Published in final edited form as:

Headache. 2012 ; 52(7): 1146–1154. doi:10.1111/j.1526-4610.2012.02098.x.

Nitric Oxide Modulation of Low-Frequency Oscillations in Cortical Vessels in FHM – a NIRS Study

Henrik W. Schytz, MD, PhD, Jakob M. Hansen, MD, PhD, Dorte Phillip, MD, Juliette Selb, PhD, David A. Boas, PhD, and Messoud Ashina, MD, DrMSc

Danish Headache Center and Department of Neurology, Glostrup Hospital, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark (H.W. Schytz, J.M. Hansen, D. Phillip, and M. Ashina); Photon Migration Imaging Laboratory, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA (J. Selb and D.A. Boas).

Abstract

Background—The pathophysiological alterations in patients with familial hemiplegic migraine (FHM) are not yet fully known. The headache characteristics in patients with FHM mutations have been examined in a series of glyceryl trinitrate (GTN) provocation studies in FHM patients, but the cortical vascular response to GTN in FHM patients has never been investigated before.

Objective—To investigate changes in spontaneous low-frequency oscillations (LFO) of cortical vessels in response to the nitric oxide donor GTN by near-infrared spectroscopy in FHM patients.

Methods—Twenty-three FHM patients without known mutations and 9 healthy controls received a continuous intravenous infusion of GTN 0.5 µg/kg/minute over 20 minutes. Using near-infrared spectroscopy, we recorded oxygenated hemoglobin (oxyHb) LFO amplitude bilateral at the frontal cortex at baseline and 15 minutes and 40 minutes after start of the GTN infusion.

Results—GTN changed oxyHb LFO amplitude in FHM patients ($P = .002$), but not in healthy controls ($P = .121$). Only in FHM patients with coexisting common migraine types did GTN infusion induced changes in LFO amplitudes ($P < .001$), where post-hoc analysis revealed an increase in LFO amplitude 15 minutes ($P = .003$) and 40 ($P = .013$) minutes after start of infusion compared with baseline. Interestingly, GTN infusion induced *no* changes in LFO amplitude in patients with a pure FHM phenotype ($P = .695$).

Conclusion—FHM patients with a mixed phenotype (coexisting common type of migraine) showed an increase in oxyHb LFO amplitude during GTN infusion, whereas FHM patients with pure phenotype showed no changes. These data suggest possible differences in frontal cortical nitric oxide vascular sensitivity between FHM patients with a mixed phenotype and patients with pure FHM.

Keywords

familial hemiplegic migraine; nitric oxide; low-frequency oscillation; cortical vessel; near-infrared spectroscopy

Familial hemiplegic migraine (FHM) is a dominantly inherited subtype of migraine with aura (MA), characterized by cortical alterations resulting in fully reversible half-sided weakness and other aura symptoms preceding or accompanying migraine headache.¹ Mutations in 3 different genes can cause the FHM phenotype.²⁻⁴ In a large proportion of FHM patients (40-80%), no mutations have been identified to date.^{5,6} Clinically, the migraine features of FHM with and without identified mutations are similar,⁷ but the differences in genetic constitution might result in different molecular migraine mechanisms. FHM1 mutations have been explored in a mouse knock-in model and revealed enhanced excitatory transmission at cortical synapses⁸ and decreased triggering threshold for cortical spreading depression (CSD).^{8,9}

It is known that FHM attacks have a statistically longer duration of visual and sensory aura than typical migraine aura attacks and motor symptoms with a mean duration close to 6 hours.¹⁰ Furthermore, in attacks of hemiplegic migraine, changes in regional cerebral blood flow have been recorded in conjunction with cerebral angiography¹¹ and showed that blood flow fluctuated rapidly possibly because of instability of cerebrovascular tone, defined as transient constriction of cerebral arterioles alternating with a normal caliber for these vessels and/or short periods of vasodilatation. When vasoconstriction was present, the blood flow decreased to values consistent with ischemia and was suggested to be the cause of the neurological deficits. Thus, FHM patients may have an altered cortical vascular regulation, which make them more susceptible to prolonged aura phenomena. Therefore, it is of key interest to study cortical vascular responses in FHM patients.

Recording of spontaneous low-frequency oscillation (LFO) amplitudes of oxygenated hemoglobin (oxyHb)¹² may be used for assessment of cortical vascular responses to glyceryl trinitrate (GTN). Hence, nitric oxide (NO) modulates LFO^{13,14} in experimental animal models, and cortical vascular changes in humans can be monitored non-invasively *in vivo* by near-infrared spectroscopy (NIRS).¹⁵

Therefore, the aim of the present study was to investigate the LFO in frontal cortical vessels in response to GTN infusion by NIRS in patients with FHM without known mutations and healthy controls.

MATERIALS AND METHODS

We recruited 23 patients with FHM (6 male/17 female, mean age 41 years, range 21-67 years) and 9 healthy controls with no personal or family history of migraine (5 male/4 female, mean age 37 years, range 22-53 years). The patients were recruited from 15 families from a population-based sample.¹⁰ Genome-wide linkage scan failed to show any new or known FHM mutations in these patients. Twelve patients exclusively had FHM (4 male/8 female, mean age 40 years, range 21-67 years), and 11 had previously had attacks of other types of migraine (2 male/9 female, mean age 42 years, range 27-63 years) (Table 1). The headache data of the study have previously been published.¹⁶ Due to technical issues, only 9 healthy subjects were studied in the present as compared with 12 in the previous study.¹⁶ The Ethics Committee of the County of Copenhagen approved the study, and written informed consent from all patients participating in the study was received. The study was registered at www.clinicaltrials.gov (NCT00541736).

Experimental Design

All subjects received a continuous intravenous infusion of GTN 0.5 µg/kg/minute over 20 minutes. The subjects were informed that GTN might induce headache, but the timing or the type of headache was not discussed. None of the subjects had previously participated in headache provocation studies. All subjects reported headache-free to the laboratory. All

procedures were performed with the subjects in the supine position, a venous catheter inserted into an antecubital vein, GTN infused over 20 minutes using a time- and volume-controlled infusion pump (B. Braun Perfusor, Melsungen, Germany). NIRS recordings lasting 5 minutes each were obtained at 3 time points: 10 minutes before the infusion, 15 minutes after the beginning of the infusion period, and 40 minutes after the beginning of the GTN infusion (ie, 10 minutes after the end of the infusion period).

NIRS Acquisition

Measurement of oxyHb LFO was performed using continuous wave NIRS (NIRS2; TechEnInc, Milford, MA, USA). The NIRS optodes were placed bilaterally on the forehead with 1 source (2 wavelengths: 690 nm and 830 nm) and 2 detectors on each side, avoiding the midline sinus. The distance between sources and detectors were 3 cm, with the detectors lateral to the source. Thus, the detectors were measuring at the frontal cortex in the territory supplied primarily by the middle cerebral artery. Optode placement and recording were performed by H.W.S. and D.P., who were blinded to the disease status and grouping of the included subjects.

Power spectra were estimated by computing the Fourier transform of the NIRS signal time series (Fig. 1). The relative LFO amplitude of oxyHb was then extracted from a narrow frequency band around 0.1 Hz. All data processing was performed in MATLAB (The MathWorks, Inc., Natick, MA, USA). NIRS intensity time series were first divided into windows of 100 seconds duration with 50 seconds overlap. Within each 100-second window, intensity variations at 690 and 830 nm were converted into relative changes in oxyHb using the modified Beer–Lambert law¹⁷ with a differential pathlength factor of 6 at both wavelengths. No correction for partial volume effect was employed because the NIRS-measured oscillations are not expected to be very much localized. The oxyHb signals were then Fourier transformed to obtain their power spectra (MATLAB function pwelch). The LFO frequency was defined as the frequency within the 0.09–0.11 Hz range. The LFO amplitude of oxyHb was extracted as the value of the oxyHb spectrum at the LFO frequency. The values of oxyHb amplitude were then averaged over all 100-second windows and over the 2 NIRS channels on each side of the head. We converted the NIRS data into oxyHb concentration. NIRS is sensitive to cerebral blood volume and oxygenation changes in the microvasculature, with mixed contribution from arterioles, capillaries, and venules. The measured oscillations in cortical oxyHb can arise from oscillations in cerebral blood flow and metabolic rate of oxygen.

Vital Signs

Heart rate (HR) and mean arterial blood pressure (MAP) were measured with an auto-inflatable cuff (ProPac Encore® Welch Allyn Protocol, Beaverton, OR, USA), and electrocardiogram (ECG) was monitored (Cardiofax V, Nihon-Kohden, Shinjuku-ku, Tokyo, Japan).

Data Analysis and Statistics

Data are presented as median values with quartiles. NIRS data processing was performed by J.S., who was blind with respect to patients (including pure FHM and FHM + MA/migraine without aura [MO] status) and controls. The subjects were divided into healthy subjects and the FHM group as a whole. The FHM subjects were also subdivided into 2 groups consisting of pure FHM and FHM + MA/MO subjects. There are no previous data on variability to allow for exact sample size calculations, but previous NIRS data from 10 healthy subjects detected amplitude changes in the LFO range following posture changes,¹⁸ which justify the chosen sample sizes in the present study.

The primary end-points were: (1) differences in LFO amplitude at baseline and following GTN infusion (15 and 40 minutes) *within* the healthy subjects and the FHM group as a whole; (2) differences in LFO amplitude at baseline and following GTN infusion (15 and 40 minutes) within pure FHM *and* FHM + MA/MO subjects.

The secondary end-points were: (1) differences in baseline LFO amplitude, HR, and MAP *between* the healthy subjects and the FHM group as a whole and also between the 3 groups of healthy subjects, pure FHM, and FHM + MA/MO; (2) differences in LFO amplitude change from baseline at 15 minutes and 40 minutes between male and female FHM patients; (3) changes in HR and MAP correlated to the change in LFO amplitude from baseline to infusion of GTN (15 minutes).

Given that the data were not normally distributed (the Shapiro–Wilk test), we used the Friedman test to test difference in LFO amplitudes within groups. If Friedman test revealed statistical significance, we performed a post-hoc analysis to test difference between baseline and after infusion by the Wilcoxon signed-rank test for paired samples. To test the first and second secondary end-points, non-parametric Mann-Whitney *U*-test for 2 independent samples on differences between groups was applied. For the third secondary end-point, Spearman's rank correlation coefficient was applied.

All analyses were performed with SPSS for Windows 17.0 (SPSS Inc., Chicago, IL, USA). Five percent ($P = .05$) was chosen as the level of significance.

RESULTS

All 32 subjects completed the study. None of the participants reported headache at baseline. In 1 subject with pure FHM, 40-minute recording was not performed due to a technical error. We found no difference between hemispheres for any group, and therefore, the amplitude was analyzed as a mean of the 2 hemispheres. There were no differences in baseline LFO amplitude ($P = .090$), HR ($P = .166$), and MAP ($P = .216$) between FHM patients and controls. There were no differences in changes of LFO amplitudes compared with baseline at 15 minutes ($P = .609$) and 40 minutes ($P = .249$) between female or male FHM patients.

LFO in FHM Patients and Healthy Subjects

Intravenous infusion of GTN induced change in LFO amplitudes in FHM patients ($P = .002$). Post-hoc analysis revealed a significant increase in LFO amplitude 15 minutes ($P = .006$) and 40 minutes ($P = .020$) after start of GTN. Intravenous infusion of GTN did not change LFO amplitudes in healthy controls ($P = .121$). To investigate the second primary endpoint, we divided FHM patients into 2 groups according to phenotype: pure FHM and FHM + MA/MO. We found significant changes in LFO amplitudes after GTN infusion in FHM + MA/MO patients ($P < .001$). Post-hoc analysis revealed a significant increase in LFO amplitude 15 minutes ($P = .003$) and 40 ($P = .013$) minutes after start of GTN infusion compared with baseline. However, there were no changes in LFO in patients with pure FHM ($P = .695$) (Table 2 and Figs. 1 and 2). Statistical analysis included visual identified outliers (Fig. 2), and removal of outliers did not change the results. Correlation analysis revealed no correlation between LFO amplitude changes from baseline to infusion (15 minutes) and HR ($r = 0.039$, $P = .831$) or MAP ($r = 0.059$, $P = .747$) changes.

COMMENTS

The major outcome of the study is that we found an increase in frontal cortical vessel LFO amplitudes in response to intravenous infusion of GTN in FHM patients without known

mutations. Interestingly, only FHM patients with coexisting common types of migraine showed an increase in cortical vessel LFO amplitudes following GTN infusion, whereas patients with the pure FHM phenotype did not. These data suggest that only FHM patients with mixed phenotype exhibit increased cortical arterial responses to GTN.

FHM patients have hemiplegic and aura symptoms that most likely are caused by CSD.^{19,20} Experimentally, a single episode of CSD in the rat frontal sensory cortex induces impaired neurovascular coupling with reduced vascular reactivity for at least 1 hour.²¹ Thus, failure of neurovascular coupling following CSD may explain the prolonged neurological deficits during hemiplegic migraine aura. Interestingly, CSD-induced vasoconstriction and reduced reactivity are ameliorated by high doses of L-arginine, the substrate for NO synthase²² or topical application of NO donors.²³ It is also known that FHM attacks have a statistically longer duration of visual and sensory aura than MA attacks and motor symptoms with a mean duration close to 6 hours.¹⁰ Surprisingly, the present study shows that pure FHM patients have the same vascular reactivity to NO as healthy controls, while in the presence of MO and MA, we found an increased cortical vascular reactivity to NO. It seems that the increased vascular reactivity is related to the common migraine phenotypes and not directly linked to the FHM phenotype. To confirm this, future studies should examine the LFO amplitude changes of cortical cephalic vessels in response to GTN in patients with typical migraine with and without aura.

GTN induces migraine attacks in 50-80% of patients with migraine with and without aura.²⁴⁻²⁶ Cerebral hemodynamics in response to GTN infusion have been extensively studied in migraineurs and healthy volunteers.^{24,25,27} Intravenous GTN infusion in humans does not alter global or regional cerebral blood flow (CBF)²⁸ and therefore changes in cerebral blood flow velocity are likely to reflect vasodilatation. In migraine patients without aura, GTN causes a more pronounced dilatation in both intra- and extracranial arteries than in controls.²⁹ These findings suggest a possible link between arterial hypersensitivity and migraine induction.³⁰ Provocation studies have revealed that FHM patients with (50% not reporting MO and/or MA) and without known mutations (52% not reporting MO and/or MA) do *not* experience more migraine attacks following GTN infusion than healthy controls.^{16,31,32} These findings raise an important question: why do the majority of FHM patients not experience migraine attacks in response to GTN? The answer is complex, and at present, we can only speculate that pathophysiological mechanisms in FHM may be distinct from the common migraine subtypes. Hence, mutations could have functional consequences that remain to be clarified.

Some interesting observations came from the provocation studies. We previously reported no difference in dilatation of intra- and extracranial arteries after GTN infusion between FHM1 and FHM2 patients and controls.^{31,32} Interestingly, GTN-induced dilatation of the superficial temporal artery was *less* pronounced in FHM2 patients compared with healthy controls.³² It seems that the FHM patients without known mutation and with coexisting MA/MO are more sensitive to the migraine-inducing effects of GTN when compared with the pure FHM phenotype and controls.¹⁶ These findings and the present data, showing that following GTN, *only* patients with FHM and coexisting MO/MA had an increase in cortical vascular low-frequency amplitude, indicate differences between FHM patients with coexisting MA/MO and patients with pure FHM in terms of both migraine *and* vascular induction to GTN. Thus, the present results indicate differences in pain as well as vascular pathways across the migraine spectrum and investigating this pathophysiological heterogeneity will be very interesting to explore in future migraine research.

This is the first time an NO donor is given systemically to detect LFO amplitude changes of cortical vessels in human. Our results are in contrast to experimental models in anesthetized

rats, where NO synthase inhibitors increase cerebral LFO amplitude.^{13,14} This difference may be explained by the fact that in the animal studies, the NO synthase inhibitor constricts cephalic vessels and thereby markedly lowers the cerebral blood flow by up to 18%¹³ and raises the systemic blood pressure up to 26%.¹⁴ The increase in LFO amplitude in the present study might have been caused by a reflex increase in sympathetic tone,¹⁸ even though we could not show a significant correlation between changes in systemic hemodynamics and the LFO amplitude changes.

Some limitations to this study need to be acknowledged. GTN infusion induces an increase in HR and a decrease in blood pressure.^{31,32} Thus, changes in cortical vessels might also reflect systemic changes. Continuous wave NIRS technique lacks precise depth resolution, which does not allow to clearly separate signals from the skin and cortex. The differential pathlength factor and the partial volume error may vary between subjects due to different head anatomy, which can introduce errors and intersubject variation in the absolute hemoglobin oscillation amplitudes we are reporting. Because NIRS is a surface measurement, it shows some level of contamination by hemodynamics in superficial tissues.³³ In this study, we have not characterized the contamination by skin vasculature, which can be a confounding effect in the results. In healthy subjects, we did not find a statistically significant increase in LFO amplitude during GTN infusion, even though visually Figure 2 on healthy subject seem to show an increase. This might be a type-2 error due to a low number of subjects or caused by outliers. The optodes might move slightly on the forehead throughout the study, which might have contributed to the high variability at the 40-minute recording. The unknown intersubject variability may account for the extreme outliers found in the study. Based on the circumstances, we chose to use the Friedman test to control for any intersubject variation. Experimental animal models have shown that cerebrovascular reactivity might be affected by estrogen via NO pathways.^{34,35} However, we found no differences in vascular responses between males and females in patients with FHM.

The present study showed that following GTN infusion, only patients with FHM and co-occurring common migraine had an increase in frontal cortical oxyHb LFO amplitude as opposed to patients with pure FHM and healthy controls. The experimental model indicates possible differences in frontal cortical NO sensitivity between FHM patients with a mixed phenotype and patients with pure FHM.

Acknowledgments

The authors thank all participating FHM patients and healthy volunteers. The authors thank Dr. Henning Piilgaard, for very useful comments to the manuscript. The study was supported by the Lundbeck Foundation via the Lundbeck Foundation Center for Neurovascular Signaling.

Study funding: Supported through the Lundbeck Foundation Center for Neurovascular Signaling.

Abbreviations

CSD	cortical spreading depression
FHM	familial hemiplegic migraine
GTN	glyceryl trinitrate
HR	heart rate
LFO	low-frequency oscillations
MA	migraine with aura

MAP	mean arterial blood pressure
MO	migraine without aura
NIRS	near-infrared spectroscopy
NO	nitric oxide
oxyHb	oxygenated hemoglobin

REFERENCES

1. IHS. The International Classification Of Headache Disorders, 2nd edition. Cephalalgia. 2004; 24(Suppl. 1):9–160. [PubMed: 14979299]
2. Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca²⁺ channel gene CACNL1A4. Cell. 1996; 87:543–552. [PubMed: 8898206]
3. Dichgans M, Freilinger T, Eckstein G, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet. 2005; 366:371–377. [PubMed: 16054936]
4. De FM, Marconi R, Silvestri L, et al. Haploinsufficiency of ATP1A2 encoding the Na⁺/K⁺ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet. 2003; 33:192–196. [PubMed: 12539047]
5. Thomsen LL, Kirchmann M, Bjornsson A, et al. The genetic spectrum of a population-based sample of familial hemiplegic migraine. Brain. 2007; 130:346–356. [PubMed: 17142831]
6. Ducros A, Joutel A, Vahedi K, et al. Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity. Ann Neurol. 1997; 42:885–890. [PubMed: 9403481]
7. Terwindt GM, Ophoff RA, Haan J, Frants RR, Ferrari MD. Familial hemiplegic migraine: A clinical comparison of families linked and unlinked to chromosome 19.DMG RG. Cephalalgia. 1996; 16:153–155. [PubMed: 8734765]
8. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, et al. A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron. 2004; 41:701–710. [PubMed: 15003170]
9. Tottene A, Conti R, Fabbro A, et al. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. Neuron. 2009; 61:762–773. [PubMed: 19285472]
10. Thomsen LL, Eriksen MK, Roemer SF, Andersen I, Olesen J, Russell MB. A population-based study of familial hemiplegic migraine suggests revised diagnostic criteria. Brain. 2002; 125:1379–1391. [PubMed: 12023326]
11. Friberg L, Olsen TS, Roland PE, Lassen NA. Focal ischaemia caused by instability of cerebrovascular tone during attacks of hemiplegic migraine. A regional cerebral blood flow study. Brain. 1987; 110(Pt 4):917–934. [PubMed: 3651801]
12. Schytz HW, Hansson A, Phillip D, et al. Spontaneous low-frequency oscillations in cerebral vessels: Applications in carotid artery disease and ischemic stroke. J Stroke Cerebrovasc Dis. 2010; 19:465–474. [PubMed: 20864356]
13. Dirnagl U, Lindauer U, Villringer A. Nitric oxide synthase blockade enhances vasomotion in the cerebral microcirculation of anesthetized rats. Microvasc Res. 1993; 45:318–323. [PubMed: 7686608]
14. Hudetz AG, Smith JJ, Lee JG, Bosnjak ZJ, Kampine JP. Modification of cerebral laser-Doppler flow oscillations by halothane, PCO₂, and nitric oxide synthase blockade. Am J Physiol. 1995; 269:114–120.
15. Obrig H, Neufang M, Wenzel R, et al. Spontaneous low frequency oscillations of cerebral hemodynamics and metabolism in human adults. Neuroimage. 2000; 12:623–639. [PubMed: 11112395]

16. Hansen JM, Thomsen LL, Olesen J, Ashina M. Coexisting typical migraine in familial hemiplegic migraine. *Neurology*. 2010; 74:594–600. [PubMed: 20157162]
17. Cope M, Delpy DT, Reynolds EO, Wray S, Wyatt J, van der Zee P. Methods of quantitating cerebral near infrared spectroscopy data. *Adv Exp Med Biol*. 1988; 222:183–189. [PubMed: 3129910]
18. Tachtsidis I, Elwell CE, Lee CW, Leung TS, Smith M, Delpy DT. Spectral characteristics of spontaneous oscillations in cerebral haemodynamics are posture dependent. *Adv Exp Med Biol*. 2003; 540:31–36. [PubMed: 15174599]
19. Olesen J, Larsen B, Lauritzen M. Focal hyperemia followed by spreading oligemia and impaired activation of rCBF in classic migraine. *Ann Neurol*. 1981; 9:344–352. [PubMed: 6784664]
20. Hadjikhani N, Sanchez del Rio M, Wu O, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc Natl Acad Sci U S A*. 2001; 98:4687–4692. [PubMed: 11287655]
21. Piilgaard H, Lauritzen M. Persistent increase in oxygen consumption and impaired neurovascular coupling after spreading depression in rat neocortex. *J Cereb Blood Flow Metab*. 2009; 29:1517–1527. [PubMed: 19513087]
22. Fabricius M, Akgoren N, Lauritzen M. Arginine-nitric oxide pathway and cerebrovascular regulation in cortical spreading depression. *Am J Physiol*. 1995; 269:H23–H29. [PubMed: 7631852]
23. Scheckenbach KE, Dreier JP, Dirnagl U, Lindauer U. Impaired cerebrovascular reactivity after cortical spreading depression in rats: Restoration by nitric oxide or cGMP. *Exp Neurol*. 2006; 202:449–455. [PubMed: 16920100]
24. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraineurs. *Pain*. 2004; 110:675–680. [PubMed: 15288408]
25. Thomsen LL, Kruuse C, Iversen HK, Olesen J. A nitric oxide donor triggers genuine migraine attacks. *Eur J Neurol*. 1994; 1:73–80.
26. Sances G, Tassorelli C, Pucci E, Ghiotto N, Sandrini G, Nappi G. Reliability of the nitroglycerin provocative test in the diagnosis of neurovascular headaches. *Cephalalgia*. 2004; 24:110–119. [PubMed: 14728706]
27. Christiansen I, Thomsen LL, Dagaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. *Cephalalgia*. 1999; 19:660–667. [PubMed: 10524660]
28. White RP, Deane C, Hindley C, et al. The effect of the nitric oxide donor glyceryl trinitrate on global and regional cerebral blood flow in man. *J Neurol Sci*. 2000; 178:23–28. [PubMed: 11018245]
29. Thomsen LL, Iversen HK, Brinck TA, Olesen J. Arterial supersensitivity to nitric oxide (nitroglycerin) in migraine sufferers. *Cephalalgia*. 1993; 13:395–399. [PubMed: 7906202]
30. Thomsen LL. Investigations into the role of nitric oxide and the large intracranial arteries in migraine headache. *Cephalalgia*. 1997; 17:873–895. [PubMed: 9453277]
31. Hansen JM, Thomsen LL, Olesen J, Ashina M. Familial hemiplegic migraine type 1 shows no hypersensitivity to nitric oxide. *Cephalalgia*. 2008; 28:496–505. [PubMed: 18384418]
32. Hansen JM, Thomsen LL, Marconi R, Casari G, Olesen J, Ashina M. Familial hemiplegic migraine type 2 does not share hypersensitivity to nitric oxide with common types of migraine. *Cephalalgia*. 2008; 28:367–375. [PubMed: 18294248]
33. Canova D, Roatta S, Bosone D, Micieli G. Inconsistent detection of changes in cerebral blood volume by near infrared spectroscopy in standard clinical tests. *J Appl Physiol*. 2011; 110:1646–1655. [PubMed: 21474700]
34. Kitamura N, Araya R, Kudoh M, et al. Beneficial effects of estrogen in a mouse model of cerebrovascular insufficiency. *PLoS One*. 2009; 4:e5159. [PubMed: 19357782]
35. Geary GG, Krause DN, Duckles SP. Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. *Am J Physiol*. 1998; 275:H292–H300. [PubMed: 9688926]

STATEMENT OF AUTHORSHIP**Category 1****a Conception and Design**

Henrik W. Schytz; Jakob M. Hansen; Messoud Ashina

b Acquisition of Data

Henrik W. Schytz; Jakob M. Hansen; Dorte Phillip

c Analysis and Interpretation of Data

Henrik W. Schytz; Juliette Selb; David A. Boas

Category 2**a Drafting the Manuscript**

Henrik W. Schytz; Messoud Ashina

b Revising It for Intellectual Content

Henrik W. Schytz; Jakob M. Hansen; Dorte Phillip; Juliette Selb; David A. Boas; Messoud Ashina

Category 3**a Final Approval of the Completed Manuscript**

Henrik W. Schytz; Jakob M. Hansen; Dorte Phillip; Juliette Selb; David A. Boas; Messoud Ashina

Fig 1.

Power spectra of the oxygenated hemoglobin oscillations at the 3 stages of glyceryl trinitrate infusion (pre-infusion, 15 minutes, and 40 minutes postinfusion) averaged over all subjects in each group: healthy controls (top), patients with pure familial hemiplegic migraine (FHM) group (middle), and FHM patients with coexisting migraine with aura (MA)/migraine without aura (MO, bottom). LFO frequency was defined as the frequency within the 0.09-0.11 Hz range.

Fig 2.

Boxplots during baseline, 15 minutes from infusion start, and 40 minutes after infusion start for familial hemiplegic migraine (FHM) + migraine with aura (MA)/migraine without aura (MO, gray boxes), pure FHM (striped boxes), and healthy controls (dotted boxes). Band near the middle is the median value. The bottom and top of the boxes is the 25th and 75th percentile. The ends of the whiskers represent the maximum and minimum of the data. y-axis units are in micromoles (μM). Outliers are shown except 1 extreme outlier in FHM + MA/MO patients (15 minutes: $233 \mu\text{M}$) and 2 extreme outliers in healthy subjects (15 minutes: $216 \mu\text{M}$; 40 minutes: $130 \mu\text{M}$).

Table 1

Patients With Pure Familial Hemiplegic Migraine (FHM) (Total Number) and FHM With Comorbidity (Total Number)

	FHM Pure (12)	FHM With Comorbidity (11)
MO	0	2
MA	0	5
MA & MO	0	4

MA = migraine with aura; MO = migraine without aura (total number).

Table 2

Median Oxygenated Hemoglobin Median Values in Micromoles

	FHM Pure	FHM + MA/MO	Healthy Subjects
Baseline	5.3 (2.1-13.3)	4.5 (2.3-11.5)	10.6 (7.2-22.9)
15 minutes after infusion start	8.4 (5.6-19.1)	14.7 (6.1-29.1)	23.2 (10.2-41.0)
40 minutes after infusion start	5.1 (4.1-31.8)	11.5 (2.6-37.2)	13.3 (7.5-27.9)

In parentheses are the 25th and 75th percentiles.

FHM = familial hemiplegic migraine; MA = migraine with aura; MO = migraine without aura.