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Headache provocation by nitric oxide in men who have never experienced a headache

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Abstract

Introduction: In the general population 4% have never experienced a headache. Freedom from headache could be due to distinctive protective mechanisms or a lack of environmental risk factors for headache. Isosorbide-5-mononitrate is an organic nitrate which in the body is metabolised to nitric oxide. The nitric oxide pathway plays a crucial role in the primary headaches. We hypothesized that people who are free from headache are protected by distinctive mechanisms in the nitric oxide pathway.

Methods: We performed an observer blinded case-control study using nitric oxide to provoke a headache. 32 headache free male participants and 26 randomly selected male controls received 60 mg Isosorbide-5-mononitrate orally on the study day. Participants fill out a headache diary with headache intensity and characteristics until 12 hours after administration of Isosorbide-5-mononitrate. Primary endpoint were areas under the curve of headache intensity score.

Results: All 58 participants completed the study. There was no significant difference in headache incidence, headache intensity score or migraine-like attack between headache free participants and controls.

Conclusion: We show that men who have never experienced a headache develop a headache when provoked with Isosorbide-5-mononitrate. This indicates that freedom from headache in men is not related to the nitric oxide pathway which is involved in the primary headache disorders.

Keywords

Provocation study, migraine, isosorbide-5-mononitrate, protective mechanisms, never had headache, pathway

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Introduction

Headache has a life-time prevalence of 91-96% in the general population (1–3). Based on the question "Do you think that you never ever in your whole life have had a headache" 4% of people say yes (4). Freedom from headache could be due to distinctive protective mechanisms or a lack of environmental risk factors for headache. Due to the high prevalence, headache is associated with a high burden of disability for both the individual and society (5). Better prevention and treatment of headache may therefore have a great impact worldwide. If people who are free from headache are protected by distinct mechanisms, this could lead to new prevention or drug development initiatives.

Isosorbide-5-mononitrate (5-ISMN) is an organic nitrate which is metabolised in the body to nitric oxide (NO) (6). Endogenous NO is released from endothelial cells and upregulates guanylate cyclase that synthesises cGMP, causing smooth muscle relaxation and vasodilation (7). The NO pathway plays a crucial role in the primary headaches (8–10). NO has been most extensively examined in migraine, where up to 80% of people with migraine experienced a migraine-like headache after provocation with glyceryl

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trinitrate (11–13). 5-ISMN induces a headache in almost all healthy individuals and a migraine-like headache in about 30–60% of healthy individuals (14–16).

We hypothesized that people who are free from headache are protected by distinctive mechanisms in the NO pathway. We tested this in an observer blinded case-control study using 5-ISMN to provoke a headache.

Material and methods

Participants

We recruited 58 male participants from the study "Pain sensitivity of headache free individuals" (ClinicalTrials.gov ID: NCT04217616) (32). For flowchart of inclusion, see Figure 1. In short, the "Pain sensitivity of headache free individuals" study is a nested case-control study with 100 male participants recruited from a cohort of voluntary Danish blood donors (17). From 2015 to 2018, 17,434 male donors had answered the question "Do you think that you never ever in your whole life have had a headache", yes or no, as part of the Danish blood donor study (4,17). Possible cases and controls were identified from this question and a sample were drawn using simple random sampling based on a random generated encrypted id. The Danish blood donor study has a migraine and headache prevalence similar to that of the general Danish population (2,4,18,19). Controls were drawn at random without selecting any specific primary headache disorder. The inclusion criteria for the "Pain sensitivity of headache free individuals" study was the same as for the present study. Inclusion criteria were: Male sex, age 18–70 years and weight of 45–95 kg. Exclusion criteria were: Serious somatic or psychiatric disease or daily medication use. We selected men only due to feasibility, as the women: men ratio in headache free individuals were 1:2.2, and to eliminate possible sex related variability (4). All participants were interviewed and underwent a medical examination by trained senior medical students, supervised by Professor Jes Olesen. Participants received a 1000 DKK (135 EUR) reimbursement for participation in the study. Participants were enrolled in the present study from November 2019 to March 2020 after completing their participation in the "Pain sensitivity of headache free individuals" study. 32 males who had never had a headache and 26 randomly selected males were included.

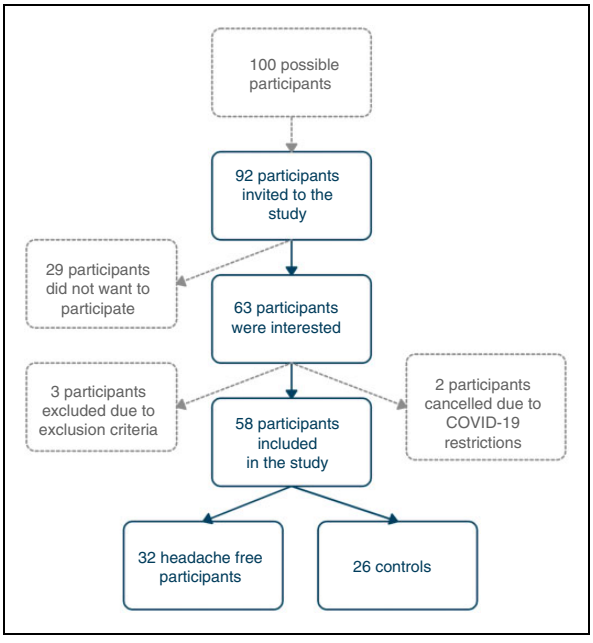

Figure 1. Flowchart of inclusion of participants.

Standard Protocol Approvals

All participants gave oral and written consent after oral and written information describing the study. The study was approved by the Ethics Committee of Copenhagen (H-19022744) and the Danish Data Protection Agency. The study was registered at Clinicaltrials.gov (ID: NCT04217668) and was conducted according to the Helsinki II declaration of 1964, 2013 version (20). The study was performed between December 2019 and March 2020. Two scheduled participants could not participate due to COVID-19 restrictions and were not replaced.

Design

This study was an observer blinded case-control study. 32 headache free male participants and 26 randomly selected male participants received 60 mg 5-ISMN orally on the study day. All 58 participants completed the headache provocation, and cases and controls were examined in a randomized order. The observers (IAO and KF) were blinded to the case-control status of the participants. The case-control status remained blinded until after data management. Blinding was performed by study investigator (TFH) who was not involved in data collection or data analyses.

Study Procedure

Participants arrived non-fasting at the Danish Headache Center, Rigshospitalet between 8.30 am and 10.45 am. Participants were without any kind of headache at least 48 hours before the study day and without any medication use at least 12 hours before the study day. The participants were instructed by a trained senior medical student not to talk about their medical history, family medical history or headache with the observers. If the participants revealed their

headache status to the observers, they would be excluded from the study. This did not happen with any participants throughout the study. The study was performed in the same room for all participants. Participants were placed in supine position, mean arterial pressure (MAP), heart rate and electrocardiogram were obtained. The participants then received 60 mg 5-ISMN (Imdur®) as one tablet orally. Participants were instructed to fill out a headache diary every 30 minutes from time 0–6 hours after administration of 5-ISMN and every hour from time 6–12 hours after administration of 5-ISMN (21). Participants stayed at the hospital until 5 hours after administration of 5-ISMN and were then discharged to fill out the rest of the diary at home. In case of severe headache, the participants were allowed rescue medication (1 g Paracetamol and 400 mg Ibuprofen) unless they had a known allergy to any of the medications. During and after the study participants could always contact the observer (IAO) on an emergency phone, if they experienced severe headaches or discomfort.

Headache Parameters

Headache and migraine-associated symptoms were recorded every 30 minutes in hospital, and every hour after discharge, until hour 12 after administration of 5-ISMN. Headache intensity was recorded using a numerical rating scale (NRS) from 0 to 10 (0: No headache. 1: A very mild headache, including a pressing or throbbing non-painful feeling. 10: Worst imaginable headache). Headache localization, pain quality (pressing or throbbing), if the headache was aggravated by physical activity, nausea, vomiting, photophobia, phonophobia and used of rescue medication were recorded.

To assess migraine, validated criteria for experimentally induced migraine-like attacks were used (22). Headache was classified as migraine-like if headache fulfilled criteria C and D for migraine without aura according to the ICHD3:

> C: Headache has at least two of the following four characteristics:
>
> Unilateral location, pulsating quality, moderate to severe pain intensity (moderate or severe pain intensity was considered >= 4 on the NRS), aggravation by routine physical activity.
>
> D: Headache accompanying by at least one of the following:
>
> 1. Nausea and/or vomiting. 2. Photophobia and phonophobia.

Statistical and data analysis

Normally distributed data were presented as mean (95% confidence interval) and non-normally distributed data as median (range). Frequencies and percentages were used for categorical variables.

The primary endpoint was area under the curve (AUC) of headache intensity score for time 0–5 hours after 5-ISMN administration between headache free participants and controls. The secondary endpoint was AUC of headache intensity score for time 0–12 hours after 5-ISMN administration between the two groups. AUC was calculated using the trapezium rule (23). One participant went to bed after 11 hours of follow up, and three participants after 10 hours of follow up. Apart from this loss to follow up, we had no missing data. For the four participants, AUC was calculated based on the available data.

Differences in AUC, peak headache score and time to peak headache scores were tested using Mann-Whitney U test. The incidence of headache, migraine and associated symptoms were analysed using Fisher's exact test. Age, weight and MAP were tested using Student's t-test. All p-values were two-sided and significance level was set at p-value <0.05. No adjustment for multiple analyses was performed. Data analysis was carried out by the observer (IAO) while she was blinded. Calculation of sample size was based on the assumption that 5-ISMN would induce a headache with a median headache intensity score difference of 2 between headache free participants and controls. With a standard deviation of 2.3, a significance of 0.05 and a power of 0.80 at least 21 participants in each group should complete the study. As there was no literature on headache provocation in headache free participants, we included additional participants to strengthen our statistical power in order to detect a difference between the groups. All statistical analyses were performed with R version 4.1.0 and Rstudio version 1.2.5001.

Results

Participants

In total, 58 male participants, 32 headache free male participants and 26 male controls, completed the study. Headache free participants had a mean age of 52.6 years (95% CI 47.2–58.1 years) and controls had a mean age of 50.7 (95% CI 45.4-56.1 years). There was no difference in age between the two groups ($P = 0.61$). There was no difference in mean weight ($P = 0.30$) between headache free participants, 81.7 kg (95% CI 78.4–85.0 kg), and controls, 84.0 kg (95% CI 80.9–87.0 kg). Headache free participants had a MAP

of 101.8 mmHg (95% CI 98.9–104.6 mmHg) and controls had a MAP of 101.1 mmHg (95% CI 97.8–104.4 mmHg) at baseline. There was no difference in MAP ($P = 0.77$) between headache free participants and controls.

Of the 26 controls, 7 (22%) had a primary headache disorder; 2 (8%) controls had episodic migraine and 5 (19%) had infrequent tension type headache. The remaining 21 controls had a history of headache but did not fulfil diagnostic criteria for a primary headache disorder at the time of inclusion in the study.

Headache induction

5-ISMN induced a mild to moderate headache in 17 (53%) headache free participants and in 19 (73%) controls, with a NRS $\geq$2 (Table 1). There was no difference in headache incidence between headache free participants and controls ($P = 0.17$). Median peak headache score was 2 (range 0–7) for headache free participants and 2 (range 0–6) for controls, ($P = 0.24$), see Figure 2 and Figure 3. Median time to peak headache score after 5-ISMN administration was 3.5 hours (range 0.5–11 hours) for headache free participants and 7 hours (range 1–11 hours) for controls. There was no statistical significant difference in median time to peak headache score between groups ($P = 0.12$).

Headache intensity

Median $AUC_{0\text{-}5h}$ for headache score was 3.75 (0–11) for headache free participants and 2.5 (0–10.5) for controls. There was no significant difference in median $AUC_{0\text{-}5h}$ for headache score ($P = 0.60$). Median $AUC_{0\text{-}12h}$ for headache score was 9.25 in headache free participants and 12.88 in controls ($P = 0.27$). Median $AUC_{0\text{-}5h}$ and median $AUC_{0\text{-}12h}$ for headache score after administration of 5-ISMN are illustrated in Figure 4.

Migraine-like attack

Two (6%) headache free participants and four (15%) controls fulfilled criteria for an experimentally induced migraine-like attack during the study (Table 1). The incidence of migraine-like attacks did not differ between headache free participants and controls ($P = 0.39$). Thirteen (76%) of the 17 headache free participants and 15 (79%) of the 19 controls who developed a headache of $\geq$2 NRS, had one or more migraine-like features to the headache. The most frequent migraine-like headache characteristics were throbbing quality and aggravation by physical activity for both headache free participants and controls. Photophobia was the most frequent accompanying symptom in headache free participants (16%) and nausea was the most frequent accompanying symptom in controls (15%). Headache characteristics and accompanying symptoms for each headache free participant and each control are presented in Table 2 and Table 3, respectively.

Discussion

Using a standardized headache provocation regime with 5-ISMN in an observer blinded, case-control design, we showed that it was possible to provoke a headache in men who have never had a headache. The headache did not differ in intensity, characteristics or accompanying symptoms when compared to controls.

Signaling pathways

Administration of 5-ISMN provokes a headache by releasing NO that in turn raises intracellular cGMP. It is possible that people who have never had a headache, are protected from headache due to mechanisms

Table 1. Headache and associated symptoms.

	Cases (n = 32)	Controls (n = 26)	*P*-value
Number of participants reporting headache (NRS range 2–7)	17 (53%)	19 (73%)	0.17
Median peak headache score (range)	2 (0–7)	2 (0–6)	0.24
Median time to peak headache (range)	3.5 (0.5–11)	7 (1–11)	0.12
Number of participants with:			
Unilateral location	7 (22%)	4 (15%)	0.74
Throbbing quality	9 (28%)	11 (42%)	0.27
Aggravation by physical activity	9 (28%)	12 (46%)	0.27
Nausea	4 (13%)	4 (15%)	1.0
Photophobia	5 (16%)	2 (8%)	0.43
Phonophobia	3 (9%)	1 (4%)	0.61
Rescue medication	1 (3%)	2 (8%)	0.58
Migraine-like attack	2 (6%)	4 (15%)	0.39

NRS, numerical rating scale

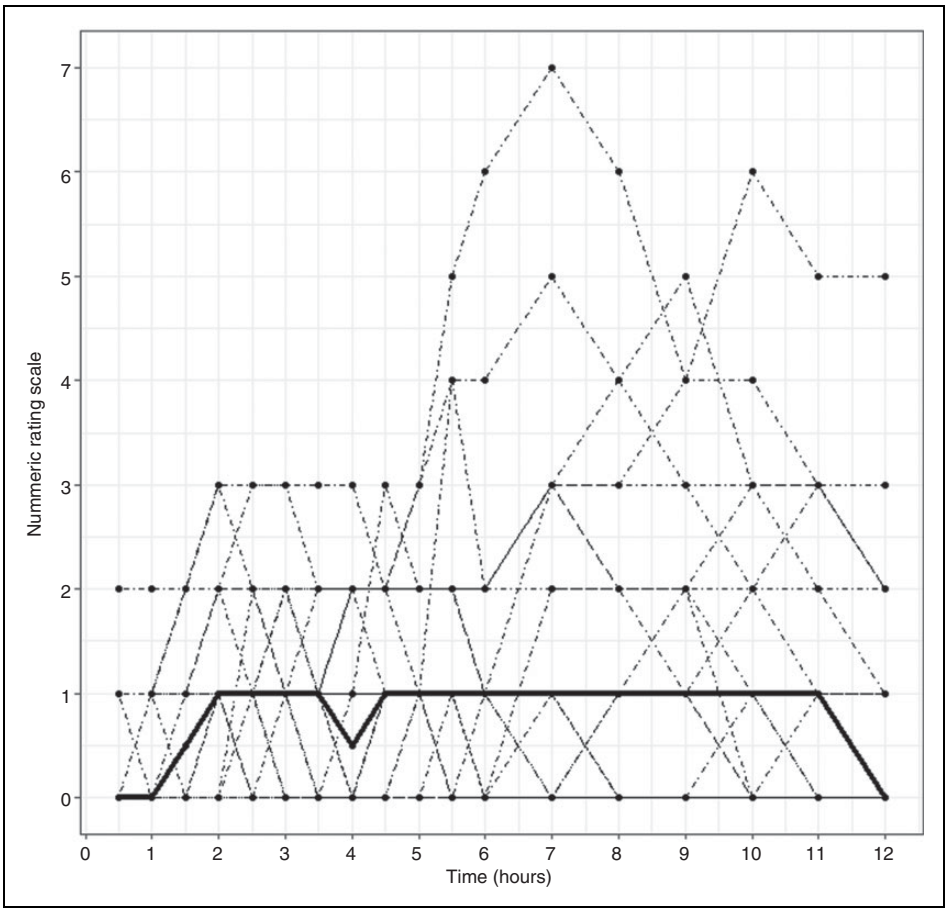

Figure 2. Headache intensity and median for headache free participants. Headache intensity measured on a numeric rating scale for each headache free participant (dotted line) and median for all headache free participants (thick line) plotted over time.

upstream of the NO-cascade induced by 5-ISMN. In the body NO is synthesized by nitric oxide synthase (NOS) that is present in three isoforms; endothelial NOS, neuronal NO and inducible NOS (24). NOS activity may play a role in the primary headache disorders. An increase NOS activity have been found in patients with chronic tension type headache (25). In spontaneous migraine attack a nonselective NOS inhibitor improved headache severity and accompanying symptoms (26). It would be interesting to examine if people who have never had a headache have a lower endogenous level of NO or a decreased activity of one or more of the NOS isoforms.

5-ISMN can provoke a headache in men who have never had a headache and the headache is probably due to raised intracellular levels of cGMP. However, other headache provoking substances, like CGRP, PACAP and Cilostazol work by increasing cAMP (27–29). Men who have never had a headache might be protected from headache by mechanisms in the cAMP pathway. CGRP, PACAP, prostanoids and opening of potassium channels are all possible candidates to study in people who have never had a headache. We hope to perform additional provocation studies in the future, using the same study design but provoking another pathway than the NO pathway. Unfortunately, this was not possible in the present study due to lack of resources.

5-ISMN in healthy volunteers

5-ISMN induced a headache in 53% of headache free participants and in 73% of controls. An earlier study also using 5-ISMN to provoke a headache in healthy volunteers, found that 100% of 30 participants developed a headache (16). Two other provocation studies also found that all participants (10 and 16) developed a headache after administration of 5-ISMN. However, in these two studies, participants were only included if they developed a headache after administration of sublingual nitroglycerin (14,15). Neither headache free participants nor controls developed a strong headache after administration of 5-ISMN in our study. Median peak headache score for both groups was 2. This is a

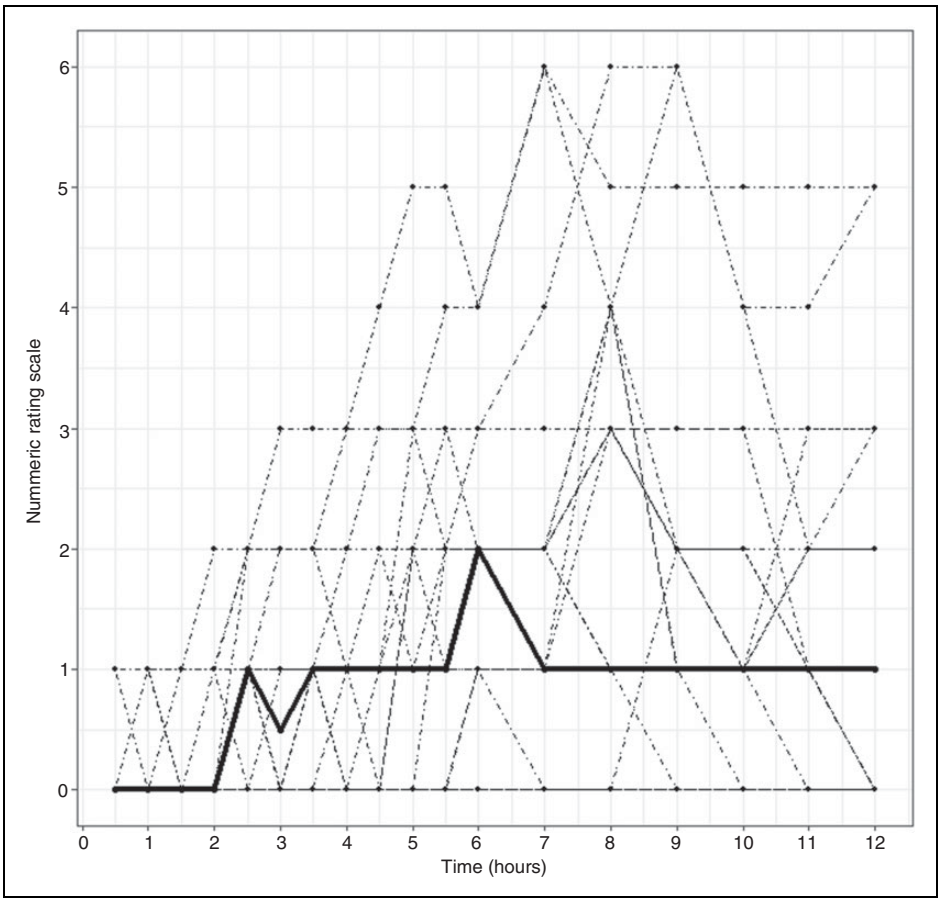

Figure 3. Headache intensity and median for controls. Headache intensity measured on a numeric rating scale for each control (dotted line) and median for all controls (thick line) plotted over time.

bit lower than other provocation studies using 5-ISMN, where median peak headache was 3–5 in healthy volunteers (14–16). The difference in headache incidence and intensity could be explained by a higher mean age (50.7-52.6 years) compared to the earlier studies (25.0–38.7 years), as pain sensitivity tends to decrease with age (14–16,30). Another reason for the lower headache incidence and intensity could be sex differences. As more men than women are free from headache and in order to reduce sex-related variability, we only included men in the present study. This is in contrast to the other provocation studies with 5-ISMN that included 50-93% female participants (4,14,16). Most provocation studies with 5-ISMN or other nitric oxide donors in healthy volunteers, have not examined if there is an age or sex difference in the response to provocation (8,14,15,31).

15% of controls and 6% of headache free participants fulfilled the criteria for an experimentally induced migraine attack. This is lower than the earlier studies with healthy volunteers where 30-66% developed an experimentally induced migraine attack after 5-ISMN (14–16). This difference could also be due to age and sex differences. However, another study found no sex difference in the response to provocation with 5-ISMN (16).

47% of headache free participants and 27% of controls did not develop a headache during the study. This difference was not significant, but it could indicate that there is a subgroup among the headache free participants and the controls who are resistant to headache even when provoked with 5-ISMN.

Strengths and limitations

There are some limitations to the present study. As it was not possible to blind study participants to their case-control status, this could affect how cases and controls experienced and responded to the headache provocation. The biggest limitation in the study is, that we did not use a crossover randomized design using 5-ISMN and placebo. We therefore do not know how big a placebo effect we see in our results. This design was chosen as participant availability and finances did not permit a crossover randomized design. We only included men in the present study and as the

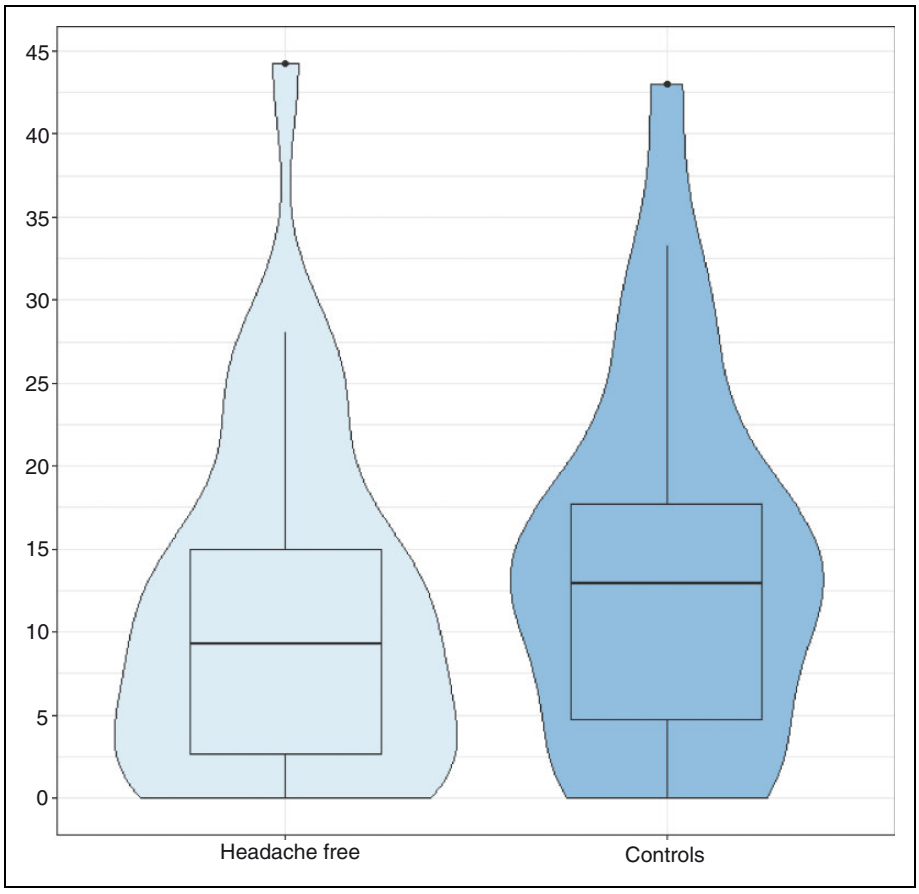

Figure 4. Violin plot of AUC 0–12 hours. Comparison of median, interquartile range and sample distribution of AUC 0–12 hours for headache free participants and controls. AUC: area under the curve of headache intensity score.

Table 2. Characteristics of headache and associated symptoms for each case.

Participant	Peak headache (onset of peak headache)[a]	Headache characteristics[b]	Associated symptoms[c]	Migraine-like attack	Rescue medication
1	0 (–)	–/–/–	–/–/–	No	No
2	4 (5.5 h)	–/+/+	–/–/–	No	No
3	3 (7 h)	–/–/+	+/–/–	No	No
6	1 (1.5 h)	–/–/–	–/–/–	No	No
9	0 (–)	–/–/–	–/–/–	No	No
14	2 (10 h)	–/–/–	–/–/–	No	No
16	0 (–)	–/–/–	–/–/–	No	No
17	3 (2 h)	–/–/+	–/+/–	No	No
19	3 (2 h)	–/–/–	–/+/+	No	No
21	2 (3 h)	–/–/–	–/–/–	No	No
22	3 (7 h)	–/+/–	–/–/–	No	No
23	1 (2 h)	–/+/–	–/+/–	No	No
27	7 (7 h)	–/+/+	+/+/+	Yes	Yes
28	1 (5.5 h)	–/+/–	–/–/–	No	No
29	1 (6 h)	–/–/–	+/–/–	No	No
30	4 (9 h)	+/–/–	–/–/–	No	No
31	0 (–)	–/–/–	–/–/–	No	No
32	1 (0.5 h)	–/–/–	–/–/–	No	No
33	2 (0.5 h)	+/–/–	–/–/–	No	No

(continued)

Table 2. Continued.

Participant	Peak headache (onset of peak headache)[a]	Headache characteristics[b]	Associated symptoms[c]	Migraine-like attack	Rescue medication
34	3 (2.5 h)	−/+/−	−/−/−	No	No
35	1 (2 h)	+/+/+	−/−/−	No	No
36	0 (−)	−/−/−	−/−/−	No	No
37	1 (1.5 h)	+/−/−	−/+/−	No	No
38	2 (3.5 h)	−/−/−	−/−/−	No	No
39	3 (11 h)	−/−/+	−/−/+	No	No
40	1 (10 h)	+/−/−	−/−/−	No	No
41	1 (1 h)	−/−/−	−/−/−	No	No
43	2 (3 h)	−/−/−	−/−/−	No	No
44	5 (7 h)	−/−/+	+/−/−	Yes	No
45	1 (0.5 h)	+/−/+	−/−/−	No	No
48	3 (10 h)	−/+/−	−/−/−	No	No
58	5 (9 h)	+/+/+	−/−/−	No	No

[a]Peak headache on the numerical rating scale. Onset of peak headache in hours after administration of 5-ISMN

[b]Headache characteristics: location (+ unilateral, − bilateral)/quality (+ throbbing, − pressing)/aggravation of headache by routine physical activity (+ present, − not present)

[c]Associated symptoms: nausea/photophobia/phonophobia (+ present, − not present).

Table 3. Characteristics of headache and associated symptoms for each control.

Participant	Peak headache (onset of peak headache)[a]	Headache characteristics[b]	Associated symptoms[c]	Migraine-like attack	Rescue medication
4	2 (5.5 h)	−/+/+	−/−/−	No	No
5	4 (8 h)	−/+/+	−/−/−	No	Yes
7	0 (−)	−/−/−	−/−/−	No	No
8	3 (4.5 h)	−/−/+	−/−/−	No	No
10	6 (7 h)	−/+/+	+/+/+	Yes	No
11	3 (8 h)	−/−/−	−/−/−	No	No
12	1 (2 h)	−/−/−	−/−/−	No	No
13	2 (2.5 h)	+/−/−	−/+/−	No	No
15	6 (7 h)	−/+/+	−/−/−	No	Yes
18	6 (9 h)	−/+/+	+/−/−	Yes	No
20	3 (8 h)	−/−/+	−/−/−	No	No
24	1 (6 h)	−/−/−	−/−/−	No	No
25	6 (8 h)	+/+/+	+/−/−	Yes	No
26	2 (5.5 h)	−/−/−	−/−/−	No	No
42	2 (9 h)	−/−/−	−/−/−	No	No
46	1 (1 h)	−/−/−	−/−/−	No	No
47	4 (8 h)	−/+/+	−/−/−	No	No
49	1 (1 h)	−/−/−	−/−/−	No	No
50	3 (11 h)	+/+/+	−/−/−	No	No
51	2 (11 h)	−/+/−	−/−/−	No	No
52	0 (−)	−/−/−	−/−/−	No	No
53	3 (5 h)	−/+/+	+/−/−	Yes	No
54	2 (4 h)	+/+/−	−/−/−	No	No
55	2 (6 h)	−/−/−	−/−/−	No	No
56	0 (−)	−/−/−	−/−/−	No	No
57	3 (8 h)	−/−/+	−/−/−	No	No

[a]Peak headache on the numerical rating scale. Onset of peak headache in hours after administration of 5-ISMN.

[b]Headache characteristics: location (+ unilateral, − bilateral)/quality (+ throbbing, − pressing)/aggravation of headache by routine physical activity (+ present, − not present).

[c]Associated symptoms: nausea/photophobia/phonophobia (+ present, − not present).

mean age of headache free participants were 52.6 years, we do not know if our results are generalizable to women or a younger cohort.

We took several steps to try and minimize bias in the study. Participants answered the question "Do you think that you never ever in your whole life have had a headache" when included in the "Pain sensitivity of headache free individuals" study and again when entering the present study, in order to minimize classification bias of case-control status. The study was by design observer blinded and study conditions were standardized to minimize bias; All participants were provoked in a random order in the same time-period of the day, in the same room, by one of only two different observers. The headache diary the participants used to report their symptoms and the criteria for identifying headache and experimentally induced migraine attacks have all been validated through numerous provocation studies at the Danish Headache Center (21). We included 58 participants in our sample, and we calculated that this sample size was large enough to detect a clinically relevant difference in headache intensity and incidence between the groups.

Conclusion

We show that men who have never experienced a headache can develop a headache when provoked with 5-ISMN. This result may indicate that freedom from headache in men is not related to the NO pathway which is involved in the primary headache disorders.

Article Highlights

- Headache free men can develop a headache when provoked with nitric oxide.
- Nitric oxide induced a headache in 53% of headache free participants and 73% of controls.
- There was no significant difference in headache incidence, intensity or migraine-like attack between headache free participants and controls.
- The results may indicate that freedom from headache in men is not related to the nitric oxide pathway.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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