The onset of the migraine attack

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Part III.

Biochemical aspects

Chapter 8.

Female sex hormones in men with migraine

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Abstract

Objective
To assess the role of oestradiol and testosterone in men with migraine.

Methods
We measured 17ß-oestradiol (E2) and calculated free testosterone (T_f) in serum of 17 medication-free men with migraine and 22 men without migraine group-matched for age and body mass index, targeted at 20-28 kg/m^2. Blood was sampled on a single, for migraineurs interictal, day at 9am, 12am, 3pm and 6pm. Migraineurs were subsequently measured 3-4 times daily until an attack occurred. Clinical androgen deficiency was assessed using the Androgen Deficiency of Ageing Men questionnaire and the Aging Males' Symptoms scale. We analysed interictal data (mean ± standard error) with repeated measurement ANCOVA and longitudinal data by Generalized Estimated Equations models.

Results
Compared to controls, men with migraine had a lower interictal T_f/E2 ratio (3.9 ± 0.4 vs. 5.0 ± 0.3; p=.03) due to higher E2 (96.8 ± 6.1 pmol/L vs. 69.1 ± 5.6 pmol/L; p=.001) and similar T_f (357.5 ± 21.4 pmol vs. 332.6 ± 18.7 pmol/L; p=.35) levels. Pre-ictal T_f levels were increased in men with migraine reporting premonitory symptoms (p=.03). Men with migraine more frequently reported symptoms of androgen deficiency (11/18 [61.1%] vs. 6/22 [27.3%]; p=.031) which were also more frequently severe (p=.006); their age- and BMI-adjusted AMS scores were higher (27.0 ± 1.2 vs. 21.0 ± 1.0; p=.002).

Conclusions
In this study, non-obese men with migraine exhibited increased levels of the sex hormone oestradiol and show clinical evidence of relative androgen deficiency. The role of oestradiol in modulating migraine susceptibility and activity in men deserves further investigations.

Introduction

Migraine is a common, disabling, episodic brain disorder, typically characterised by recurrent attacks of severe headache, associated features and, in one third of patients, aura [1,2]. In up to two-thirds of migraineurs, attacks may be preceded by affective and physical premonitory symptoms [3,4]. Migraine prevalence and the frequency, duration and severity of migraine attacks are highly dependent on age, sex and, in women, events which are associated with marked fluctuations in female reproductive hormones [5-7]. In the fertile period, three times more women (24%) than men (8%) have active migraine and their attacks are on average more frequent, longer and more severe [5,7]. Furthermore, fluctuations in female sex hormones during puberty, menstruation, pregnancy, breast-feeding, menopause and post-menopause are associated with changes in attack frequency [5-7]. Additional evidence that sex hormones might modulate migraine risk and activity is coming from other observations [6,7]: migraine prevalence is higher in obese individuals with elevated oestrogen levels [8] possibly due to increased conversion from testosterone in adipocytes [9]; starting or stopping using oral contraceptives can be associated with either onset or disappearance of migraine attacks [10]; many male-to-female transsexuals develop migraine after starting oestrogen and anti-androgen therapy [11]; testosterone administration was associated with reduction in migraine frequency and severity in some women [12]; certain polymorphisms in sex hormone receptor genes were associated with increased risk of migraine [13]; and menstrual cycle-related changes in oestrogen levels influence the activity of the trigeminovascular system which is responsible for causing migraine headache [14,15]. Finally, sex-related differences and experimental manipulation of sex hormone levels modulated neurogenic vasodilatation and cortical spreading depression in animal migraine models [15,16], surrogate markers for migraine susceptibility. It is unknown whether sex hormones might modulate migraine risk and activity in men. Here we assessed interictal, pre-ictal and ictal levels of 17ß-oestradiol, free testosterone and the free testosterone to 17ß-oestradiol ratio in men with migraine, hypothesising that levels of 17ß-oestradiol and the ratio would be higher at baseline and would increase further towards the attack.

Material and Methods

Participants

We selected males between age 18 and 74 years, N = 18 with episodic migraine without aura according to the criteria of the International Classification of Headache Disorders (ICHD-IIIb)[17] and N = 24 controls without migraine themselves or in first degree relatives group-matched for age and body mass index (BMI). When we use the word controls, we refer to healthy controls without a history of recurrent headaches. Controls could in addition not have any other type of headache on ≥ 2 days per month. Migraineurs were excluded if: (i) they were unable to differentiate migraine from other headaches; (ii) had headache or were using acute headache medication on ≥10 days per month; and (iii) were daily using migraine prophylactic medication. Exclusion criteria for both migraineurs and controls were: (i) BMI <20 or >28; (ii) smoking during participation; (iii) frequent consumption of spicy foods; (iv) hypertension (defined as blood pressure >150/90mmHg or use of antihypertensive

medication; (v) intake of high-fat foods immediately before a measurement; (vi) history of hypogonadism; (vii) using any medication or supplements which could affect hormone levels; (viii) any liver or kidney condition; and (ix) coagulopathies such as haemophilia or a compromised immune system.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was conducted as part of the Leiden University Medical Center Neuro Analysis Programme (see below) [18]. LUMINA and the present study were approved by the local medical ethics committee and all participants provided written informed consent prior to participation.

LUMINA programme

Both migraine patients and controls were recruited via nationwide public announcement, advertising in lay press and via the research website, and were considered eligible after a two-step inclusion process using validated questionnaires via the especially designed LUMINA website. Additionally, patients from our outpatient headache clinic were invited to participate by a letter. On the website, patients were asked to fill out a screening questionnaire that has been validated previously1. Firstly, if patients fulfilled the screening criteria, they were sent a web-based extended migraine questionnaire, based on the ICHD-II criteria(14, 15). This questionnaire was validated before by performing a semi-structured telephone interview in 1,038 patients who had filled out the extended migraine questionnaire[14]. The specificity of the questionnaire was 0.95. We consider the cohort a well-defined web-based cohort, with 4% of subjects included from our dedicated headache outpatient clinic, 87% of the participants having been diagnosed as migraineurs previously by a medical doctor. In addition to questions that were necessary to diagnose migraine accurately, the extended questionnaire also included items on demographic factors, acute and prophylactic headache medication use, migraine attack frequency and allodynia. Participants without the needed internet skills were able to fill out the questionnaires on paper. Non-headache individuals willing to participate had to pass a screening questionnaire online via the research website. If this screening questionnaire did not show any indication for having migraine, cluster headache, chronic tension type headache or medication overuse headache, individuals were sent a subsequent in depth questionnaire. This second questionnaire again assessed possible headache complaints, together with demographic variables. Only individuals that fulfilled both the criteria of 'non-headache' in the screening and in depth questionnaire were considered eligible controls and were approached for this questionnaire study.

Study Design

Blood samples were collected on a single (for migraineurs interictal) day at 9am, 12am, 3pm and 6pm. Participants with migraine were in addition measured three to four times daily on the following days until an attack occurred. The interictal (baseline) day was planned at least three days after the last migraine attack and within ten days from the next expected attack (based on the participant's historical attack frequency).

Hormone Level Assessment and Sample Storage

Levels of 17ß-oestradiol (E2; pmol/L), sex hormone-binding globulin (SHBG; nmol/L)

and albumin (g/L) were assessed using ECLIA immunoassays (Roche Diagnostics GmbH, Mannheim, Germany). Inter-assay coefficients of variability mean ± sd) were 5.3 ± 1.7 (SHB), 1.5 ± 0.5 (albumin), 2.1 ± 1.4 (oestradiol) and 5.1 ± 1.4 (total testosterone) respectively. The free testosterone fraction (T_f; pmol/L) was calculated using SHBG and albumin levels [19]. Previous studies have revealed that female sex hormone levels in men show only little inter- and intra-individual variability[20,21]. Serum total testosterone (T_t; nmol/L) was assessed by coat-a-count® radioimmunoassay (Siemens, Camberley, UK). Main outcome measure was the T_f/ E2 ratio. Prior to analysis, samples had been stored for a mean of 85.3 ± 38.5 days.

Migraine and Premonitory Symptoms

Using a standardised interview, we assessed at each measurement the presence and characteristics of headache and premonitory symptoms, including less-frequent micturition, ankle or wrist oedemas, changes in defecation, thirst, changes in appetite, craving for specific food, stiffness of limbs and/or face, stiff neck, difficulty with concentrating, mental agitation, physical agitation, fatigue, excessive yawning, hyperirritability, and mood changes such as depression [3]. The premonitory phase was defined as presence of ≥ 1 of the above symptoms which was then followed by migraine headache within 24 hours. Measurements were labelled afterwards as "baseline" (which in participants with migraine refers to interictal), "pre-ictal with or without premonitory symptoms", or "headache".

Items Relevant to the Reproductive System

We collected the following items that are relevant to the reproductive system and secondary sex characteristics: frequency of shaving of facial hair, age of dropping of voice in puberty, number of children, unwanted childlessness, delay in parenthood despite of attempts, help in fertilisation (in vitro fertilisation; sperm donation; surrogacy), and cryptorchidism.

Questionnaires on Androgen Deficiency

To assess clinical androgen deficiency, we used two validated questionnaires. The Androgen Deficiency of Ageing Men (ADAM) questionnaire contains 10 items regarding the most common symptoms observed with age-related decline in androgens (corresponding to bioavailable testosterone levels <70ng/dL) [22]. All questions are answered with *yes* or *no*. If question 1, 7, or any 3 other questions are answered positively, the results indicate an androgen-deficient state with a sensitivity of 0.88 and specificity of 0.60 [22]. The Aging Males' Symptoms (AMS) scale contains 17 items on ageing and clinical testosterone deficiency with per-item scores ranging from 1-5 (none to severe) [23]. Sum-scores range from to 17-85 with higher scores indicating lower health related quality of life and suggesting lower free testosterone levels. Scores can be categorized into no/few symptoms (17-26 points), mild (27-36 points), moderate (37-49 points), and severe symptoms (>50 points). Test-retest reliability is 0.8-0.9 for the total score [23]. Both questionnaires were filled out once prior to the first measurement.

Sample Size Calculations

The sample size was based on log [T_f/E2], since the ratio between these two hormones is a commonly used parameter in clinical studies. We based our sample size on intra-individual comparison, assuming a change of 25% to be clinically relevant [24]. We furthermore assumed

a 10% day-to-day variation in testosterone and 15% day-to-day variation in oestrogens [25]. With α set at 0.05 and β at 0.10, an intra-individual paired sample-size calculation resulted in 15 participants.

Statistics

General characteristics were compared between participants with migraine and controls using Student's t-tests for continuous variables and Chi square tests for categorical data. Baseline hormonal levels (both using the 9 am measurement and the average over the four baseline measurements of 9am, 12am, 3pm, 6pm) were compared between participants with migraine and controls using ANCOVA allowing for adjustment for age and BMI. Hormonal changes prior to an attack were assessed using a generalised estimated equations (GEE) model run with timing (baseline/ pre-ictal [<24 hours from beginning of attack] / headache) as dependent variable without additional correcting for age and sex [26]. Separate analyses were done for pre-ictal measurements with and without premonitory symptoms. Measurements up to 72 hours prior to the migraine headache were used in the analyses and measurements performed after acute migraine medication was taken were excluded. For research and financial reasons, we chose to only analyse migraine data from the baseline day, a prodromal day, and the headache phase. All hypothesis tests were 2-sided. Data analyses were performed using SPSS 17.0 (SPSS inc., IBM, USA). The statistical threshold was set at $p<.05$.

Data availability

All data, methods and materials used to conduct this research are mentioned in this article.

Results

Study Population and Measurements

We included 42 males, 18 with migraine without aura and 24 controls. Demographic characteristics were similar (Table 1). Data from two controls had to be excluded, one because he later turned out to have ever suffered from a migraine aura without headache and the other because of very low levels of LH suggestive of recreational use of androgen. Of one participant with migraine, no baseline interictal measurements were performed. Thus, interictal/baseline data were available for 17 participants with migraine and 22 controls. Follow-up data were available for 14 participants with migraine over a period of 2-11 days with 8-41 measurements per participant. In total 149 serum samples were analysed in 18 participants with migraine: interictal n=95; pre-ictal (<24h before onset headache) n=43 of which n=36 with premonitory symptoms; and during the headache phase n=18.

Female Sex Hormone Levels in Migraineurs

We assessed interictal/baseline levels in two ways: as a single measurement at 9 am and as mean of all 4 measurements on the interictal/baseline day. Interictal/baseline 17ß-oestradiol levels (9 am) were higher in participants with migraine (96.8 ± 6.1) compared to non-migraine controls (69.1 ± 5.6 pmol/L; p=.001) while free testosterone levels were similar (357.5 ± 21.4 vs. 332.6 ± 18.7 pmol/L; p=0.35; Figure 1). As a result the adjusted T_f/E2 ratio was lower in participants with migraine (3.9 ± 0.4) compared to controls (5.0 ± 0.3;

p=.03; Figure 1). Similar results were obtained for the means of the 4 measurements on the interictal/baseline day for 17ß-oestradiol (migraine: 92.5 ± 5.3 vs. controls: 67.6 ± 4.8 pmol/L; *p*=.002), free testosterone (migraine: 312.6 ± 17.2 vs. controls: 318.0 ± 15.5 pmol/L; *p*=.82), and adjusted T_f/E2 ratio (migraine: 3.6 ± 0.3 vs. controls: 4.9 ± 0.3; *p*=.007).

Table 1. Baseline Characteristics of the Study Population.

Variable	Men with Migraine (N=18)		Male Controls without Headache (N=22)	
Demographics				
Age, mean (SD), year	46.9	(16.4)	48.5	(17.2)
BMI, mean (SD), kg/m²	24.7	(4.1)	23.4	(1.8)
Clinical testosterone items				
Shaving frequency/week, mean (SD)	5.2	(2.1)	6.3	(2.4)
Age of dropping voice, mean (SD), year	13.5	(1.5)	13.8	(1.4)
Cryptorchidism, n (%)	1	(6)	1	(5)
Reproductive items				
Number of children, mean (SD)	0.9	(1.1)	1.3	(1.2)
Longer than expected time-to-conception, n (%)[a]	2	(20)	1	(7)
Unwanted childlessness, n (%)[b]	3	(30)	3	(19)
Aid in fertilisation, n (%)[b]	1	(10)	0	(0)
Migraine characteristics				
Migraine subtype MO, n (%)	11	(61)	-	
Attack frequency/month, mean (SD)	2.8	(1.6)	-	
Acute medication, n (%)	18	(100)	-	
Prophylactic medication, n (%)	0	(0)	-	

M = male; MO = migraine without aura; n.a. = not applicable. The section 'Reproductive items' only applies to those participants with a child wish, and therefore the total number is smaller than the entire group.

[a] In total n=24 participants had at least one child: n=14 controls and n=10 migraineurs

[b] In total n=26 participants (n=16 controls and n=10 migraineurs) had childwish

Pre-Ictal and Ictal Changes in Sex Hormone Levels

To analyse whether hormone levels change during or shortly before attacks compared to interictal/baseline we intra-individually compared the interictal/baseline, pre-ictal and ictal measurements in two ways. First, for all participants with migraine, irrespective of whether or not they had experienced premonitory symptoms in the pre-ictal phase, and second only for those who reported premonitory symptoms during the pre-ictal phase (Table 2; Figure 2). While there were no differences when analyzing all participants (Tf: *p*=.19; 17ß-oestradiol: *p*=.09; Tf/E2 ratio: *p*=.10), when only analyzing migraine participants with premonitory symptoms, Tf levels (*p*=.03) were different, but not the 17ß-oestradiol levels (*p*=.15) or Tf/E2 ratio (*p*=.08). Rerunning the prospective analyses using a linear mixed model approach yielded similar results. Post-hoc analysis revealed that pre-ictal T_f (358.6 ± 21.1 vs. 293.6 ±

23.0 nmol/L; *p*=.03) and 17ß-oestradiol (96.3 ± 7.7 vs. 73.2 ± 6.7; *p*<.001) levels were higher in migraine participants with premonitory symptoms compared to those without. Rerunning the analyses including only patients >50 years of age yielded similar results.

Clinical Evidence of Androgen Deficiency

The age and BMI adjusted mean ± SE AMS scores were higher in men with migraine (27.0 ± 1.2 vs. 21.0 ± 1.0; *p*=.002; pearson correlation with T_f levels: r=-0.05; *p*=.74), suggesting relative functional deficiency of T_f. Compared to controls, men with migraine did not report symptoms of androgen deficiency more frequently (ADAM questionnaire): 11/18 [61.1%] vs. 6/22 [27.3%]; *p*=.053, but they were more often mild/severe (AMS scale): 10/18 (55.6%) vs. 2/22 9.1%; *p*=.006 (Table 3).

Table 2. Hormone Levels during Different Phases of the Migraine Attack.

Hormone		Baseline		Pre-Ictal		Headache		*P* overall	*P* post-hoc pre-ictal
T_{free}, mean (SE), pmol/L	All	313.5	(18.5)	336.1	(17.0)	355.7	(32.3)	.19	
	PS+	313.2	(17.8)	354.1	(21.4)	354.8	(31.4)	.03	.03
	PS-	312.5	(18.1)	306.4	(24.8)	352.6	(32.7)	.41	
E2, mean (SE), pmol/L	All	94.2	(6.1)	90.3	(6.3)	98.6	(8.1)	.09	
	PS+	90.5	(6.1)	93.4	(7.1)	101.5	(8.4)	.15	<.001
	PS-	93.3	(5.7)	87.8	(6.5)	97.2	(7.3)	.08	
T_f/E ratio, mean (SE)	All	3.3	(0.2)	4.1	(0.4)	4.1	(0.5)	.10	
	PS+	3.3	(0.2)	4.2	(0.4)	4.1	(0.4)	.08	.98
	PS-	3.3	(0.2)	3.9	(0.4)	4.0	(0.4)	.25	
T_{total}, mean (SE) nmol/L	All	19.4	(1.3)	20.5	(0.9)	21.0	(1.5)	.59	
	PS+	19.4	(1.2)	21.8	(1.2)	21.2	(1.5)	.04	.005
	PS-	19.4	(1.3)	19.3	(1.0)	20.9	(1.6)	.45	

Variables are depicted as mean ± standard error (SE), derived from generalized estimated equation models. *P* values are not adjusted for multiple comparisons per endpoint / multiple endpoints.

Pre-ictal = 24 hours prior to begin of migraine headache.

PS+ = pre-ictal measurements with presence of premonitory symptoms.

PS- = pre-ictal measurements without presence of premonitory symptoms

T_{free} = Testosterone$_{free}$ (pmol/L)

T_{total} = Total testosterone, free and bound (nmol/L)

E2 = 17β-oestradiol (pmol/L)

P overall = comparison between baseline, pre-ictal, and headache phases

P pre-ictal = comparison between pre-ictal measurements with premonitory symptoms present vs pre-ictal measurements without premonitory symptoms

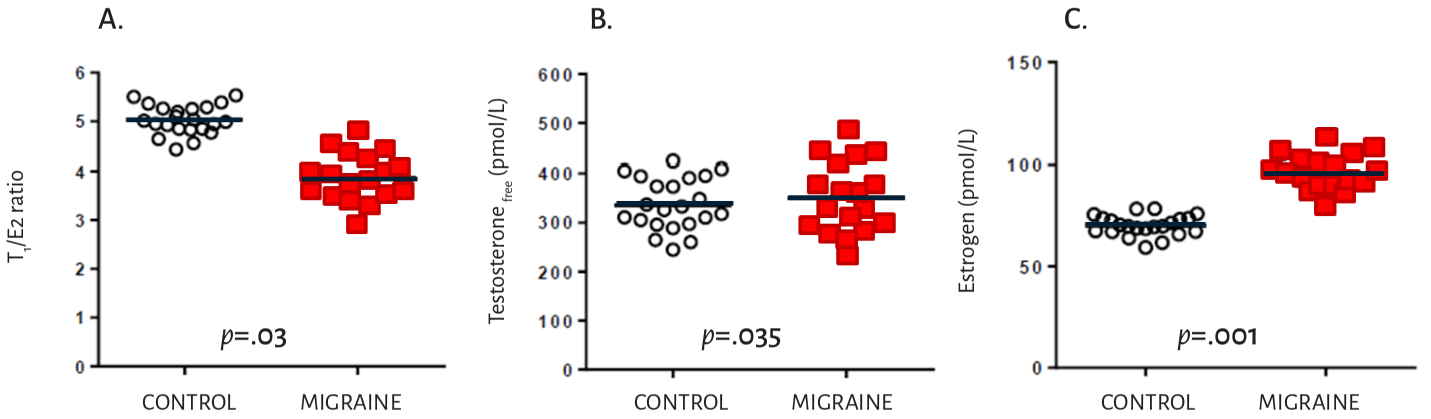

Figure 1. Tf/E2 Ratio and Levels of Free Testosterone and 17ß-Oestradiol in Men with Migraine and Non-Headache Controls at Baseline.
Depicted levels are adjusted for age and BMI. Tf:E2 ratio = free testosterone (pmol/L) / 17β-oestradiol (pmol/L) ratio

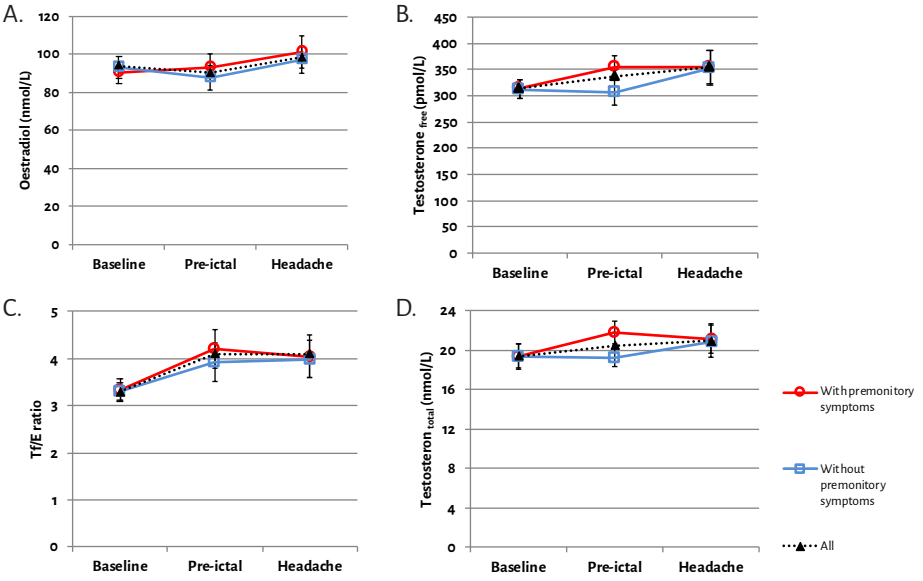

Figure 2. Hormone Levels during Different Phases of the Migraine Attack.
Depicted levels are means ± standard error of the mean. Tf:E2 ratio = free testosterone (pmol/L) / 17β-oestradiol (pmol/L) ratio

Table 3. Scores on Aging Males' Scale Score and on the Androgen Deficiency of Ageing Men questionnaire in male migraineurs and controls.

Variable	Men with Migraine (N=18)		Male Controls without Headache (N=22)		*p*
AMS score[a]					
Mean (SE)	27.0	(1.2)	21.0	(1.0)	0.001
AMS category[b]					0.006
No / few, n (%)	8	(44.4%)	20	(90.9%)	
Mild, n (%)	9	(50.0%)	2	(9.1%)	
Moderate, n (%)	0	(0%)	0	(0%)	
Severe, n (%)	1	(5.6%)	0	(0%)	
ADAM category[c]					0.053
Androgen deficiency	6	(23%)	7	(38.9%)	
No androgen deficiency	16	(72.7%)	11	(61.1%)	

[a] Adjusted for BMI and age
[b] no /few versus (mild / moderate / severe)
[c] Fisher's exact test
AMS = Aging Males's Scale
ADAM = Androgen Deficiency of Ageing Men questionnaire

Discussion

We prospectively assessed sex hormone levels in 17 well characterised non-obese men with migraine (with moderate attack frequency) and 24 controls without headache who were matched for age- and BMI on group level. Use of medications that could potentially affect hormone levels was carefully excluded. Men with migraine had increased oestrogen plasma levels, both absolute and relative to free testosterone, and reported higher scores on the ADAM and AMS scales reflecting functional androgen deficiency. Those who reported premonitory symptoms showed pre-ictal increase in testosterone and possibly also oestradiol.

While there is ample, though still circumstantial, clinical evidence that female sex hormones might modulate migraine susceptibility in women (see introduction and [5-7], little is known whether sex hormones have similar effects in males. We are aware of one study in which testosterone was measured in eight men with migraine during and outside attacks; in accordance with our study, no differences were found [27]. The pre-ictal rise in testosterone in our study might be related to a general stress-response anticipating the impending attack [28].

Unfortunately, our sample size is too small and the migraine characteristics are too homogeneous for additional analyses by headache frequency, intensity or duration. We can also not fully exclude that, possible due to self-selection, our results may only apply to patients with e.g. severe migraine. However, as the clinical characteristics of our study population seem rather typical for the average male migraineur (see Table 1), we believe such an alternative interpretation is unlikely.

There are several possible mechanisms through which changes in reproductive hormone levels might modulate migraine susceptibility. Women with menstrual-related migraine attacks seem to have a more rapid late-luteal phase drop in 17ß-oestradiol compared to women without menstrual-related migraines [7,29]. This might cause imbalance between long-lasting genomic effects of nuclear oestradiol receptors and short-lasting non-genomic effects via intra-membranous G-protein-coupled oestradiol receptors [30]. This imbalance might activate a cascade leading to neuronal sensitisation and ultimately triggering of migraine attacks [30]. Unfortunately, although in clinical trials with oestradiol-releasing skin creams [31-33] or oestrogen supplements [34], all aimed at preventing or delaying abrupt oestrogen-withdrawal, promising results were obtained, in clinical practice these measures are considered of only limited value[35].

In animal models, female and male sex hormones differentially affect two important basic mechanisms likely involved in migraine pathogenesis: susceptibility to cortical spreading depolarization (CSD), a putative surrogate marker of migraine susceptibility [15], and activation of nociceptive transmission within the trigeminovascular system [15,36]. The threshold for inducing CSD was lower and the velocity and frequency of CSD were higher in female compared to male transgenic mice carrying a human pathogenic *CACNA1A* familial hemiplegic migraine type 1 (FHM1) mutation[37]. Moreover, ovariectomy revoked

and orchiectomy enhanced these sex-related differences [37]. Female gonadal hormones enhanced CSD susceptibility, possibly by increasing cortical hyperexcitability, whereas male hormones had the opposite effect. These differential hormonal effects were only observed in mice carrying an FHM1 mutation and not in wild-type mice, supporting the hypothesis that female hormonal fluctuations affect migraine activity in genetically predisposed individuals [15,38]. In addition to their effects on CSD, sex hormones might also regulate sensitization of trigeminal neurons by modulating expression of nociceptive mediators such as calcitonin gene-related peptide (CGRP) and by affecting serotonin synthesis, dural mast cell density, and intracellular downstream signalling. Oestrogens seem to have a positive and androgens a suppressing effect on nociceptive transmission, with higher oestrogen levels reflecting higher activation states of these mechanisms [39]. Overall, the differential effects on CSD susceptibility and trigeminovascular activity might well explain, at least partly, why migraine is so much more prevalent among women, and why periods of major sex-hormonal fluctuations are so often associated with marked changes in migraine activity[6,7].

Taken together, in our current study non-obese men with migraine exhibited increased levels of the sex hormone oestradiol and show clinical evidence of relative androgen deficiency. Further studies in larger and additional populations are needed to validate these findings. What exactly the role is of oestradiol in men with migraine and whether fluctuations in oestradiol levels, like in women, might be associated with changes in migraine activity, deserves further intra-individual follow-up studies over multiple attack cycles.

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