Food intake regulating hormones in adult craniopharyngioma patients

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Abstract

Introduction: Patients with craniopharyngioma (CP) have disturbances of the hypothalamic–pituitary axis and serious comorbidities such as obesity. We hypothesized that the secretion of hormones regulating the nutritional status is altered in adult patients with CP compared with patients with non-functioning pituitary adenoma (NFPA).

Methods: We included 40 CP (50% males, mean age: 49.6 ± 14.3 years) and 40 NFPA (72.5% males, mean age: 63.4 ± 9.8 years) patients. We measured glucose, insulin, leptin, total ghrelin, peptide-YY (PYY) and cholecystokinin (CCK) during oral glucose tolerance test (OGTT). Fat mass (FM) was determined by dual X-ray absorptiometry.

Results: Gender distribution was not significantly different, but CP patients were significantly younger ($P < 0.001$). CP patients had significantly higher BMI and FM than NFPA patients (BMI 32 ± 8 vs 28 ± 4 kg/m², $P = 0.009$ and FM 37 ± 9 vs $33 \pm 9\%$, $P = 0.02$). Fasting glucose level (84 ± 12 vs 78 ± 11 mg/dl, $P = 0.03$), leptin (27.9 ± 34.2 vs 11.9 ± 11.6 µg/l, $P = 0.008$) and leptin levels corrected for percentage FM (0.66 ± 0.67 vs 0.32 ± 0.25 µg/l%, $P = 0.005$) were significantly higher in CP than in NFPA patients, whereas ghrelin was significantly lower (131 ± 129 vs 191 ± 119 ng/l, $P = 0.035$). Insulin, PYY and CCK did not differ significantly between groups. After glucose load, leptin decreased significantly in CP patients ($P = 0.019$). In both groups, ghrelin decreased significantly during OGTT (both $P < 0.001$). The percentage decline was significantly smaller for CP. PYY and CCK increased equally after glucose in both groups.

Conclusion: Our patients with CP have more metabolic complications than our patients with NFPA. The levels of leptin and ghrelin at fasting status and after glucose seem to be altered in CP, whereas changes in insulin, PYY and CCK do not seem to be responsible for the metabolic changes in these patients.

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Introduction

Patients with craniopharyngioma (CP) often suffer from obesity and features of the metabolic syndrome. One reason seems to be the location of the tumor which lies in the pituitary–hypothalamic region. Especially, suprasellar growth and damage of the medial ventral part of the hypothalamus leads to disturbances in food regulation and weight gain (1, 2). Significant weight gain is on the one hand caused by the tumor location itself (3), on the other hand by consecutive treatment, surgery and radiation of the tumor (4). This kind of obesity is called

hypothalamic obesity and is defined by a rapid, unyielding weight gain, which is almost untreatable (5).

By now, several circulating peptides such as insulin, leptin, ghrelin, peptide-YY (PYY), glucagon-like peptide 1 and cholecystokinin (CCK) are known to influence weight regulation by affecting the hypothalamic nuclei (6, 7). It is assumed that in patients with CP, the central response to these peptides is disturbed due to hypothalamic damage leading to obesity (5, 8). Hyperinsulinemia, which is thought to be one reason for obesity in humans, might be

due to increased vagal tone caused by lesions in the ventromedial nuclei (9). Leptin is produced in fat cells and correlates positively with the amount of fat mass (FM) (10). Leptin resistance of the hypothalamus and high circulating leptin levels have been shown in general obesity and in patients with CP, who have a greater leptin resistance compared with obese controls (11). Ghrelin is a hunger hormone and is produced in the endocrine X/A-like cells of the gastric mucosa (12). Ghrelin levels are high during fasting and in lean subjects and low after food intake and in obesity (13). Patients with CP seem to have neither changes in ghrelin levels compared with obese controls (14) nor hypoghrelinemia and reduced post-meal response compared with patients with general obesity (15). PYY is a strong appetite-restricting gut peptide, which is secreted by the L cells of the lower intestine (16). PYY-knockout mice suffer from hyperphagia and obesity and exogenous replacement of PYY can stop uncontrolled food intake (17). In obese and normal-weight CP patients, the increase in PYY after a meal was weaker compared with obese and normal-weight controls respectively (15). Neither have significant differences in secretion pattern reported when comparing obese CP patients with obese controls (18). Also in adult patients with tumors in the hypothalamic region – mostly patients with CP – PYY secretion was not changed after a mixed meal compared with healthy controls (19). CCK is produced in the intestinal tract (20); it is secreted after food intake and also restrains appetite.

We performed this cross-sectional study in a large cohort of patients with CP to get more information about the pattern of the appetite-regulating hormones such as insulin, leptin, ghrelin, PYY and CCK in these patients. Patients with non-functioning pituitary adenoma (NFPA) were used as a control group, because they also have a central lesion lying in the pituitary–hypothalamic region.

As patients with CP have greater damage of the pituitary–hypothalamic area than patients with NFPA, we hypothesized that they should have more disturbances in hormones influencing the nutritional status as well as more signs of the metabolic syndrome.

Subjects and methods

Subjects

We informed all patients with CP, treated at the Medizinische Klinik und Poliklinik IV of the Ludwig-Maximilians-Universität (LMU) Muenchen, and the Max-Planck-Institute (MPI) of Psychiatry, both

Munich, Germany, and the Department of Neurosurgery, Istituto Scientifico San Raffaele, Università Vita-Salute, Milano, Italy, about this cross-sectional study. A total of 33 patients from Germany and seven patients from Italy agreed to participate. Nine patients had childhood onset and 31 patients had adult onset of CP. Mean age at diagnosis was 35 ± 19 years. In addition, 40 patients with non-functioning pituitary macroadenomas of the LMU and the MPI were asked to take part in the study as a control group. The study protocol was reviewed and approved by the Ethics Committee of the Medical Faculty of the LMU Muenchen. All patients gave informed consent to participate. Patients with diabetes mellitus (DM) currently on or requiring insulin therapy were excluded from the study. All patients with corticotrophic insufficiencies and thyrotrophic axis as well as patients with diabetes insipidus were on permanent, sufficient substitution (hydrocortisone 15–25 mg/day, thyroxine (T_4) titrated by free T_4 level, desmopressin controlled by serum electrolytes and clinical symptoms). Male patients with insufficiency of the gonadotrophic axis received adequate testosterone substitution and female patients with insufficiency of the gonadotrophic axis sex steroids until reaching postmenopausal age. Growth hormone (GH) substitution was offered to all GH-deficient patients who did not have contraindications for substitution (Table 1).

Methods

All evaluations of the German patients were performed in the Medizinische Klinik und Poliklinik IV, of the Italian patients in the Istituto Scientifico San Raffaele, Università Vita-Salute according to the same protocol. The patients were asked to arrive at the hospital between 0700 and 0900 h after overnight fasting. We first determined clinical parameters and body composition and then baseline laboratory values. Afterwards, we performed a 3-h oral glucose tolerance test (OGTT, time points –15, 0, 30, 60, 90, 120, 150 and 180 min) with 75 g glucose (Dextro O.G.T., Roche) according to the World Health Organization (WHO) criteria and measured glucose and insulin at all time points in all patients ($n=80$), serum leptin and serum total ghrelin at time point –15 min, baseline, and at 60 min in all patients ($n=80$), and PYY and CCK at time point –15 min, baseline, and at 60 min in 23 German patients with CP and 32 German patients with NFPA. Leptin, ghrelin, PYY and CCK was measured only 60 min after glucose load at this time point has been shown to be the most important during OGTT (15, 21, 22). Blood samples were immediately chilled and centrifuged at 4 °C

Table 1 Characteristics and variables of patients with CP and NFPA. Data are shown in mean $\pm$ s.d. Comparisons were calculated using Fisher's exact test and independent two samples *t*-test.

	CP	NFPA	P value
Patients (n)	40	40	
Age (years)	49.3 $\pm$ 14.3	63.4 $\pm$ 9.8	<0.001
Gender (f (%)/m)	20 (50)/20	11 (27.5)/29	0.066
Pituitary insufficiencies			
Corticotrope (n/%)	34/85	30/75	NS
Thyrotrope (n/%)	34/85	29/73	NS
Gonadotrope (n/%)	36/90	31/78	NS
Somatotrope (n/%)	36/90	28/70	<0.05
Diabetes insipidus (n/%)	24/60	5/13	<0.001
GH substitution (n/%)	21/52.5	12/30	NS
Number of operations = 1	25/62.5%	25/62.5%	NS
Number of operations > 1	15/37.5%	14/35%	NS
Pituitary radiation (n/%)	9/22.5%	18/45%	NS
BMI (kg/m ²)	32 $\pm$ 8	28 $\pm$ 4	<0.05
Fat mass (%)	37 $\pm$ 9	33 $\pm$ 9	<0.05
Waist:hip ratio	0.94 $\pm$ 0.08	0.96 $\pm$ 0.07	NS
Glucose (mg/dl), baseline	84 $\pm$ 12	78 $\pm$ 11	<0.05
Glucose (mg/dl), 120 min	127 $\pm$ 51	117 $\pm$ 42	NS
HbA1c (%)	5.9 $\pm$ 0.9	5.7 $\pm$ 0.4	NS
IGF1 (μ g/l)	130 $\pm$ 95	102 $\pm$ 58	NS
Hypertension (n/%)	16/40%	28/70%	<0.05
DM2 (n/%)	7/18%	4/10%	NS
DM2 on oral antidiabetics (n/%)	7/18%	2/5%	NS

n, number of patients; f, female; m, male; IGF1, insulin-like growth factor 1; DM, diabetes mellitus; NS, not significant.

and serum aliquots were frozen until assayed. Blood samples for leptin and ghrelin from Italy were sent to Munich deep frozen to be measured by the same assay.

Evaluation of clinical parameters

Weight of patients was measured by using bioelectric impedance analysis scale (Tanita, Firma Soehnle, Model Volga TR51025, Nassau, Germany) wearing only underwear and without shoes; height, waist and hip circumference were measured by tape; heart rate by palpation of pulse and blood pressure by a commonly used blood pressure cuff. For evaluation of total body FM, body composition was determined by dual energy X-ray absorptiometry (Lunar Prodigy, General Electric (Nuernberg, Germany), software version Encore 9.30).

Laboratory values

Glucose levels were determined by an automated glucose analyzer (Care Eco solo I, Care Diagnostic, Voerde (Niederrhein), Nordrhein-Westfalen, Germany); insulin

levels were measured by the Adaltis Italia insulin RIA (S.p.A; Casalecchio di Reno, Italy). HbA1c levels were analyzed from the whole blood, standardized to IFCC and calculated according to DCCT/NGSP (Integra 700, Roche). Insulin-like growth factor 1 (IGF1) concentrations were measured using an automated chemiluminescent immunoassay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA).

Leptin concentration in serum was measured using an immunofluorometric in-house assay (IFMA) as described previously (23). The lower limit of quantification was 0.1 μ g/l and the linear working range was 0.2–40 μ g/l. Samples measured above this range have been re-assayed after dilution in 0-standard. Intra-assay variability was 7.4, 4.3 and 5.6% at leptin concentrations of 0.8, 2.5 and 15.3 μ g/l respectively. Inter-assay variability at the same concentrations was 8.3, 5.2 and 5.9% respectively. Spiking experiments revealed that this IFMA for leptin measures total leptin independently from the formation of leptin/leptin receptor complexes.

Immunoreactive total human serum ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA). Serum ghrelin was measured by using ¹²⁵I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. All samples from individual subjects were measured by the same assay. Intra- and inter-assay coefficients of variation (CV) were 5.3 and 13.6% respectively.

Immunoreactive total human plasma PYY was measured by a commercially available RIA (LINCO Research, MO, USA) using ¹²⁵I-labeled bioactive PYY as a tracer and a PYY antiserum to determine the level of active PYY by the double-antibody technique. The PYY antibody is raised in guinea pigs and recognizes both the PYY 1–36 and PYY 3–36 forms of human PYY. All samples from individual subjects were measured by the same assay. The minimal detectable dose of PYY is 10.0 pg/ml. Intra- and inter-assay CV were 5.3 and 7.0% respectively.

Plasma CCK concentrations (pmol/l) were determined by a sensitive and specific RIA (24). In short, the antibody (CH40IX), raised in rabbits, was specifically directed to the biologically active site of CCK, including the sulfated tyrosyl residue at position 7 from the C-terminal end, and showed no cross-reactivity with unsulfated CCK-8, unsulfated gastrin-17 or unsulfated gastrin-34. The cross-reactivity to sulfated gastrin-17 was <1%. The mean minimal detectable concentration in extracted plasma samples was 0.3 $\pm$ 0.1 pmol/l. The intra-assay CV was between 5.6% (0.7 pmol/l) and 7.2% (15.1 pmol/l).

Statistical analysis

We used IBM SPSS Statistics (version 20.0, IBM Corp., Armonk, NY, USA) for data analysis. Results were expressed in mean and s.d. unless otherwise stated. Baseline levels were defined as mean of the value at time point –15 min and the value at time point 0 min. For comparison between the two groups, the independent two-sample *t*-tests were used; for comparison within a group, paired two-sample *t*-tests were used. Furthermore, data for leptin, ghrelin, PYY and CCK were compared by ANCOVA, using age and gender as cofactors. Moreover, leptin was corrected for percentage FM and compared using independent two-sample *t*-tests and ANCOVA. Comparison for dichotomized data was conducted using Fisher's exact test. Correlations between variables were calculated using the Pearson's correlation coefficient. Waist:hip ratio was calculated by dividing waist circumference by hip circumference. Diabetes was defined by patients either taking oral antidiabetics or according to the WHO criteria. Hypertension was defined by patients either taking medications for hypertension or having a blood pressure above 140 mmHg systolic or above 90 mmHg diastolic, calculating the mean out of three consecutive measurements during the visit. A *P* value <0.05 was considered as the nominal level of significance.

Results

Fifty percent of CP patients (*n*=20) were male and 50% female (*n*=20), whereas 72.5% of NFPA patients (*n*=29) were male and 27.5% (*n*=11) female. The gender distribution between both groups was not significantly different (*P*=0.066). The mean age in the CP group was 49.6 years (± 14.3 years), and in the NFPA group 63.4 years (± 9.8 years); this difference was highly significant (*P*<0.001). BMI and body FM were significantly higher in CP than in NFPA patients (Table 1).

Slightly significantly more patients with CP had insufficiency of the somatotrophic axis (*P*<0.05), highly significantly more had diabetes insipidus (*P*<0.001), whereas the prevalence of the other hormonal pituitary insufficiencies (thyreotropic, gonadotropic and corticotropic) did not differ significantly between groups (Table 1). At the time of diagnosis, 34 CP patients had suprasellar, in two only intrasellar tumor growth and in four patients tumor growth was not known. Glucose levels at baseline were significantly higher for CP than for NFPA patients, but did not differ at 120 min after glucose intake. The prevalence of DM was not significantly different in

patients with CP (*n*=7) compared with patients with NFPA (*n*=4) (18 vs 10%, *P*=NS). Significantly more patients with NFPA had hypertension (*n*=28 (70%) vs *n*=16 (40%), *P*=0.013). For more patients' characteristics and variables see Table 1.

Comparing CP patients with childhood onset with that of adult onset, differences in HbA1c could be found. Patients with childhood onset had lower HbA1c levels (5.5% ($\pm 0.50\%$)) compared with adult onset patients (6.0% ($\pm 0.96\%$), *P*<0.05). No differences were seen in pituitary insufficiencies, GH substitution, number of operations, number of radiations, glucose levels, insulin levels, leptin, ghrelin, CCK, PYY, BMI, waist:hip ratio and FM.

Insulin levels at baseline and after glucose load did not differ between CP and NFPA patients. Leptin levels at baseline and 60 min after glucose intake were significantly higher in patients with CP compared with patients with NFPA. When adjusted for gender and age, the difference in leptin levels did not persist. Leptin levels decreased significantly after 60 min in patients with CP (*P*=0.019), whereas in patients with NFPA leptin levels tended to decrease without reaching statistical significance (*P*=0.089). The percentage decline was not significantly different between CP and NFPA patients (Table 2). Leptin levels corrected for percentage FM at baseline and after 60 min were significantly higher in patients with CP compared with patients with NFPA (baseline: CP, 0.66 $\mu\text{g/l\%}$ ($\pm 0.67 \mu\text{g/l\%}$) and NFPA, 0.32 $\mu\text{g/l\%}$ ($\pm 0.25 \mu\text{g/l\%}$), *P*=0.005 and after 60 min: CP, 0.60 $\mu\text{g/l\%}$ ($\pm 0.60 \mu\text{g/l\%}$) and NFPA, 0.30 $\mu\text{g/l\%}$ ($\pm 0.25 \mu\text{g/l\%}$), *P*=0.008). When adjusted for gender and age, the differences in percentage FM corrected for leptin did not persist.

Ghrelin levels at baseline and after 60 min were significantly lower in CP compared with NFPA patients. Correcting for age and gender eliminated the difference at baseline, whereas ghrelin levels at 60 min remained significantly lower for CP. In both groups, ghrelin levels decreased significantly after glucose load (both *P*<0.001). The percentage decline was significantly lower for CP. PYY and CCK at baseline, as well as after 60 min did not differ significantly between the two groups. PYY (CP, *P*=0.039 and NFPA, *P*=0.002) and CCK (for both *P*=0.001) significantly increased after glucose load in both groups. The percentage increase for CP and NFPA did not differ significantly between groups for PYY and CCK (Table 2).

Despite the well-known correlations for leptin and insulin with FM and BMI, we were able to detect the following significant correlations: in the CP group,

Table 2 Insulin, leptin, ghrelin, PYY and CCK values for patients with CP and NFPA. Data are shown in mean $\pm$ s.d. Comparisons were calculated using independent two samples *t*-test and ANCOVA corrected for age and gender.

	CP (n=40)	NFPA (n=40)	t-test	ANCOVA adjusted for age/gender
Insulin (μ E/ml)				
0 min	22 $\pm$ 15	19 $\pm$ 12	NS	NS
120 min	106 $\pm$ 87	114 $\pm$ 75	NS	NS
Leptin (μ g/l)				
0 min	27.9 $\pm$ 34.2	11.9 $\pm$ 11.6	<i>P</i> =0.008	NS (<i>P</i> =0.395)
60 min	25.3 $\pm$ 29.8	11.2 $\pm$ 12.1	<i>P</i> =0.008	NS (<i>P</i> =0.402)
Decrease (%)	7.2 $\pm$ 13.7	9.8 $\pm$ 13.3	NS	NS
Leptin/fat mass (μ g/l%)				
0 min	0.66 $\pm$ 0.67	0.32 $\pm$ 0.25	<i>P</i> =0.005	NS (<i>P</i> =0.341)
60 min	0.60 $\pm$ 0.60	0.30 $\pm$ 0.25	<i>P</i> =0.008	NS (<i>P</i> =0.333)
Ghrelin (ng/l)				
0 min	131 $\pm$ 129	191 $\pm$ 119	<i>P</i> =0.035	NS (<i>P</i> =0.067)
60 min	95 $\pm$ 73	134 $\pm$ 72	<i>P</i> =0.018	<i>P</i> =0.039
Decrease (%)	18 $\pm$ 16	26 $\pm$ 12	<i>P</i> =0.011	<i>P</i> =0.035
PYY (ng/l)				
0 min	208 $\pm$ 106	178 $\pm$ 74	NS	NS
60 min	240 $\pm$ 132	203 $\pm$ 65	NS	NS
Increase (%)	15 $\pm$ 34	18 $\pm$ 24	NS	NS
CCK (pmol/l)				
0 min	0.8 $\pm$ 0.7	1.2 $\pm$ 1.0	NS	NS
60 min	2.5 $\pm$ 0.9	2.7 $\pm$ 0.7	NS	NS
Increase (%)	428 $\pm$ 514	311 $\pm$ 453	NS	NS

n, number of patients; NS, not significant; decrease (%), percentage decrease from baseline to 60 min; increase (%), percentage increase from baseline to 60 min.

a significant correlation for age and baseline glucose ($r=0.438$, $P<0.01$) and in the NFPA group, a significant correlation for PYY and baseline insulin ($r=0.586$, $P<0.001$) and for BMI and basal glucose ($r=0.445$, $P<0.01$). No further correlations were found, especially not for the gut hormones.

Discussion

The main results of our study were that: i) leptin levels were significantly higher at baseline and after glucose intake and decreased significantly in our patients with CP compared with our patients with NFPA; ii) baseline and post-meal ghrelin levels were significantly lower and decrease after glucose load was significantly smaller in our patients with CP than in our patients with NFPA; and iii) insulin levels, PYY and CCK did not differ significantly in fasting condition and the secretion after glucose intake was not different between our two groups. The differences in leptin and ghrelin levels at baseline and the post-meal differences for leptin did not persist when correcting by age and gender.

BMI, FM and fasting glucose levels were significantly higher in our CP patients, despite the younger age of this patient group. Thus, our patients with CP have more metabolic complications and higher FM than our patients with NFPA. The reason for more patients with NFPA having hypertension can be explained by the higher mean age of this patient group.

It might be argued that one influencing factor of these findings could be the prevalence of pituitary insufficiencies. Particularly, GH deficiency is a risk factor for developing a metabolic syndrome (25). Slightly more patients with CP had GH deficiency. But the amount of patients with GH deficiency having no GH replacement did not differ between the two groups (CP, $n=15$ and NFPA, $n=16$) and the IGF1 levels were not significantly different between groups. The prevalence of other insufficiencies of the anterior pituitary was not significantly different between the two groups and all patients were on permanent, adequate hormone substitution. Hence, we do not think that pituitary insufficiencies did have a significant influence on the metabolic differences observed between our CP and NFPA patients.

It is not clear whether the changes in leptin and ghrelin levels at baseline and after glucose load are the reason for or the consequence of higher FM and BMI. Interestingly, the baseline differences in leptin and ghrelin levels and the post-meal leptin levels did not persist when correcting by age and gender. The fact that patients with CP were younger than patients with NFPA would rather strengthen the noticeable results of higher leptin levels in CP, as FM is known to increase with age, and leptin positively correlates with FM (10). It can be speculated that the tendency – even if not significant – of more female patients in the CP group might lead to the loss of significant differences in leptin levels after correcting for age and gender. Females are described to have more FM and thus higher leptin levels (26, 27). Furthermore, female hypopituitary patients have a higher risk of vascular disease than females with a normal function of the same age, which could also bias the results (28). The change in the baseline result for ghrelin levels after correcting for age and gender could also be explained by the younger age and the higher number of women in the CP group, as ghrelin levels are higher in women and young people (29). Changes in leptin and ghrelin should be evaluated in comparison with an obese healthy control group to further clarify whether they are due to the disease or the obesity.

Increased leptin levels in CP patients have been reported before (11, 30). Two causes could account for this: on the one hand, the higher FM of CP patients and on the other hand, a leptin resistance caused by the damage of the hypothalamus (31).

In our CP patients, leptin levels decreased after glucose intake. A tendency toward a decrease was also seen in patients with NFPA, but without reaching statistical significance. We reported before that leptin levels decrease during OGTT in patients with pituitary disease, though not in healthy controls (21, 22, 32). We hypothesized that this phenomenon is due to a central dysregulation caused by the damage of the pituitary region. As we first saw this phenomenon in patients with acromegaly, we speculated that it could be due to non-physiological high GH levels during active disease (22). This is underlined by the fact that some of the GH cells in the pituitary contain leptin granules and have leptin receptors (33). But the exact nature of the hypothesized central dysregulation is not known. Apart from that, a leptin decline during OGTT has been described after carbohydrate restriction and following a high-fat meal in obese men (34, 35) and healthy men (36). The reason why leptin levels decrease in response to glucose is not known. Jenkins *et al.* (35)

speculated the fall in serum leptin in association with reduced carbohydrate intake before substantial loss of body fat, which suggests a role for leptin in defending the body's carbohydrate stores and implicates leptin in the satiating effects of carbohydrate. Imbeault *et al.* (34) hypothesized that obesity is related to an impaired regulation of leptin by insulin, because leptin levels increased in lean men but decreased in obese men following a high-fat meal. Poppitt *et al.* reported a decrease in leptin levels after high-fat meals in healthy men. They mentioned that a decrease in circulating leptin over 24 h may represent an adverse risk for obesity, but this remains controversial and recombinant leptin weight loss therapy in hyperleptinemic obese subjects has been met with limited success (36). In some normal subjects, leptin levels have been reported to not change after food intake and in some obese women an increase in leptin levels has been seen (26). In CP patients, the tumor often lies in the suprasellar region invading hypothalamic structures, whereas in patients with NFPA suprasellar tumor parts rarely damage the hypothalamus. Considering this, we can speculate that the observed, more pronounced reduction in leptin levels after glucose load in patients with CP is a consequence of the hypothalamic damage rather than the involvement of the pituitary. Still, we cannot rule out that this finding is only due to a statistical calculation. The range of leptin values was wide in our patients and the reduction of leptin after glucose was low so that this finding could only be statistically calculated without causality.

Our data showed reduced baseline and post-glucose ghrelin levels in patients with CP. The reduced ghrelin levels can be explained by the positive energy balance shown by the high-FM and BMI in patients with CP, as ghrelin levels are well known to be lower in obese and higher in lean subjects (37). The ghrelin decrease after glucose load was significantly lower in patients with CP (18%) than in patients with NFPA (26%). However, both declines were lower compared with healthy control subjects (28 and 34% respectively) (13, 38) and similar to acromegalic patients (19 and 26% respectively) (22, 39). Accordingly, Roth *et al.* (15) reported reduced ghrelin levels and a reduced ghrelin response after meals in patients with CP compared with obese controls, and Goldstone *et al.* (18) described lower fasting and postprandial ghrelin levels in obese patients with CP than in normal controls. Previously, it has been postulated that ghrelin levels might be high in patients with CP, as hyperghrelinemia was found in Prader-Willi syndrome (18), a genetic cause of hypothalamic obesity. But in the

mentioned study, ghrelin levels were low in patients with CP compared with patients with Prader–Willi syndrome. However, similar ghrelin secretion compared with obese controls has also been reported (18, 19). It seems as if reduced ghrelin response after meals is due to obesity itself as it has been seen in obese patients with other diseases, too (18, 40).

Our patients with CP had equal levels of baseline and post-glucose insulin compared with patients with NFPA. The reason for that finding is not clear. We had expected patients with CP to have higher insulin levels either as a consequence of an increased vagal tone caused by lesions of the ventromedial nuclei (9) or as an effect of obesity itself. One reason of similar insulin levels might be the younger age of patients with CP, as women above 50 years have been shown to present higher HOMA-IR levels than younger women (41). Previously, it has been described that CP patients have high insulin levels (42, 43). Conflicting results have been reported regarding insulin levels in patients with CP compared with other obese subjects: Roth *et al.* (15) did show higher insulin levels in patients with CP than in obese controls and Simoneau-Roy *et al.* (44) also reported increased insulin secretion compared with BMI-matched controls, whereas Shaikh *et al.* (30) described similar insulin levels in patients with CP compared with other obese patients and controls.

Fasting levels of PYY and CCK were not significantly different between our two patient groups, nor did the secretion pattern of PYY and CCK after glucose intake differ. One study has reported that the response of PYY after meals was weaker in patients with CP compared with normal controls (15), but another study did not reveal any differences in secretion pattern of PYY (18). As far as we know, dynamics of CCK after food intake have not yet been studied in patients with CP. It was assumed that an intact vagal–brainstem–hypothalamic pathway is required for gut hormones to influence food intake (15). Hence, we suggested that in CP patients with a damaged hypothalamic region PYY and CCK secretion would be impaired. However, it might be concluded from our data that PYY and CCK do not seem to have any influence on the development of obesity in our patients with CP.

The study has some limitations. In particular, the heterogeneous study population and many cofounders, such as different age at diagnosis, different amount of pituitary insufficiencies, and metabolic differences could possibly affect hormonal profile, independent of disease status. Treatment differences in the extent of surgery are possible and cannot be ruled out due to the number of different surgical centers. Moreover, due to the

cross-sectional design the causality of our results cannot be proven and the descriptive nature of this study might limit the conclusions. Furthermore, we did not assess acylated and desacylated ghrelin levels but only total ghrelin levels.

In summary, we could show in a large cohort of patients with CP that they have more signs of the metabolic syndrome like obesity, higher FM, and higher glucose levels compared with patients with NFPA, although they are younger. Moreover, our results point into the direction that patients with CP have disturbances of food regulation hormones in terms of higher leptin levels and lower ghrelin levels as well as a significant leptin decline and a reduced ghrelin response after glucose intake compared with our patients with NFPA. But the differences in baseline leptin, post-glucose leptin and baseline ghrelin levels did not persist when adjusting for age and gender. Levels of PYY and CCK were not altered in our patients with CP. Thus, they do not seem to be responsible for the metabolic changes in these patients. We want to point out that the results were found in our patient groups and therefore may not be universally valid for all patients with CP and NFPA.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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