduced.[26–31]

The main stimulant of CCK release is the presence of amino acids and fatty acids in the duodenum.[32] Thus, the macronutrient composition of the meal influences the different outflow of CCK after ingestion, and a protein protease inhibitor could then exert its corresponding effects on the released hormone. The diet of our study participants at the time of enrolment consisted of 17–20% protein, 31–33% fat, 43–46% carbohydrates and 3.6% alcohol. During the weight loss period, protein intake increased to 27–28%, with a decrease in carbohydrate intake and a slight decrease in fat and alcohol intake. The test meal in our study was high protein and low carbohydrate. Therefore, we suggest that the composition of the weight loss diet and the high protein test meal induced adjustments for the CCK response, since CCK is released locally and enters plasma after the intraluminal presence of nutrient digestion products from intestinal endocrine cells.

An effect of PI2 on appetite evaluation was observed at test meals. Although study participants reported a higher satiety sensation in response to PI2 before the first test meal, a significant effect was observed around the second test meal at week 10 of the intervention. This result corresponds to the higher basal and postprandial release of CCK after 10 weeks of diet and may be due to changes in macronutrients. However, weight loss per se leads to changes in hormones and thus to CCK concentrations.[18] In our study, ghrelin, GLP-1, leptin and insulin were also affected by weight loss, all synthesized in different parts

of the body. The identification of the underlying mechanisms for these changes during weight loss are questions for future research.

Our results revealed no association between CCK and VAS sensations, but positive corresponding levels were observed for ghrelin and insulin concentrations with VAS scales. Possibly, the released CCK concentration was too low to achieve a significant result, since with the exact formulation of our test meals and in regard to limited intake of macronutrients (especially fat) and calories, this may be the reason that no correlation between CCK and appetite scales could be shown. CCK is postulated as the best-established gastro-intestinal endocrine satiation signal in humans.[33] However, attempts to relate endogenous CCK levels with subjective measure of appetite have been less informative and in the few studies available on the relationship between CCK and VAS, the results have always been weak.[33] It has been suggested that CCK does not appear to play a unique independent role in satiety/satiation, but rather acts in conjunction with other peptides and the action of the stomach.[34] Thus, altered concentrations of ghrelin, leptin or insulin may have influenced appetite ratings in conjunction with CCK.

A substantial influence of PI2 on appetite rating was observed by the combined evaluation of the four datasets summarized over 19 weeks. PI2 showed a significant effect on appetite one hour after intake and thus shortly before lunch. But major differences between the two study groups were also observed one hour before dinner and just before dinner. In our study on test-meal days we observed a higher basal and postprandial sustained release of CCK during weight loss for at least three hours after food intake. The inhibition of CKK-degradation induced by the PI2-inhibitor may have led to higher satiety ratings. Further, as outlined above, the observed changes in ghrelin and insulin concentrations could have contributed to the observed increase in VAS scales during the weight reduction phase.

Weight-loss was not different between the two study groups, although subjects receiving PI2 reported a significant positive influence on all appetite measures in the course of the intervention. At the beginning of the study calorie intake was specified for each individual and the control by food diaries showed high compliance to the prescription. Since no unlimited food intake was proposed in the study and the prescribed energy intake was not allowed to be undercut, the weight loss was the same in both groups. Usually, during weight-loss programs, weight reduction and changes in appetite feelings are in favor of increased appetite.[18] Therefore, the findings of our study indicate that the intake of PI2 could facilitate the adherence to weight-reduction diets by enhancing satiety feelings.

GLP-1, ghrelin, and leptin were not affected by the PI2 inhibitor. Consideration of GLP-1 concentrations in our study shows that there exists also controversy as to whether basal and postprandial GLP-1 concentrations in obese subjects are similar to those with normal weight.[18] Postprandial, we observed an increase in GLP-1 levels after the first test meal, which was in the range of an increase previously observed in normal weights.[35] Other findings show a lower post-prandial response in obese subjects after test meals.[35,36] It has also been suggested for GLP-1 concentrations that obesity-related postprandial GLP-1 responses may be specific to the type of meal challenge that may contribute to the understanding of the different study results.[35] The GLP-1 release in our study was lower at test meal 2, a finding consistent with previous studies which reported a significant decrease in GLP-1 concentration after 62 weeks of diet, which was less pronounced after 10 weeks.[29]

The weight reduction in our study resulted in higher overall ghrelin concentrations. Dietary weight loss in obese subjects is associated with an increase in ghrelin levels, as several studies have shown.[29,37] Nutrient-specific effects have been reported for this gastrointestinal hormone.[38] In response to a meal, the release of ghrelin is suppressed by the intake of macronutrients, but the extent and duration appear inconsistent.[38] A similar or reduced release of ghrelin compared to other macronutrients was reported with high protein intake.[38] A meta-analysis showed that protein-rich meals over longer periods of time lead to more reduced ghrelin-plasma concentrations than high carbohydrate intake, although some studies observed an increase in ghrelin concentration after a protein-rich meal.[39,40]

In our study we did not find an effect of PI2 on glucose or insulin concentrations in response to a test meal, neither before nor during the weight loss diet. This finding is in contrast to previous studies showing a significant reduction in post-prandial glucose and insulin levels.[15,41] Our test meal contained a relatively high amount of fiber (10 g). This leads to a delayed increase in blood glucose and may explain why we could not detect any effects of PI2 on post-meal blood glucose and hence insulin levels.

The lower leptin and insulin levels reported in our study after weight loss are consistent with findings that serum leptin is strongly correlated with the body mass index,

with further evidence that leptin levels decrease during fasting, similar to insulin release from pancreatic islets.[42]

Conclusion

This study showed that PI2 exerted a significant effect of appetite rating, which is probably caused by the higher CCK concentration. Weight loss was similar in all subjects. Yet it should be considered that study participants also had a prescribed reduction diet and were obligated adhering to the compulsory reduced energy intake while relative protein- and fiber content increased. Therefore, it would be interesting to investigate whether PI2 may be used preventively in individuals to avoid further weight gain in ad libitum diets.

Clinical Trial Information

Deutsches Register Klinischer Studien, registration trial DRKS00015821.

Data Sharing Statement

Data can be requested from the corresponding author.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Disclosure

MFM: Nutrition advisory board member (honoraria), Herbalife Nutrition, Inc. All other authors have no conflicts of interest to declare in this work.

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