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The cross-sectional relation between medically unexplained physical symptoms (MUPS) and the Cortisol Awakening Response

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ABSTRACT

Objectives: We aimed to assess the cross-sectional relation between levels of cortisol and specific symptom clusters, symptom severity and duration of symptoms in patients with medically unexplained physical symptoms (MUPS).

Methods: Baseline data of a cohort of MUPS patients were used. We chose the Cortisol Awakening Response (CAR) as a cortisol parameter, using saliva samples. We used confirmatory factor analysis for the identification of 4 specific symptom clusters: (1) gastro-intestinal symptoms; (2) pain; (3) cardio-pulmonary symptoms; and (4) fatigue. For this factor analysis we used the Physical Symptom Questionnaire (PSQ), which assesses the occurrence and frequency of 51 physical symptoms. Symptom severity was measured with the Patient Health Questionnaire-15 (PHQ-15). Duration of symptoms was based on self-reported duration of top 3 symptoms. We performed multiple linear regression to assess relations between CAR and individual factor scores on symptom clusters, symptom severity and duration of symptoms.

Results: Data from 296 patients (76% female) were included in the analyses. The majority of patients suffered from symptoms in multiple organ systems. Factor analysis confirmed that the model with 4 symptom clusters fitted our data. For the total study population, we found no significant relation between CAR and participants' factor scores on any of the symptom clusters. We also found no significant relations between CAR and severity or duration of symptoms.

Conclusion: Our results suggest that within a heterogeneous MUPS population there is no relation between CAR and symptom severity and duration. However, more studies are needed to confirm our findings.

1. Introduction

In all health care settings many patients present with persistent physical symptoms for which no sufficient somatic explanation is found after proper medical examination. Such symptoms are called persistent medically unexplained physical symptoms (MUPS). In some cases persistent MUPS fit criteria of specific functional somatic syndromes such as Fibromyalgia (FM), Irritable Bowel Syndrome (IBS) or Chronic Fatigue Syndrome (CFS). However, the existence of these syndromes as distinct entities (instead of being an artefact of medical specialization) has been up to debate [1,2]. Patients with persistent MUPS have a greater risk of psychosocial disability and experience more psychological distress than patients with explained physical symptoms [3].

Apart from psychological and social mechanisms, physiological mechanisms are thought to play a role in the development and persistence of MUPS. One of these mechanisms is a disturbed hypothalamic pituitary adrenal axis (HPA axis). It has been firmly established that stress (physical or psychological) influences the bodily hormonal stress system. When exposed to stress the HPA axis is initially up regulated resulting in higher levels of the stress hormone cortisol. However, prolonged stress may lead to a “burnout” response, resulting in HPA axis down regulation and reduced cortisol production [4]. As a result of the reduced cortisol production stress sensitivity increases, which is thought to contribute to the development and persistence of MUPS such as pain or fatigue [5–8].

The cross-sectional relation between cortisol levels and several

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functional somatic syndromes has been studied before. A meta-analysis of 85 studies showed that hypocortisolism was found in CFS and possibly in FM, but not in IBS [9], indicating that the presence and extent of cortisol disturbances within MUPS populations may vary between different symptom clusters. However, results were heterogeneous and the review also included studies in which normal or high cortisol concentrations were found. Furthermore, a limitation of this meta-analysis is that only studies concerning three specific functional somatic syndromes were included (CFS, IBS and FM).

As most studies in this field of research evaluated cortisol levels within populations suffering from specific functional somatic syndromes, knowledge about cortisol levels within heterogeneous MUPS populations (that do not necessarily fit criteria of specific functional syndromes) is scarce. In addition, it is unclear whether severity and duration of symptoms play a role in the relation between MUPS and cortisol levels.

It is important to increase our knowledge about the relation between cortisol levels and all sorts of MUPS, as this may shed light on the issue whether cortisol disturbances are symptom specific or whether they exist in all MUPS patients. This knowledge would provide guidance in the unravelling of the (general or symptom specific) pathophysiology of MUPS. Given the described gaps in current knowledge, we formulated the following research question:

What is the cross-sectional relation between cortisol levels and (1) the presence of symptoms from specific symptom clusters, (2) symptom severity and (3) duration of symptoms in a heterogeneous population of MUPS patients?

Based on the results of earlier studies among patients with functional somatic syndromes, we hypothesized that reduced cortisol levels (as a marker for HPA axis down regulation) are symptom specific and will only be seen in patients with certain specific symptoms (e.g. patients with fatigue). We also hypothesized that reduced cortisol levels are more prevalent among patients with severe symptoms and a long duration of symptoms.

2. Methods

2.1. Study design and subjects

For this study data of the PROSPECTS study were used. This is an on-going prospective cohort study, following patients with MUPS in multiple health care settings. Participants were included between September 2013 and March 2015. They were recruited in general practices and in specialized MUPS programmes of secondary and tertiary care organizations across the Netherlands. Participating MUPS patients were between 18 and 70 years old. For this analysis we only used baseline data.

We have defined MUPS as the presence of physical symptoms, which have lasted at least several weeks and for which no sufficient explanation is found after proper medical examination by a physician.

In primary care, electronic medical records were searched to select patients who visited their general practitioner (GP) twice or more in the last 3 months with one or more physical symptoms without a matching diagnosis. The list of selected patients was checked for exclusion criteria by the GP. In secondary and tertiary care all newly referred patients with MUPS as the reason for referral were screened for exclusion criteria by the physician performing the intake consultation.

Exclusion criteria were a sufficient medical explanation for the symptoms or incomplete diagnostic evaluation, according to the physician, insufficient command of the Dutch language, a cognitive or visual impairment that prohibits participating in a questionnaire survey, severe psychopathology (e.g. psychotic disorder, bipolar disorder), pregnancy, cancer diagnosed in 5 years prior to inclusion, or another life threatening condition or a short life expectancy.

In all setting, patients who did not meet exclusion criteria received by mail the Patient Health Questionnaire 15 (PHQ-15 [10,11]), which is considered an adequate measure for somatic symptom severity, as it assesses somatic symptoms regardless of their aetiology. Patients who returned the questionnaire and had a score of 2 for at least one symptom (indicating that the symptom was bothering a lot) were considered eligible and were approached for informed consent and inclusion.

Further details about the study design have been published elsewhere [12]. The Medical Ethics Committee of VU University Medical Center Amsterdam approved the study protocol and we obtained written informed consent from all participants.

2.2. Measures

2.2.1. Salivary cortisol samples

Cortisol levels vary greatly during the day: they are lowest at the beginning of the night and then increase, reaching a peak level during the first 30–45 min after the awakening (a natural stressor) in the morning [13,14]. This peak is called the Cortisol Awakening Response (CAR). The steepness of this response is thought to be related to stress reactions [15]. We used the Cortisol Awakening Response as a parameter for measuring cortisol levels, as it forms a discrete entity superimposed on the circadian cycle and therefore shows higher intra-personal stability than solitary measurements [16]. The CAR does not seem to be significantly influenced by age, duration of sleep, time of awakening or the use of an alarm and seems to be stable over time [13].

We measured the CAR using saliva samples. This method is commonly used, because of the non-invasiveness and the ability to sample in patients' natural environment. Salivary cortisol levels correspond well with cortisol levels in plasma [17]. Participants collected saliva samples at awakening time (T0), and 30 (T1) and 60 min (T2) afterwards [18,19]. They collected saliva at home using Salivettes® (Sarstedt, Etten-Leur, The Netherlands). We provided a comprehensive written sampling manual, according to the guideline of the manufacturer. Samples were stored in home refrigerators and returned by mail as quickly as possible (mostly within 1 day). Returned swabs were stored at − 20 °C and centrifuged and analysed with all samples in one batch using liquid chromatographic methods coupled with mass spectrometry (LC-MS/MS, using the Acquity UPLC system and the Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA) [20]).

2.2.2. Questionnaires

At baseline, patients answered questions about personal characteristics (general, socioeconomic and medical characteristics) and a subset of validated and widely used questionnaires concerning outcome measures and relevant covariates.

Outcome measures included the presence of symptoms from specific symptom clusters, symptom severity and duration of symptoms. We measured the presence of symptoms from specific symptom clusters with the Physical Symptom Questionnaire (PSQ [21]), which assesses the occurrence and frequency of 51 physical symptoms that are described in the DSM–III classification [22]. For each symptom, participants scored the occurrence in the past week (never/sometimes/often/most of the time, scoring respectively 0/1/2/3 points). The PSQ covers most organ systems and has been used in earlier MUPS studies [3,23]. Symptom severity was assessed using the PHQ-15 questionnaire. Finally, participants reported a top 3 list of their most prominent symptoms, including the duration of these symptoms. The self-reported duration of the most long-lasting top 3 symptom was used to assess the duration of MUPS.

Based on earlier research, we selected potential confounders of the relation between cortisol levels and MUPS [24,25]. These included characteristics (sex, alcohol use, smoking and obesity), but also relevant medication use and received treatments, which were assessed with the Trimbos/iMTA questionnaire for Costs associated with Psychiatric Illness (TIC-P, [26]). Relevant medication use was defined as the use of

medication potentially affecting the HPA axis: oral corticosteroids, oral contraceptives, oral oestrogen replacement therapy and antidepressants. Further potential confounders were levels of depressive symptoms and anxiety (Quick Inventory of Depressive Symptomatology, QIDS-SR [27] and Beck Anxiety Inventory, BAI [28,29]), stressful life events (Life Events Questionnaire, LEQ [30]) and physical inactivity (International Physical Activity Questionnaire, IPAQ [31,32].

2.3. Statistical analysis

Data analyses were performed using SPSS (version 20.0) and Mplus version 7 [33].

For CAR analysis we calculated the area under the curve (AUC) to incorporate the cortisol levels on all 3 time points after awakening (T0, T1 and T2) into one variable. First, we calculated the area under the curve with respect to the ground (AUC_G), as this value estimates the total cortisol secretion over the first hour after awakening. Secondly, we calculated the area under the curve with respect to the increase (AUC_I). This AUC_I only concerns the AUC above the level at T0 and therefore forms a measure of time-dependent change. We performed these calculations using the formulas described by Pruessner et al. in 2003 [34].

The PSQ questionnaire was used to assess the presence of symptoms from specific symptom clusters. For the analysis of symptom prevalences, symptoms were rated as present if scored 2 or 3, indicating that they were "bothersome often or most of the time during the last week" [23]. We performed confirmatory factor analysis in order to test the fit of previously postulated symptom clusters. Several valid cluster models have been described in literature [35–39]. We decided to use the model published by Witthöft et al., as this model most closely resembles symptomatology of the most common functional somatic syndromes, as described in the introduction. The model describes 4 symptom clusters: gastro-intestinal symptoms, pain, cardio-pulmonary symptoms and fatigue. Included PSQ items for all 4 clusters are presented in Table 1. The analyses of the measurement models were conducted with the robust mean- and variance-adjusted weighted least squares (WLSMV) estimation. Goodness-of-fit was assessed using the following fit indices: χ^2 test (preferably non-significant), Comparative Fit Index (CFI, preferably > 0.95) and root mean square error of approximation (RMSEA, preferably < 0.05) [40]. For all participants, factor scores for each of the symptom clusters were calculated and used for further analyses.

All variables were tested for normality and log-transformed if necessary. For all cortisol analysis, extreme values of AUC_G and AUC_I (i.e. at least 2 standard deviations from the mean) were excluded. As there seems to be a relation between negative cortisol slope and non-adherence to the sampling protocol (due to a probable delay between awakening and saliva collection), we also excluded cases with negative cortisol sloping from AUC_I analyses [41].

Multiple linear regression was used to assess whether AUC_G and AUC_I were cross-sectionally associated with specific symptom clusters (based on individual factor scores), symptom severity (i.e. PHQ15 score) and duration of symptoms (i.e. duration of most long-lasting symptom) at baseline. If the scatter plot showed no linear relation between cortisol levels and these variables, we transformed them into categorical variables.

For registered duration of symptoms, 17.5% of data was missing. Therefore we imputed missing data before further analysis steps, using multiple imputation with Predictive Mean Matching (as symptom duration was not normally distributed). We performed 20 imputations and 50 iterations and produced an iteration plot to confirm sufficiency of these numbers [42]. Note that in an earlier publication using baseline data of the PROSPECTS study we did not use these imputation techniques [38], resulting in differences in reported duration of symptoms. We adjusted for potential confounders using a stepwise forward algorithm. We also adjusted for potential effect modification by sex, depression and anxiety.

Table 1
Included symptoms in the confirmatory factor analysis (as retrieved from the PSQ questionnaire).

Symptom	Factor 1 (GI symptoms)	Factor 2 (pain)	Factor 3 (CP symptoms)	Factor 4 (fatigue)
Obstipation	*			
Stomach pain	*			
Nausea	*			
Pain during sexual intercourse	*			
Back pain		*		
Limb pain		*		
Headache		*		
Dizziness/light-headedness			*	
Shortness of breath without exertion			*	
Palpitations			*	
Chest pain or pressure			*	
Generalized fatigue or apathy				*
Insomnia				*
Excessive sleeping				*

GI symptoms: gastro-intestinal symptoms; CP symptoms: cardio-pulmonary symptoms.

3. Results

3.1. Patient characteristics

Information about the inclusion process of the PROSPECTS study has been published elsewhere [43]. After inclusion 325 patients completed the baseline questionnaires. 296 of these patients (91.1%) also provided a complete set of 3 saliva samples that could be analysed. For 8.9% (N = 29) a complete set of saliva samples was missing. Thirteen participants did not provide saliva samples at all, while for 16 participants 1 or 2 samples were not evaluable due to insufficient quantity. We decided to include only patients with complete saliva samples in our analyses (N = 296).

Socio-demographic and medical characteristics are provided in Table 2, including salivary cortisol levels for all sampling moments with and without 1 outlier, based on a AUC_G value deviating more that 2SD from the mean.

As shown in Table 2, almost all participants described one specific symptom as most prominent (99.3%, N = 294). Three participants reported unexplained symptoms that bothered them a lot at the moment of inclusion, but had resolved when they filled out the baseline questionnaire (on average 2 weeks after inclusion). This was reflected by a PHQ-15 score under 2 at baseline (indicating that they did not suffer from a single symptom which was bothering a lot). These 3 participants remained included in all analyses, as we know that severity of symptoms can fluctuate and so (temporary) recovery can be part of the natural course of MUPS.

Most participants suffered from multiple symptoms originating from various organ systems. An overview of the prevalence figures of all reported symptoms (data derived from PSQ scores) is provided in Table 3.

3.2. Cortisol Awakening Response

The results of the AUC_G were normally distributed with a mean value of 12.29 h∗nmol/L (SD 20.44). After exclusion of one outlier (AUC_G deviated more than 2SD from the mean) we found a mean value of 11.15 h∗nmol/L (SD 5.71). We excluded the outlier in all regression analyses concerning the AUC_G. The results of the AUC_I were also normally distributed with a mean value of 1.06 h∗nmol/L (SD 31.67). After exclusion of all negative values (N = 58, 19.6%) we found a mean

Table 2
Socio-demographic and medical characteristic of study population.

	Overall group Mean (SD) Number (%)	Median (IQR)[a]	Range	N
Age	46.44 (12.52)		19–70	296
Female sex	225 (76,0%)			296
Nationality				296
Dutch	253 (85.5%)			
Other	43 (14.5%)			
Educational level				296
No education	4 (1.4%)			
Elementary school	11 (3.7%)			
Intermediate vocational education	167 (56.4%)			
Higher education	88 (29.7%)			
Academic education	26 (8.8%)			
Work status				296
Employed	176 (59.5%)			
Unemployed	23 (7.8%)			
Long term sick leave	80 (27.0%)			
Retired	17 (5.7%)			
Marital status				296
Married or cohabiting	179 (60.5%)			
Unmarried	80 (27.0%)			
Divorced	29 (9.8%)			
Widow	8 (2.7%)			
PHQ-15 score (scale 0–30)	12.08 (5.28)		0–28	296
Score 0–9	104 (35.1%)			
Score 10–14	93 (31.4%)			
Score 15–30	99 (33.4%)			
Duration of symptoms (years)	10.53 (11.51)	6.00 (16.00)	0.02–62	247
< 1 year	52 (21.1%)			
1–5 years	71 (28.7%)			
5–10 years	38 (15.4%)			
> 10 years	86 (34.8%)			
QIDS-SR score (scale 0–27)	9.21 (4.91)		1–26	294
BAI score (scale 0–63)	12.45 (9.62)	11.00 (12.00)	0–51	278
Cortisol values in nmol/L				296
Directly after awakening	10.9 (51.2)		1.0–879.9	
30 min afterwards	13.0 (12.8)		1.5–207.2	
60 min afterwards	10.5 (7.5)		1.4–101.3	
Cortisol values in nmol/L (excluding 1 outlier based on AUC_G[b])				295
Directly after awakening	7.97 (8.5)		1.0–127.6	
30 min afterwards	12.40 (6.1)		1.5–37.7	
60 min afterwards	10.22 (5.4)		1.4–30.6	
Most prominent symptom				294
Musculoskeletal	139 (47.0%)			
Fatigue	50 (16.9%)			
(Pseudo-)neurological	45 (15.2%)			
Gastro-intestinal	35 (11.8%)			
Other	25 (8.5%)			

SD: standard deviation; IQR: interquartile range; PHQ-15: Patient Health Questionnaire-15; QIDS-SR: Quick Inventory of Depressive Symptomatology; BAI: Beck Anxiety Inventory; AUC_G: area under the curve with respect to the ground.

[a] When not normally distributed.

[b] Cortisol values of outlier: 879.9 nmol/L directly after awakening, 207.2 nmol/L 30 min afterwards and 101.3 nmol/L 60 min afterwards.

value of 4.52 h∗nmol/L (SD 3.65). As described in the method section, we excluded the negative values in all regression analyses concerning the AUC_I. There were no positive outliers.

Table 3
Prevalence of the 51 physical symptoms assessed by the Physical Symptom Questionnaire (PSQ).

Symptom	Prevalence (n)	% (of 296)
Generalized fatigue or apathy	201	67.9
Back pain	175	59.1
Limb pain	169	57.1
Joint pain	168	56.8
Muscle tension	165	55.7
Easily fatigued after little exertion	164	55.4
Muscle pain or stiffness	157	53.0
Other pain symptoms	123	41.6
Insomnia	122	41.2
Headache	118	39.9
Forgetfulness	104	35.1
Flatulence	101	34.1
Tingling sensations (e.g. tingling hands)	99	33.4
Bloating	94	31.8
Cold poorly tolerated	90	30.4
Increased urination	83	28.0
Walking problems	82	27.7
Excessive sleeping	79	26.7
Dizziness/Light-headedness	74	25.0
Dry mouth	73	24.7
Excessive sweating	71	24.0
Hot flushes with sweating	65	22.0
Obstipation	65	22.0
Stomach pain	64	21.6
Cold shivers	64	21.6
Sexual indifference	52	17.6
Certain food types poorly tolerated	51	17.2
Heat poorly tolerated	49	16.6
Trembling	43	14.5
Diarrhea	43	14.5
Shortness of breath without exertion	40	13.5
Heartburn	36	12.2
Nausea	34	11.5
Palpitations	34	11.5
Blurry or double vision	30	10.1
Chest pain or pressure	29	9.8
Decreased appetite	28	9.5
Muscle weakness or palsy	25	8.4
Burning sensation genitals or anus	22	7.4
Pain during sexual intercourse	21	7.1
Choking	21	7.1
Deafness	21	7.1
Swallowing problems	20	6.8
Loss of voice	14	4.7
Weight loss (in the last month)	12	4.1
Urination problems	10	3.4
Vomiting	10	3.4
Painful urination	8	2.7
Fainting	2	0.7
Fits or epileptic seizures	1	0.3
Blindness	0	0.0

3.3. Confirmatory factor analysis

We defined a gastro-intestinal factor, a pain factor, a cardio-pulmonal factor and a fatigue factor, including symptoms as described in Table 1. The fit of this model was reasonable [χ^2(df = 71) = 101.814, p-value = 0.0097; CFI = 0.958; RMSEA = 0.038].

3.4. Cross-sectional relation between cortisol and individual scores on specific symptom clusters

Linear regression analyses of the relation between AUC_G and AUC_I and participants' scores on the specific factors revealed no significant relations in both unadjusted analyses and analyses adjusted for confounders (Table 4). For AUC_G, depressive symptoms were a significant effect-modifier (p = 0.025) of the relation between individual scores on factor 4 (fatigue) and AUC_G. For a subpopulation of patients with moderate or severe depressive symptoms (i.e. a QIDS-SR score of 11 or

Table 4
Regression analyses on the relation of individual scores on factors (symptom clusters) and AUC_G/AUC_I.

	Unadjusted				Adjusted			
	β	SE	p-Value	OR (CI)	β	SE	p-Value	OR (CI)
AUC_G (min∗nmol/L)								
Symptom cluster								
Factor 1 (gastro-intestinal)	− 1.01	0.59	0.085	0.36 (0.11–1.16)	− 0.61	0.77	0.430	0.54 (0.12–2.46)
Factor 2 (pain)	− 1.06	0.65	0.104	0.35 (0.10–1.24)	− 0.28	0.87	0.743	0.76 (0.14–4.16)
Factor 3 (cardio-pulmonal)	− 1.08	0.57	0.062	0.34 (0.11–1.04)	− 0.01	0.80	0.986	0.99 (0.21–4.75)
Factor 4 (fatigue)	− 0.72	0.49	0.140	0.49 (0.19–1.27)	0.11	0.64	0.859	1.12 (0.32–3.91)
AUC_I (min∗nmol/L)								
Symptom cluster								
Factor 1 (gastro-intestinal)	0.05	0.42	0.906	1.05 (0.46–2.39)	− 0.19	0.55	0.724	0.83 (0.28–2.43)
Factor 2 (pain)	0.20	0.46	0.660	1.22 (0.50–3.00)	0.08	0.61	0.902	1.08 (0.33–3.58)
Factor 3 (cardio-pulmonal)	0.41	0.40	0.310	1.51 (0.69–3.30)	0.65	0.54	0.228	1.92 (0.66–5.52)
Factor 4 (fatigue)	0.21	0.34	0.540	1.23 (0.63–2.40)	0.16	0.47	0.735	1.17 (0.47–2.95)

AUC_G: area under the curve with respect to the ground; AUC_I: area under the curve with respect to the increase; SE: standard error; OR: odds ratio; CI: 95% confidence interval.

higher, N = 117) we found a significant relation between factor 4 (fatigue) and the AUC_G, [β − 2.11; SE 0.88; p-value 0.019], while for the subpopulation of patients without these symptoms (i.e. a QIDS-SR score of 10 or lower, N = 176) no significant relation was found [β 1.22; SE 0.92; p-value 0.184]. There was no effect-modification by sex or anxiety. For AUC_I, there was no effect-modification by sex, depression or anxiety.

3.5. Cross-sectional relation between cortisol and severity of symptoms

As the scatter plot showed no clear linear relation between AUC_G/AUC_I and severity of symptoms, we performed the analyses with dummy variables, using symptom severity as a categorical variable based on clinically relevant cut-off values reflecting mild, moderate and severe symptoms (i.e. PHQ-15 score of respectively 0–9, 10–14 and 15–30) [10]. For both the AUC_G and the AUC_I unadjusted analyses showed no significant relations (Table 5). The analyses adjusted for identified confounders also showed no significant relations. Sex was a significant effect modifier of the relation between the AUC_I and severity of symptoms ($p < 0.001$), so we performed additional separate analyses for both men and women. Although the direction of the relations differed, for none of the analyses results were significant.

3.6. Cross-sectional relation between cortisol and duration of symptoms

As duration of symptoms was not normally distributed within the study population and as the scatter plot showed no clear linear relation between the AUC_G/AUC_I and duration of symptoms, we performed the analyses with dummy variables, using duration of symptoms as a categorical variable (i.e. duration of symptoms < 1 year, 1–5 years, 5–10 years and > 10 years) (Table 6). As we used multiple imputation techniques for these analyses, pooled results of the imputed data sets are displayed. Analyses showed no significant relations, and no effect-modifiers could be identified.

4. Discussion

In this study, we analysed cross-sectional relations between Cortisol Awakening Response (CAR) and individual scores on specific symptom clusters, symptom severity and duration of symptoms within a heterogeneous population of patients with all sorts of MUPS. We found that CAR values varied widely in our study population. For the total study population we found no significant cross-sectional relations between CAR (AUC_G/AUC_I) and the described symptom characteristics. We found that depression was a significant effect-modifier of the relation between individual scores on factor 4 (fatigue) and AUC_G. As this was the only specific relation influenced by depression, this finding is most likely to be fortuitous.

4.1. Comparison with literature

Few studies have assessed cortisol levels in heterogeneous populations of patients with MUPS. We found 2 relevant studies.

Janssens et al. studied the relation between cortisol responses and functional somatic symptoms in 715 adolescents [44]. They also used factor analysis to create symptom clusters and they studied the relation with the CAR. They identified two symptom clusters and found that a cluster of overtiredness, dizziness and musculoskeletal pain was associated with a low AUC_G and that a cluster of headache and gastrointestinal symptoms was associated with a low AUC_G during a stress

Table 5
Regression analysis of symptom severity and AUC_G/AUC_I, using PHQ-15 score 0–9 (mild symptoms) as reference group.

	Unadjusted				Adjusted			
	β	SE	p-Value	OR (CI)	β	SE	p-Value	OR (CI)
AUC_G (min∗nmol/L)								
Symptom severity								
PHQ-15 score 10–14 (moderate)	− 0.67	0.81	0.41	0.51 (0.10–2.50)	− 0.90	0.89	0.32	0.41 (0.07–2.33)
PHQ-15 score 15–30 (severe)	− 1.33	0.80	0.10	0.26 (0.06–1.27)	− 0.82	0.98	0.40	0.44 (0.06–3.00)
AUC_I (min∗nmol/L)								
Symptom severity								
PHQ-15 score 10–14 (moderate)	− 0.01	0.58	0.98	0.99 (0.32–3.09)	− 0.58	0.63	0.36	0.56 (0.16–1.92)
PHQ-15 score 15–30 (severe)	0.58	0.57	0.32	1.79 (0.58–5.46)	− 0.23	0.74	0.75	0.79 (0.19–3.39)

AUC_G: area under the curve with respect to the ground; AUC_I: area under the curve with respect to the increase; PHQ-15: Patient Health Questionnaire-15; SE: standard error; OR: odds ratio; CI: 95% confidence interval.

Table 6
Regression analysis of duration of MUPS and AUC_G/AUC_I (with symptoms < 1 year as reference group).

	Unadjusted				Adjusted			
	β	SE	p-Value	OR (CI)	β	SE	p-Value	OR (CI)
AUC_G (min∗nmol/L)								
Duration of symptoms								
1–5 years	− 0.85	1.02	0.41	0.43 (0.06–3.16)	− 0.80	1.11	0.47	0.45 (0.05–3.96)
5–10 years	− 1.24	1.43	0.39	0.29 (0.02–4.78)	− 0.87	1.46	0.55	0.42 (0.02–3.96)
> 10 years	− 0.80	0.97	0.41	0.45 (0.07–3.01)	− 0.83	1.06	0.43	0.44 (0.05–3.48)
AUC_I (min∗nmol/L)								
Duration of symptoms								
1–5 years	0.49	0.74	0.51	1.63 (0.38–6.96)	0.82	0.80	0.30	2.27 (0.47–10.89)
5–10 years	0.07	0.99	0.94	1.07 (0.15–7.47)	0.23	1.03	0.82	1.26 (0.17–9.48)
> 10 years	0.32	0.70	0.65	1.38 (0.35–5.43)	0.83	0.78	0.29	2.29 (0.50–10.58)

AUC_G: area under the curve with respect to the ground; AUC_I: area under the curve with respect to the increase; SE: standard error; OR: odds ratio; CI: 95% confidence interval.

test. However, it is difficult to compare their results with ours, due to differences in population characteristics (adolescents instead of adults) and measurement methods of the CAR.

Tak et al. studied the relation between cortisol levels and a range of functional somatic symptoms [45]. They used a population-based cohort of 741 adults with all sorts of functional symptoms. Results of this study are in line with ours: no cross-sectional or longitudinal relations were found between altered HPA-axis function and several clusters of functional somatic symptoms. However, in their study 24 h urinary free cortisol was used as an index for HPA-axis function. Urinary cortisol levels differ from salivary levels and as they can be influenced by cortisol clearance by kidneys and liver [46,47]. As a consequence the results of Tak et al. cannot be directly compared to ours.

4.2. Strengths and limitations

Our study has a few limitations that may have led to a possible underestimation of the relation between the CAR and symptom characteristics in MUPS patients.

A first limitation concerns our selection procedure. We relied on medical examinations as performed in routine care (instead of a standardized medical examination) to rule out medical explanations for the presented symptoms. Additionally, total number and severity of symptoms were assessed with self-rated questionnaires. In these questionnaires patients may have reported additional symptoms, which they had not presented to their physician. For these symptoms the unexplained nature may not have been confirmed by a medical examination.

A second limitation concerns our cortisol sampling procedure. When we developed our study protocol, we based our cortisol sampling procedure on earlier studies in this field, as no guideline or 'gold standard' was available. However, recently an expert consensus guideline about assessment of the CAR was published [48]. Unfortunately, as our study was conducted before publication of this guideline, some aspects of our cortisol sampling procedure are not in line with the guideline recommendations., The authors advise to collect samples on more days (instead of only 1 day). Another advice is to use objective monitoring techniques such as polysomnography (instead of self-report) to assess timing of sampling, as self-report techniques have shown to be associated with sampling non-adherence.

The choice to exclude participants with a negative AUC_I from further AUC_I analyses is also in line with earlier studies [9]. However, this choice is up to debate and therefore forms a possible third limitation [48]. A descriptive study showed that negative AUC_I values can be present in up to 23% of the normal adult population [13]. Another study showed that a delay in sampling only leads to significantly higher awakening cortisol values (and consequently a risk of a negative AUC_I) if the delay is > 15 min [49]. We can conclude that negative AUC_I values are not necessarily a result of non-adherence to the protocol. This conclusion is strengthened by the fact that within our population AUC_I values were normally distributed. By excluding negative values, the lower tail of this distribution was cut off. The choice to exclude negative AUC_I values had important consequences for our study findings as analyses in which negative AUC_I values were included showed divergent results. Therefore, the presented AUC_I results need to be interpreted with caution.

Despite the described limitations, we believe that our study provides new information as it is one of the first studies evaluating cortisol levels within a heterogeneous MUPS population, additionally including analyses concerning the relation with specific symptom clusters as well as severity and duration of MUPS. We also included a relatively large sample size, enhancing the robustness of our findings.

4.3. Considerations

We found that many participants showed high scores on several symptom clusters. This finding was strengthened by the fact that most patients reported symptoms from various organ tracts in their symptom top 3. The co-existence of symptoms from different symptom clusters has been described before, and tends to support the theory that the similarities between symptom clusters or functional syndromes may outweigh the differences (the 'lumpers'-theory) [1]. Following this theory, it may be no surprise that we did not find relations between the CAR and scores on individual symptom clusters (as symptom typology seems to be indistinctive).

The question remains why earlier studies did find relations between cortisol and specific functional syndromes. It is possible that these studies included specific subgroups of patients that only suffered from symptoms from one specific symptom cluster. Within such homogeneous patient groups, a relation between the specific cluster and the CAR may be present. However, it is also possible that the identified relations between cortisol and specific functional somatic syndromes were not a consequence of the characteristics of the symptoms themselves, but a consequence of the different behavioural aspects (such as sleeping habits or medication use) or co-morbidities (such as anxiety or depression) related to these symptoms [9].

In the light of these considerations, and in line with the earlier results of Tak et al. [45], we believe that it is plausible that within heterogeneous MUPS populations, with symptoms from several organ tracts, there is no relation between cortisol disturbances and the presence of symptoms from specific symptom clusters.

5. Conclusion

Within our total population of patients with MUPS we did not find evidence for a relation between the CAR and symptom characteristics,

indicating that cortisol disturbances in MUPS populations are not symptom specific. The absence of a relation could be explained by the fact that most participants showed no cluster specific symptom pattern. However, more studies, using comprehensive cortisol measurement methods, are needed within heterogeneous MUPS populations to confirm our findings.

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