Altered endocannabinoid-dynamics in craniopharyngioma patients and their association with HPA-axis disturbances

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European Journal of Endocrinology

Abstract

Objective: Patients with craniopharyngioma (CP) frequently suffer from morbid obesity. Endocannabinoids (ECs) are involved in weight gain and rewarding behavior but have not been investigated in this context.
Design: Cross-sectional single-center study.
Methods: Eighteen patients with CP and 16 age- and sex-matched controls were included. Differences in endocannabinoids (2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA)) and endocannabinoid-like molecules (oleoyl ethanolamide (OEA), palmitoylethanolamide (PEA), and arachidonic acid (AA) were measured at baseline and following endurance exercise. We further explored ECs-dynamics in relation to markers of HPA-axis activity (ACTH, cortisol, copeptin) and hypothalamic damage.
Results: Under resting conditions, independent of differences in BMI, 2-AG levels were more than twice as high in CP patients compared to controls. In contrast, 2-AG and OEA level increased in response to exercise in controls but not in CP patients, while AEA levels decreased in controls. As expected, exercise increased ACTH and copeptin levels in controls only. In a mixed model analysis across time and group, HPA measures did not provide additional information for explaining differences in 2-AG levels. However, AEA levels were negatively influenced by ACTH and copeptin levels, while OEA levels were negatively predicted by copeptin levels only. There were no significant differences in endocannabinoids depending on hypothalamic involvement.
Conclusion: Patients with CP show signs of a dysregulated endocannabinoid system under resting conditions as well as following exercise in comparison to healthy controls. Increased 2-AG levels under resting conditions and the missing response to physical activity could contribute to the metabolic phenotype of CP patients.

European Journal of Endocrinology (2021) **185**, 231–239

Introduction

Craniopharyngiomas (CP) are epithelial tumors arising along the path of the craniopharyngeal duct. With an overall incidence of 1.3 cases per 1 000 000 person-years they are extremely rare (1). Despite their histologically benign character, they are associated with a high degree of morbidity due to their location and growth pattern, which commonly results in panhypopituitarism and hypothalamic dysfunction (2). Depending on the

https://eje.bioscientifica.com
https://doi.org/10.1530/EJE-21-0178

Published by Bioscientifica Ltd.

extension and the hypothalamic nuclei affected, sequela may include homeostatic imbalances such as disturbed thermoregulation, impaired circadian rhythm, and most commonly morbid obesity (2, 3). Despite growing understanding of hypothalamic functioning, the exact mechanisms for weight gain in these patients are incompletely understood (4), but there is a clear association with the extent of hypothalamic involvement (5). While hyperphagia does not seem to be the main culprit in these patients, autonomic dysfunction (6) resulting in reduced sympathetic activity (7) has been reported, potentially explaining a reduction in energy expenditure (8, 9). In addition, patients often complain about persistent fatigue (10) and show reduced physical activity (9, 11).

A potential mediator of hypothalamic obesity not yet investigated in this context is the endocannabinoid (EC) system, as it might explain reduced energy expenditure as well as reduced physical activity. ECs are abundantly expressed in the CNS, including the hypothalamus (12) as well as in various peripheral tissues (13), and it might play a crucial role in central and peripheral regulation of energy metabolism. They modulate, for example, food intake, energy expenditure, lipogenesis and glucose homeostasis (14), both via central and peripheral mechanisms (15, 16). Furthermore, ECs likely affect reward behavior (17, 18) and have been shown to increase during physical exercise (19, 20). The main ECs are 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (anandamide; AEA). ECs are synthesized on demand and can either act in an autocrine or paracrine manner or be secreted into the bloodstream. There are two major endocannabinoid receptors. CB1 receptors are found predominantly in the central and peripheral nervous system, while CB2 receptors are expressed predominantly in the immune system (21). AEA is the main ligand for CB1 receptors, while 2-AG acts as a full agonist on both CB receptors (13). CB1 blockage in the forebrain as well as in sympathetic neurons increases energy expenditure by activating the peripheral sympathetic nervous system (22) and subsequently increasing noradrenaline excretion (23). While AEA and 2-AG have been associated with the development of metabolic syndrome and thus have been targets for pharmacological weight loss drugs such as the CB1 blocker rimonabant (24), other EC-related compounds such as oleoylethanolamide (OEA) (25) and N-palmitoylethanolamide (PEA) have been associated with more favorable metabolic health outcomes (26). Due to their widely distributed expression, the exact source of circulating ECs is not completely understood; however, evidence demonstrates that systemic 2-AG and/or AEA levels are increased in people with obesity (27), and these elevated levels correlate specifically with visceral fat mass (28). Finally, ECs also show a close interaction with the activation of the hypothalamus–pituitary–adrenal (HPA) axis (29), with peripheral levels rising in response to a psychological stressor (30). Matching this, an exercise-induced rise of peripheral ECs has been shown to correlate with cortisol levels (20). Importantly, the interaction between ECs and the HPA axis seems to be bidirectional (31). While glucocorticoids can, for example, stimulate hypothalamic EC release (32), ECs, in turn, are involved in adrenal steroid synthesis (33). The integration of EC signaling is complex, and receptive brain regions include among others, the hypothalamus, prefrontal cortex, brain stem and amygdala (14).

In the present study, we hypothesized endocannabinoid function in CP patients to be dysregulated, since the hypothalamus is both a crucial source as well as signaling target for ECs. In detail, we hypothesized that independent of BMI, baseline ECs might be higher in CP patients than controls, and furthermore that CP patients show a blunted EC-response to a stimulus, specifically to physical activity, which is known to substantially increase EC release and might contribute to beneficial effects of exercise (18). Lastly, due to the known interaction of EC signaling with the HPA axis on the one hand and the high prevalence of pituitary insufficiency in CP patients, including the corticotropic axis on the other, we were interested if blunted EC-release during exercise would be explained by a reduced endogenous HPA-axis activity, as measured via ACTH and copeptin levels.

Methods

Study design and participants

Patients were recruited from the Neuroendocrine Outpatient Unit of the Max-Planck Institute of Psychiatry (MPIP) and the Endocrine Department of the Medical Clinic IV, Ludwig Maximilian Universität, Munich, Germany. In this study, 18 adult patients participated, and 16 adult controls were recruited by public advertising and were included only if they had not been using hormonal contraceptive medication in the 6 months before the study. For further clinical characteristics of CP patients, see Table 1. The study was approved by the local ethics committee and conducted in accordance with the 2013 Declaration of Helsinki. All participants gave their written informed consent.

Table 1 General characteristics. Data are presented as mean ± S.D. or *n* (%).

	CP	Controls	*P*
n	18	16	
Age (years)	39.1 ± 10.6	34.9 ± 15.7	0.377*
BMI (kg/m²)	28.8 ± 7.6	24.0 ± 6.5	**0.036***
Years since diagnosis	13.7 ± 9.4	NA	NA
Age at diagnosis	25.2 ± 11.9	NA	NA
Sex			0.716
Women	9 (50.0%)	9 (56.3%)	
Men	9 (50.0%)	7 (43.8%)	
Smoking			0.451
Never	14 (77.8%)	13 (81.3 %)	
Current	0 (0.0%)	1 (6.3%)	
Former	4 (22.2%)	2 (12.5%)	
Alcohol consumption			0.917
Never	3 (16.7%)	2 (12.5%)	
Less than once a week	9 (50.0%)	9 (56.3%)	
1–3 times a week	6 (33.3%)	5 (31.3%)	
Sports activity			0.691
Never	4 (22.2%)	3 (18.8%)	
Less than once a week	4 (22.2%)	1 (6.3%)	
1–3 times a week	3 (16.7%)	3 (18.8%)	
3–4 times a week	6 (33.3%)	7 (43.8%)	
More than 4 times a week	1 (5.6%)	2 (12.5%)	
Surgery			
Transsphenoidal	15 (83.3%)	NA	NA
Transcranial	3 (16.7%)	NA	
Radiation	3 (16.7%)	NA	NA
MRI grading		NA	NA
Missing	2 (11.1%)		
0	6 (33.3%)		
1	5 (27.7%)		
2	5 (27.7%)		
Diabetes insipidus	10 (55.6%)	NA	NA
Any pituitary insufficiency	17 (94.4%)	NA	NA
Pituitary axes affected		NA	NA
0	1 (5.6%)		
1	1 (5.6%)		
2	1 (5.6%)		
3	2 (11.1%)		
4	13 (72.2%)		
Complete anterior hypopituitarism	13 (72.2%)	NA	NA
Corticotropic insufficiency	14 (77.8%)	NA	NA
Thyrotropic insufficiency	16 (88.9%)	NA	NA
Gonadotropic insufficiency	15 (83.3%)	NA	NA
Substituted	13 (72.2%)	NA	NA
Somatotropic insufficiency	16 (88.9%)	NA	NA
Substituted	11 (61.1%)	NA	NA

T-test and X^2-test: *log-transformed.
NA, not applicable.

Hormone and endocannabinoid measurements

Participants arrived at the outpatient unit of the MPIP at 8:30 h in fasting state (food > 12 h, water > 1 h). Patients suffering from hypopituitarism were asked to take their morning hydrocortisone and L-thyroxin dose approximately 1 h before arrival. Blood was drawn before and directly after exercise (see below). Blood samples were placed on ice, plasma was separated immediately, and aliquots were stored at −80°C until analysis. Endocannabinoids, endocannabinoid-like molecules oleoyl ethanolamide (OEA), palmitoylethanolamide (PEA) and arachidonic acid (AA) levels from 100-mL plasma samples were extracted and measured by liquid chromatography/multiple reaction monitoring (LC/MRM) according to the protocol previously described (34, 35). Cortisol was measured by RIA (DRG International, Inc., USA) with an intra-assay CV of 8.6% and an interassay CV of 10.8 and 8.7%, respectively. The lower detection limit for cortisol was 0.9 µg/L. ACTH was measured by RIA (MP Biomedicals, Solon, USA) with an intra-assay CV of 6.8% and an interassay CV of 10.7%, and the lower detection limit was 5.7 pg/mL. Copeptin was determined using a commercially available luminescence assay device (BRAHMS Kryptor, Berlin, Germany). Intra- and interassay CVs were below 8%, respectively (36).

Grading of hypothalamic lesions

MRI classification was based on patients' most recent post-surgical MRI-data and classified by a board-certified radiologist blinded to the clinical data who assessed the degree of individual hypothalamic damage according to a previously applied grading system (37). Patients were assigned to one of the following three groups: grade 0 = no hypothalamic lesion, grade 1 = anterior hypothalamic lesions sparing mammillary bodies, or grade 2 = anterior and posterior hypothalamic lesion, including mammillary bodies.

Exercise paradigm

A detailed description of the exercise paradigm has been published elsewhere (32). Summarized in brief, participants exercised on a bicycle ergometer (Kettler Ergometer TXl, Germany). Lactate in capillary blood was measured repeatedly (Lactate Pro2, Arkray, Japan) to standardize for individual exertion. Briefly, participants exercised for > 7 min, and then stopped either (a) whenever lactate levels reached > 4 mmol/L (considered to

be the anaerobic threshold) or (b) if lactate continuously remained < 4 mmol/L when participants either reached physical exhaustion or after overall 25 min of exercise.

Statistical analysis

Data were checked for normality using Q–Q plots. Non-normally distributed variables were log-transformed before further analysis. For clarity, untransformed values are reported. Comparisons of groups at baseline were conducted by t test (for continuous variables) and X^2 tests (categorical variables). Group scores are presented as mean ± S.D. or as percent. For correlation analyses, Pearson correlation was used. To evaluate the effects of exercise on different outcome variables, group, time, and interaction effects were examined using linear mixed-effects model analysis with time and group effects as fixed, and subject effects as random and an unstructured covariance structure. To ascertain whether changes in ECs-outcome variables occurred independently of differences in BMI, BMI was added as a covariate. In addition, separate models were built. including ACTH, cortisol and copeptin, to explore potential effects of HPA-activity on EC levels across time and groups. Statistical analysis was performed with SPSS 24 ® for Windows (IBM Corporation), with $P < 0.05$ considered statistically significant.

Results

CP patients had a significantly higher BMI than controls (28.8 ± 7.6 kg/m² vs 24.0 kg/m² ± 6.5; $P = 0.036$), while there were, as intended, no significant differences in sex ($P = 0.716$) or age ($P = 0.377$). Patients and controls did also not differ regarding alcohol consumption ($P = 0.917$), smoking habits ($P = 0.451$) or regular physical activity (=0.691) (Table 1). Regarding the exercise paradigm, there was also no difference in maximal lactate value reached ($P = 0.115$), total exercise duration in minutes ($P = 0.263$) or the proportion of those having reached predefined (200 W minus age) submaximal wattage goals ($P = 0.311$) (Table 2).

HPA-axis measures

ACTH levels were higher in controls than in patients (group: $F_{(1, 34.5)} = 9.246$; $P = 0.004$) and rose significantly more (6.0% vs 27.8%) in response to exercise (group × time: $F_{(1, 34.5)} = 12.622$; $P = 0.001$). A similar pattern was seen for copeptin levels which increased only 12.3% in patients but 75.9% in controls (group: $F_{(1, 28)} = 10.214$; $P = 0.003$), time: $F_{(1, 28)} = 17.030$; $P < 0.001$), group ×time interaction: $F_{(1, 28)} = 8.154$; $P = 0.008$). Cortisol levels did not differ between patients and controls and were only affected by BMI, independent of group ($F_{(1, 33.4;} = 6.918$; $P = 0.013$).

Table 2 Exercise measures. Data are presented as mean ± S.D. or as *n* (%).

	CP	Controls	*P*
Submaximum exhaustion level reached			0.331
No	5 (27.8%)	7 (43.8%)	
Yes	13 (72.2%)	9 (56.3%)	
Max Lactate (mmol/L)	4.3 ± 1.1	4.8 ± 0.7	0.115
Duration of exercise (min)	14.9 ± 3.7	13.4 ± 4.0	0.263
Wattage reached (W)	157.2 ± 34.1	156.1 ± 24.0	0.919

*Log transformed.

Endocannabinoids

At baseline, there was a significant positive correlation of BMI with all endocannabinoid measures (AA: R = 0.649, $P = 0.004$; AEA: R = 0.656, $P = 0.003$; PEA: R = 0.626, $P = 0.005$; OEA: R = 0.708, $P = 0.001$), except for 2-AG (R = 0.339, $P = 0.169$) in CP patients but not in controls (Fig. 1). When one patient with noticeably high 2-AG levels despite a relatively low BMI was excluded from analysis 2-AG correlated also significantly with BMI (R = 0.579; $P = 0.015$).

For AEA we found a trend for a time effect ($F_{(1, 34)} = 3.20$; $P = 0.082$) and a significant time ×group interaction ($F_{(1,34)} = 13.46$; $P = 0.001$), as AEA decreased in controls (−13.7%) but remained relatively stable in patients. Moreover, a significant effect of BMI ($F_{(1, 34)} = 11.55$; $P = 0.002$) was found. 2-AG levels were higher in patients at baseline (1.7 ± 1.2 pmol/L vs 0.8 ± 0.2 pmol/L) but did only significantly rise in patients in response to exercise (+60.2%) (group: $F_{(1, 34.6)} = 10.55$; $P = 0.003$; time: $F_{(1, 34.6)} = 10.55$; $P = 0.003$; time ×group: $F_{(1, 34)} = 5.08$; $P = 0.031$). There were significant effects of time ($F_{(1, 34)} = 14.66$; $P = 0.001$) and BMI ($F_{(1, 34)} = 9.14$; $P = 0.005$) for AA, without significant interactions, indicating a slight rise in both groups. There was a significant group × time interaction for PEA levels ($F_{(1, 34)} = 5.189$; $P = 0.029$) which decreased in controls but not in patients. In addition, there was an effect for BMI ($F_{(1, 34)} = 5.41$; $P = 0.026$). There was a trend for a group effect for OEA which tended to be lower at both timepoints in patients ($F_{(1, 34.4)} = 3.92$; $P = 0.056$) and a significant group × time interaction ($F_{(1, 34)} = 6.74$; $P = 0.014$) demonstrating increasing levels in patients (+13%) but decreasing levels in controls (−6.8%) (Table 3). There was no significant difference in baseline or stimulated ECs measures depending on the hypothalamic involvement as

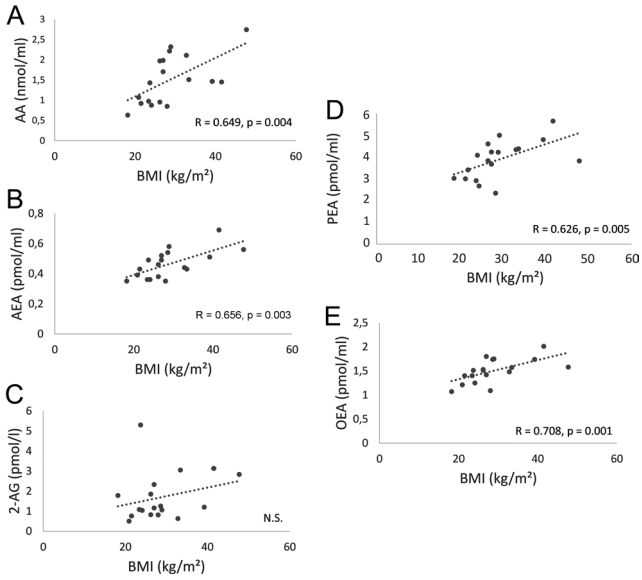

Figure 1

Correlation of EC measures with BMI in CP patients. At baseline, there was a significant positive correlation of BMI with all endocannabinoid measures (AA: R = 0.649; P = 0.004; AEA: R = 0.656; P = 0.003; PEA: R = 0.626; P = 0.005; OEA: R = 0.708; P = 0.001), except for 2-AG (R = 0.339; P = 0.169) in CP patients but not in controls (Fig. 1). When one patient with noticeably high 2-AG levels despite a relatively low BMI was excluded from analysis 2-AG correlated also significantly with BMI (R = 0.579; P = 0.015).

graded on MRI (data not shown). However, these groups were small reducing statistical power.

Effect of HPA-axis measures on ECs

Independent of the effect of BMI, across time and group, AEA levels were influenced by ACTH levels ($F_{(1,46.1)}$ = 4.26; P = 0.045, t = −2.064) and copeptin levels ($F_{(1,49,179)}$ = 4.99; P = 0.030; t = −2.234), indicating that HPA-axis activation has negative effects on AEA release. In contrast, HPA measures did not provide additional information for explaining 2-AG and AA levels. There was a positive effect of cortisol levels on PEA levels ($F_{(1,46.5)}$ = 8.48; P = 0.005; t = 2.912). Independent of a group effect, OEA levels were negatively predicted by copeptin levels ($F_{(1, 52.1)}$ = 8.10; P = 0.006; t = −2.846) but not by ACTH or cortisol (Supplements).

Discussion

Patients with CP show differences in ECs compared to healthy controls both under resting conditions and after

Table 3 EC and HPA measures during exercise. Linear mixedeffects model, all models included BMI as a covariate.

	CP			Controls			Group		Time		Time × group	
	Baseline	Post-exercise	%	Baseline	Post-exercise	%	F	P	F	P	F	P
AEA (pmol/mL)	0.46 ± 0.09	0.49 ± 0.10	5.16	0.51 ± 0.12	0.44 ± 0.12	−13.70	1.23	0.276	3.20	0.082	13.46	**0.001**
2-AG (pmol/mL)*	1.70 ± 1.23	1.78 ± 0.68	4.78	0.76 ± 0.23	1.22 ± 0.37	60.15	10.55	**0.003**	22.03	**<0.001**	5.08	**0.031**
AA (nmol/mL)	1.51 ± 0.60	1.86 ± 0.57	23.05	1.62 ± 0.52	1.82 ± 0.44	12.76	1.70	0.201	14.66	**0.001**	0.96	0.334
PEA (pmol/mL)	3.89 ± 0.88	4.10 ± 0.80	5.52	4.14 ± 1.37	3.69 ± 0.90	−10.89	0.25	0.619	0.65	0.424	5.19	**0.029**
OEA (pmol/mL)	1.50 ± 0.25	1.70 ± 0.28	13.01	1.94 ± 0.66	1.80 ± 0.50	−6.97	3.912	0.056	0.20	0.655	6.74	**0.014**
ACTH (pg/mL)	43.83 ± 16.77	46.47 ± 19.14	6.03	51.03 ± 15.50	77.19 ± 27.79	51.27	9.25	**0.004**	18.94	**<0.001**	12.62	**0.001**
Cortisol (µg/L)*	138.60 ± 83.31	157.73 ± 96.17	13.80	158.87 ± 39.73	138.15 ± 62.80	−13.04	0.0	0.974	0.01	0.930	2.36	0.134
Copeptin (pmol/L)*	2.52 ± 0.96	2.82 ± 1.28	12.26	3.68 ± 2.12	6.47 ± 3.99	75.88	10.21	**0.003**	17.03	**<0.001**	8.15	**0.008**

*Log-transformed.

exercise. Although BMI has a well-established effect on ECs that was also evident in our study, it did not solely explain most of the observed differences. The most striking result of our study was that while 2-AG levels were significantly elevated at resting conditions in CP patients, they did not respond to exercise compared to controls. Other ECs and EC-like molecules were also showing signs of dysregulation in CP patients, however, to a smaller degree than 2-AG.

It has been shown before that there is an increase in AEA (19, 20, 38), 2-AG (19, 39), PEA (19) and also OEA levels (39) following different exercise regimes. These findings were however not consistent across all studies, potentially due to differences in duration and intensity that seem to affect ECs response (38). The relatively short bout of exercise in the present study may not have been sufficient to increase AEA levels as most previous studies used exercise regimes that lasted considerably longer (24, 25).

We initially hypothesized that the EC system in CP patients might be impaired as the hypothalamus is crucial as a source as well as a target for ECs (14). However, our study failed to show differences in EC depending on the estimated extend of hypothalamic damage.

Due to the complex interaction of ECs with metabolism, the correlation of BMI and fat mass with EC measures (28) is not necessarily causal. On the one hand, ECs are synthesized in adipose tissue (40), and correlation with fat mass is therefore obvious, but on the other hand, they also promote fat mass accumulation by decreasing energy expenditure and promoting lipogenesis (14). That the correlation of all ECs with BMI was restricted to CP patients and not observed in controls, may be due to differences in fat mass distribution that was not separately assessed in our study. It has been shown that, in particular, visceral fat mass correlates with EC measures (28). Nonetheless, differences in resting 2-AG levels as well as the following exercise were independent of BMI.

2-AG in general favors energy preservation. It has been shown to increase fat accumulation (41) and reduce energy expenditure in mice (34) and correlates negatively with energy expenditure in humans (35). Likewise blocking the 2-AG receptor CB1 in the forebrain and sympathetic neurons increases energy expenditure via elevating the peripheral sympathetic activity (22) and subsequently increasing noradrenaline excretion (23). The observed baseline increase of 2-AG in our patient group matches the previously reported decrease in energy expenditure and sympathetic activity (7) in CP patients (9, 11). Of course, other causes for this generally decreased energy expenditure in CP patients are possible. Previous research, for example, suggested damage of the PVN and its autonomous efferences as a potential mechanism (5). However, until now, studies have failed to find a clear association between autonomous imbalances and hypothalamic lesion in CP (36).

In addition, 2-AG mediates the consumption of palatable food (42), increases during fasting (43) and is associated with feelings of hunger (39). While most, but not all studies (44), could not confirm an increase in food intake in CP patients (9, 11), there is at least evidence for an increase in hunger perception (10).

Lastly, as a rise during exercise has been associated with the rewarding effect of exercise (18, 19), a blunted release of ECs during exercise might reduce motivation for physical activity in the long-term as it is often observed in CP (9, 11). In the present study, however, patients and controls did not differ in average weekly sports activity, keeping in mind that duration, intensity and time spent per session was not in detail documented.

Lower baseline OEA levels in CP patients may also reflect a negative impact on metabolic health, as OEA has been associated with beneficial health effects (26), for example, by enhancing lipolysis (25) and reducing appetite (45). OEA as well as PEA are expressed in subcutaneous adipose tissue (40) but are also derived from the intestines (46). While OEA levels increased in CP patients following exercise and may therefore potentially indicate health benefits of physical activity in these patients, they did not reach the baseline levels seen in controls.

As there is a well-established, though complex interaction between the HPA axis and ECs functioning (29) and due to the fact that an increase in ECs during exercise has been attributed to HPA-axis activation (20), we were interested if the expected high prevalence of secondary (tertiary) adrenal insufficiency in CP patients would affect ECs dynamics. As it was not justifiable to perform the strenuous exercise in CP patients without them taking their morning hydrocortisone (HC) dosage, measured cortisol levels in patients primarily reflect pharmacokinetics following HC-intake. We were, therefore, more interested in the effects of markers of endogenous HPA-axis activation, namely ACTH and copeptin as a marker of antidiuretic hormone (ADH) release that acts synergistically with CRH on promoting ACTH release from the pituitary. While ADH is also an important regulator of water balance, copeptin levels may serve as a surrogate marker for the activity of the hypothalamic level of the HPA axis (47, 48).

As expected, healthy controls responded to exercise with an increase in ACTH and copeptin that was absent in CP patients. The fact that cortisol levels as opposed to copeptin and ACTH levels in controls did not change following exercise might most likely be explained by a

delayed cortisol response that was not detectable in our samples. As only four patients in the CP group were not suffering from adrenal insufficiency, subgroup analysis regarding ECs response during exercise was not useful. We, therefore, explored if surrogate measures of HPA-axis activity, in particular ACTH and copeptin, could explain differences in ECs.

With regard to AEA, there was a negative association with ACTH and copeptin, indicating that HPA-axis activation has actually negative effects on AEA release across groups. This is in contrast to the study by Heyman *et al.* that speculated on a positive effect of cortisol on AEA release during exercise (20). ECs are an important part of the negative feedback loop of the HPA axis (32). In particular, AEA serves as a kind of gatekeeper. A rise in CRH results in a decrease in central AEA levels (49, 50) by inducing its degradation in the amygdala (50) in order to disinhibit the HPA axis (14). Although we did not measure CRH directly due to its instability, the inverse relationship of AEA with copeptin and ACTH is in line with these findings. While stress also induces central 2-AG synthesis, these changes occur later than for AEA (51). We, however, have to keep in mind that, in particular, the source of peripheral ECs during exercise is not completely understood (18, 31) and peripheral and central EC levels do not necessarily correlate (52) and even inverse correlation of hypothalamic and peripheral EC levels has been described in rodents (16). Thus, the source of circulating ECs in response to exercise is still a puzzle as previous studies have also not been able to identify where they are mainly produced and released (22).

Lastly, we cannot exclude that differences in baseline EC levels between patients and controls may be due to differences in circadian rhythm that can be compromised in CP patients (53). It has in particular been shown that 2-AG levels follow a circadian rhythm with 2-AG concentrations peaking between 00:00 h and 15:00 h. that do not follow HPA-axis activation patterns (54). The difference between nadir and peak can be up to 300%. In line, sleep restriction increases 2-AG levels by 80% in the morning (39).

Limitations

Although our study is unique and was performed in a well-characterized patient cohort, there are several limitations that have to be kept in mind. First, our cohort was not large enough to control for potential confounding effects of further pituitary insufficiency. However, the majority of patients was on a stable substitution therapy (two women were not receiving sex hormone substitution due to postmenopausal age and five patients showed unsubstituted GH-deficiency). A further subgroup of patients with isolated pituitary insufficiency without hypothalamic involvement would also have helped to disentangle the effects of tertiary and secondary hormonal deficiencies as well as hypothalamic involvement. It would also be further interesting to investigate potential differences in EC dynamics between different subtypes of CP, namely papillary and adamantinomatous tumors. Pathological sub-classification was not available in all our patients and given the rather small sample size of our study it would be interesting to investigate this issue in more detail in a follow-up study, including more patients. Papillary and adamantinomatous CP are regarded as distinct histological entities and although we assume that potential differences in hypothalamic function and ECs would be due to differences in mass effects, it is also thinkable that differences in the tumoral microenvironment (55) may have an independent effect on EC released. Lastly, we did not collect data on body composition, in particular visceral and subcutaneous fat mass, that may have different effects on ECs independent of BMI.

Conclusion

Patients with CP present with differences in ECs under resting conditions as well as following exercise in comparison to healthy controls. In particular, the increase in 2-AG under resting conditions and the missing response to physical activity could contribute to the metabolic phenotype of CP patients. While these disturbances were independent of BMI and measures of HPA-axis activity, we could further demonstrate that there is an inverse association between the stress response and peripheral AEA levels as postulated on the basis of rodent models.

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.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
The authors thank Madlen Lahne for her help in the recruitment of CP patients and all participants of this study.

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Received 22 February 2021
Revised version received 30 April 2021
Accepted 1 June 2021