Trigger factors and mechanisms in migraine

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CHAPTER 7

MIGRAINE HEADACHE IS NOT ASSOCIATED WITH CEREBRAL OR MENINGEAL VASODILATATION - A 3T MAGNETIC RESONANCE ANGIOGRAPHY STUDY

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Abstract

Background

Migraine headache is widely believed to be associated with cerebral or meningeal vasodilatation. Human evidence for this hypothesis is lacking. 3 Tesla Magnetic resonance angiography (3T MRA) allows for repetitive, non-invasive, sensitive assessment of intracranial vasodilatation and blood flow. Nitroglycerine (NTG) can faithfully induce migraine attacks facilitating pathophysiological studies in migraine.

Methods

Migraineurs (n=32) randomly received NTG (IV 0.5 µg/kg/min for 20 min; n=27) or placebo (n=5; for blinding reasons). Using 3T MRA, we measured: a) blood flow in the basilar (BA) and internal carotid (ICA) arteries and b) diameters of the middle meningeal (MMA), external carotid (ECA), ICA, middle cerebral (MCA), BA and posterior cerebral (PCA) arteries at three timepoints: i) at baseline, outside an attack; ii) during infusion of NTG or placebo; and iii) during a provoked attack or, if no attack had occurred, at 6 hours after infusion.

Findings

Migraine headache was provoked in 20/27 (74%) migraineurs who received NTG, but in none of the five patients who received placebo. The headache occurred between 1.5 – 5.5 hrs after infusion and was unilateral in 18/20 (90%) responders. During NTG (but not placebo) infusion, there was a transient 6.7% – 30.3% vasodilatation ($p<0.01$) of all blood vessels. During migraine, blood vessel diameters were no different from baseline, nor between headache and non-headache sides. There were no changes in BA and ICA blood flow during either NTG infusion or migraine.

Interpretation

In contrast to widespread belief, migraine attacks are not associated with vasodilatation of cerebral or meningeal blood vessels. Future antimigraine drugs may not require vasoconstrictor action.

Introduction

Migraine is a neurovascular disorder typically characterised by attacks of severe, throbbing, unilateral headache, associated autonomic symptoms, and, in one third of patients, focal neurological aura symptoms [75]. Since the seminal work by Wolff and colleagues [198], showing that stimulation of cerebral and meningeal arteries caused headache, there is a widespread belief that vasodilatation of intracranial blood vessels is the underlying mechanism for migraine headache [221]. This hypothesis was further fed by a number of other observations. Balloon dilatation of the middle cerebral artery (MCA) may cause migraine-like headache [222]. Vasoactive substances such as the nitric oxide (NO) donor nitroglycerin (NTG) [78] and calcitonin gene related peptide (CGRP) [62] can trigger migraine in susceptible subjects. In fact, the recent development of novel CGRP antagonists for treating migraine attacks was at least partly based on the hypothesis that prevention or reversal of vasodilation would block migraine headache [223,224]. Animal and *in situ* pharmacological experiments [75,225] and human *in vivo* studies using transcranial Doppler (TCD) [204,226,227] have shown that acute antimigraine agents (ergots and triptans) constrict cerebral and meningeal blood vessels [228]. In fact, the triptan class was specifically designed to selectively constrict intracranial blood vessels [221].

The role of vasodilatation in migraine has been vividly debated in the past (for review see: [229]) and more recently [75,87]. Some researchers view vasodilation of meningeal or cerebral blood vessels as a primary trigger for migraine headaches, and consider vasoconstriction necessary for acute antimigraine efficacy [230]. Others feel that vasodilation is a secundary phenomenon, due to activation of the trigeminovascular system and release of vasoactive neuropeptides. Vasodilation would primarily be involved in sustaining and worsening of the headache during migraine attacks [79]. A third line of thinking holds that vasodilation is irrelevant or, at best, "an innocent bystander" in the pathogenesis of migraine headache. Consequently, vasoconstriction may not be needed to treat migraine headaches [231 232,233]. This would be an enormous advantage as the currently available most effective antimigraine agents, triptans and ergots, all possess (sometimes strong and sustained) vasoconstrictor activity [234]. They may cause myocardial and cerebral ischaemia in patients with (risk factors for) vascular disease [235]. Novel antimigraine agents, which are devoid of vasoconstrictor activity, would be safer and could thus also be used by the many migraineurs with vascular disease.

Remarkably, the three opposing views on the role of vasodilation in migraine are all primarily based on extrapolations of observations in experimental animal models, with

very little evidence from human studies. This is primarily due to lack, until recently, of sensitive non-invasive imaging techniques to directly and reliably assess intracranial blood flow and blood vessel diameters in humans. Previous studies have used invasive methods such carotid angiography [236], or could only indirectly estimate diameter changes of cerebral blood vessels using TCD [204,237] Meningeal blood vessels proved too small to be investigated quantitatively. With the advent of 3 Tesla Magnetic Resonance Imaging (3T MRA) a sensitive and non-invasive imaging technique has become available to reliably measure intracranial blood flow and diameter changes of cerebral and meningeal blood vessels [238] as small as the middle meningeal artery (MMA) [110].

Infusion of NTG can reliably and faithfully provoke migraine headaches in migraineurs [55,83,210]. The response to NTG infusion is typically biphasic: an initial, brief and mild bilateral headache during the infusion in nearly all migraine and non-migraine study subjects [83], followed by a typical migraine, 4 to 5 hours later, in 60% to 80% of migraine. but not in non-migraine study subjects. [55,78] The symptomatology of provoked attacks is no different from that of spontaneous attacks of migraine without aura [78], including premonitory symptoms [56], response to anti-migraine drugs [239], and increase of CGRP, a marker for activation of the trigeminovascular system [124]. This provocation model has greatly facilitated the logistics of studying pathophysiological changes during migraine attacks.

In the present study we used 3T MRA to intra-individually compare: a) blood flow in the basilar (BA) and internal carotid (ICA) arteries; and b) the diameters of the external carotid (ECA), internal carotid (ICA), middle cerebral (MCA), BA, posterior cerebral (PCA) and middle meningeal arteries (MMA) between three conditions: i) at baseline, outside an attack; ii) during infusion of NTG or placebo (to assess the immediate vascular effects of NTG); and iii) during NTG-provoked migraine attacks or, if no attack had occurred, at 6 hours post infusion (to assess whether migraine attacks are associated with vasodilatation). We will demonstrate that there is no detectable vasodilation of cerebral or meningeal blood vessels during NTG-provoked migraine attacks, suggesting that vasoconstriction may not be required to treat migraine headaches.

Methods

Subjects

In total 32 migraine patients (n = 5 with aura; n = 27 without aura) were recruited from the neurology outpatient clinic of Leiden University Medical Centre. Inclusion criteria were: i) age between 18 and 55 years; ii) diagnosis of migraine according to the diagnostic criteria of the International Headache Society [3]; iii) an average attack frequency between 1 - 8 attacks per 2 months in the six months prior to the study; and iv) moderate or severe headache during spontaneous migraine attacks. Exclusion criteria included: i) more than 10 days of headache per month; ii) inability to differentiate between migraine and other forms of headache; iii) contra-indications for the use of triptans; iv) current use of vasoactive drugs; and v) MRI-specific contra-indications (such as claustrophobia). The study was approved by the local medical ethics committee and the subjects gave informed consent prior to the start of the study.

Experimental procedure and NTG provocation

All subjects arrived at the hospital between 8 and 10 a.m. on the day of the study. No medication, coffee, tea or alcohol was allowed in the 12 hours prior to the start of the experiment. From one hour before the experiments until the very end of the experiments, study subjects were not allowed to smoke. Patients had to be free of migraine for at least the three days prior to the study day and they could not have any form of headache at the beginning of the experiment.

Migraine patients (n=32) were scanned: i) at baseline (outside an attack; ii) during randomly allocated and double-blind infusion of NTG (0.5 µg/kg/min over 20 min; n=27) or placebo (n=5); and iii) during an ensuing migraine attack or, if no migraine had occurred, at 6 hours after infusion. The duration of the scan sessions was approximately 25 minutes. The study subjects remained in the scanner between the baseline and the NTG or placebo infusion scanning sessions which began 10 minutes after onset of the infusion. Heart rate and blood pressure were monitored during the experiments. Two days after the experiment, subjects were contacted by telephone to check whether a migraine attack had occurred beyond the 6-hour time window [221].

Placebo administration was included in the protocol to minimise patient and observer's bias for diagnosing whether or not NTG infusion had provoked a migraine headache (as this diagnosis is based on subjective assessment of symptoms [3]). We choose for an unequal and incomplete allocation to receiving NTG or placebo mainly for two

reasons. First, NTG administration was only used as a well established tool to provoke migraine attacks. Our study objective was primarily to assess intra-individual changes from baseline, rather than comparing the effect of NTG with that of placebo. Secondly, we wanted to minimise the number of patients who would contribute only very little to the study results (placebo was only given for masking reasons) to reduce unnecessary burden to patients, investigators, and MRI scanning time (the study protocol was very time consuming).

Magnetic resonance angiography

The MR investigations were performed on a 3.0-Tesla whole-body system (Philips Medical Systems, The Netherlands). The MRA protocol consisted of two parts, one to assess blood vessel diameter changes and one to assess blood flow changes.

The "blood vessel diameter protocol" consisted of a thick two-dimensional phase contrast (2D PC) sagittal localiser survey through the circle of Willis, followed by a three-dimensional time-of-flight (3D TOF) MRA sequence to visualise the BA and ECA, ICA, PCA and MCA on both sides. This scan had the following imaging parameters: repetition time / echo time (TR/TE): 22 ms / 3.5 ms; flip angle 15^{o}; field of view: 220 x 220 mm; number of excitations: 1; slice orientation: transverse; slice thickness: 0.65 mm; number of slices: 200; scan percentage 100%, matrix reconstruction size: 512 x 512 resulting in a nominal voxel size (x,y,z) of 0.43 x 0.43 x 0.65 mm; total acquisition time: 4min 30sec. Based on the reconstruction of this 3D-TOF a second 3D-TOF with a higher spatial resolution was performed to visualise the extra- and intracranial parts of the MMA on both sides. This scan had the following imaging parameters: TR/TE: 15 ms / 2.1 ms; flip angle 15o; field of view: 200 x 200 mm; number of excitations: 1; slice orientation: transverse; slice thickness: 0.25 mm; number of slices: 130; scan percentage 100%, matrix reconstruction size: 512 x 512 resulting in a nominal voxel size (x,y,z) of 0.39 x 0.39 x 0.25 mm; total acquisition time: 8min 31sec.

For the "blood flow protocol", a 2-dimensional phase-contrast (2D-PC) section was positioned on the basis of two thick slab localiser MRA scans in the coronal and sagittal plane at the level of the skull base, perpendicular on the ICA and BA, to measure the flow volume. The MRA flow volume measurements in the present study are derived from previously developed and optimized protocols [211-214]. Acquisition parameters: repetition time / echo time (TR/TE): 16 ms / 8.5 ms; flip angle 10^{o}; field of view: 150 x 150 mm; number of excitations: 20; slice orientation: transverse; slice thickness: 5.0 mm; number of slices: 1; scan percentage 100%; PC velocity encoding: 140 cm/s; matrix

reconstruction size: 256 x 256 resulting in a nominal voxel size (x,y,z) of 0.59 x 0.59 x 50 mm; total acquisition time: 56sec. Figure 1 illustrates the positioning of the 2D PC section through the ICA and BA. On an independent workstation, quantitative flow values were calculated in each vessel by integrating across manually drawn regions of interest that enclosed the vessel lumen closely.

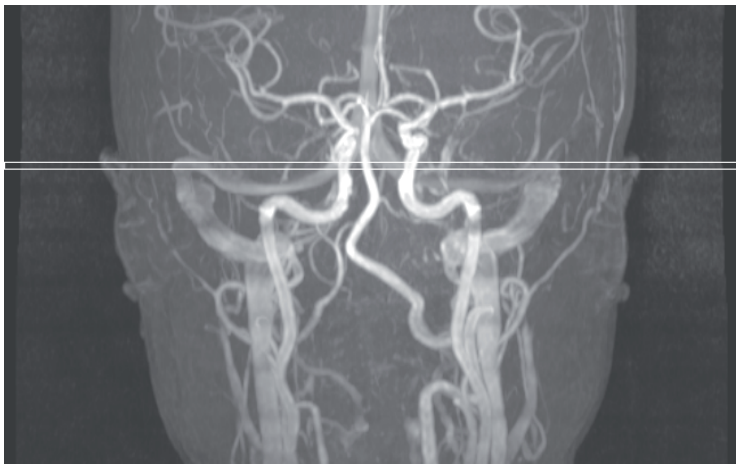


Figure 1 Magnetic resonance angiography, coronal maximum intensity projection. Horizontal line indicates the positioning of the 2-dimensional phase-contrast section through the ICA and the BA.

Image post processing: diameter calculations

All MRA images were transferred to a remote workstation for quantitative analysis using the Quantitative-MRA (QMRA) software package developed at our institution. A full description of the contour detection methods used and the validation have been described previously [215]. The software provides automated contour detection and quantification of the luminal boundaries in selected vessel segments in 3D MRA datasets. The only user interaction required is the definition of the vessel segment of interest by placing a proximal and distal point in the 3D dataset. Subsequently, the software detects a 3D path line following the centre of the vessel lumen and cross-sectional multiplanar recontructions (MPR's) are generated perpendicular to the centreline at 0.5 mm intervals. In each of these MPR's a contour around the vessel lumen is detected automatically. From these contours, based on the assumption of circular vessel cross-sections, the average diameter of the selected vessel segment is derived. Blood vessel segments were selected as follows: A) the MMA was measured in an extra-cranial segment (from the origin at the maxillary artery to the end, 5 to 6 mm distally; Figure2); B) the ECA from

the origin at the superficial temporal artery to the end, 10 mm proximally; C) the ICA from just proximally of the syphon to the end, 15 mm distally; D) the MCA, onset after A1 segment and end 8 mm distally; E) the BA, from the origin at the PCA to the end 12 mm proximally; F) the PCA, beginning at the origin at BA and end 8 mm distally). Location of measured vessel segments were kept constant within subjects.

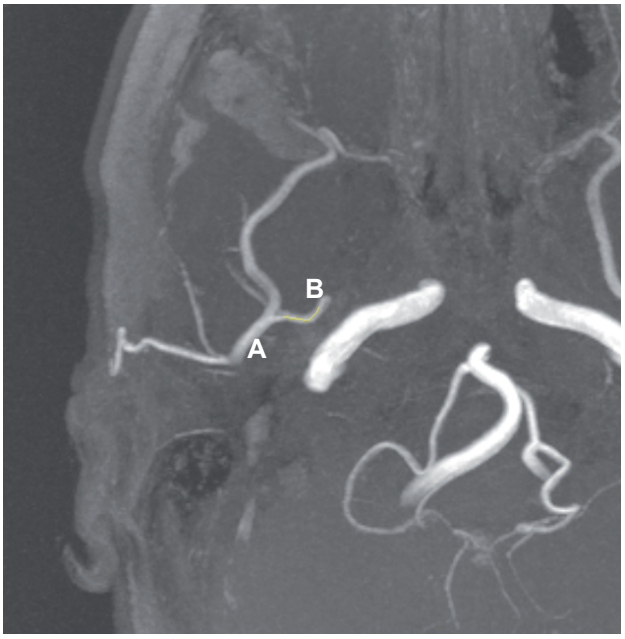


Figure 2 Magnetic resonance angiography of the MMA region and position of the measured segment: (A) maxillary artery, (B) middle meningeal artery (MMA).

Statistical analysis

We first tested the left-to-right differences in diameters for bilateral blood vessels (MMA, ICA, ECA, MCA and PCA) using paired t-tests. Since the differences were not statistically significant, we only present the mean diameters for the right and left blood vessels throughout the manuscript. The effect of NTG and migraine attack on blood vessel diameters and blood flow were tested using a linear mixed model. Patients with a migraine attack (n=20) were compared to patients without an attack after NTG (n=7). Data from patients receiving placebo were not used for statistical testing. $P<0.05$ was considered statistically significant.

Results

Clinical effects of infusion of NTG or placebo

In total 32 migraine patients were randomly infused with either NTG (N=27) or placebo (N=5). Demographic characteristics of the study population are summarised in Table 1. No attack occurred after placebo (0/5). In contrast, infusion of NTG provoked a migraine attack (all without aura) in 20/27 (74%) migraine patients after a median time of 3.75 hours (range: 1.5 - 5.5 hours). In 18/20 attacks the headache was unilateral (left: n=9; right n=9). The clinical characteristics of the patients who developed a migraine attack in response to NTG and the clinical features of the provoked attacks are summarised in Supplemental Table s1.

Table 1 Demographic characteristics of study participants

	Migraine (n=32)		
Intervention	NTG (27)		Placebo (n=5)
Attack	Yes (n=20)	No (n=7)	No
Age in years (SD)	45.5 (8.5)	34 (8.9)	44.8 (13.3)
Ratio female to males	15 : 5	7 : 0	3 : 2
Ratio MO to MA	17 : 3	6 : 1	4 : 1
Attack frequency; mean (SD)	2.6 (1.0)	2.1 (0.38)	2.4 (1.1)

MO denotes migraine without aura, MA migraine with aura.

Side to side differences for blood vessel diameters

There were no ($p>0.05$) right-to-left differences for the diameters of the four bilateral blood vessels (MMA, ICA, ECA, MCA, PCA) in any of the three conditions (data not shown), except for the MCA during session three ($p=0.024$). This difference was considered not significant after correction for multiple testing. Similarly, in the 18 patients with a unilateral headache, there were no significant ($p>0.05$) differences between the diameters on the headache and the non-headache side (Supplemental Table s4). Therefore, the mean diameters of the right and left blood vessels are presented throughout the paper.

Diameter and blood flow changes during infusion of NTG or placebo

During NTG infusion there was a significant vasodilatation of all blood vessels compared to baseline (Figures 3A to F and Supplemental Table s2; $p<0.01$ for all blood vessels). The diameter increase was greatest in the extra-cerebral blood vessels (MMA and ECA), ranging from 16.4% to 30.3%, as compared to 6.7% - 20.7% diameter increase in the

intra-cranial blood vessels (ICA, MCA, BA and PCA). During infusion of placebo, there were no changes in diameter for any of the blood vessels. There were no changes in ICA or BA blood flow during infusion of NTG or placebo (Figure 4A – B and Supplemental Table s3).

Figure 3A -F Mean blood vessel diameter changes (mean of left and right in bilateral vessels) in six selected intracranial blood vessels at baseline, during infusion of nitroglycerin (NTG) or placebo, and during an NTG-provoked migraine or, if no attack had occurred, at 6 hours after infusion. (● Migraine patients (NTG) with a provoked attack, ▲Migraine patients (NTG) without an attack, **X** Migraine patients (placebo) without an attack).

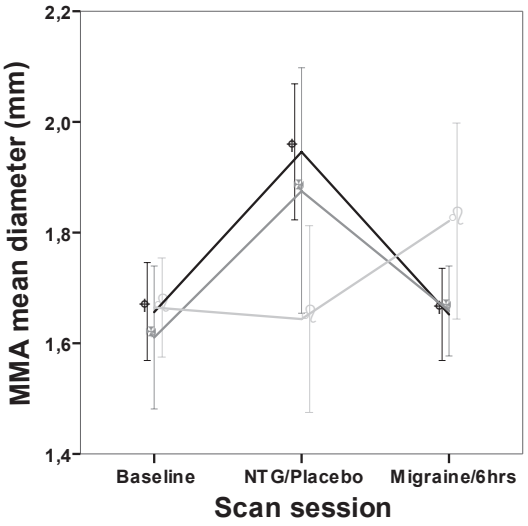
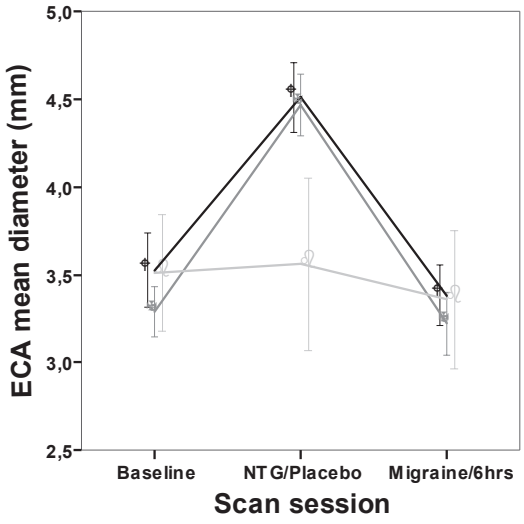


Figure 3A



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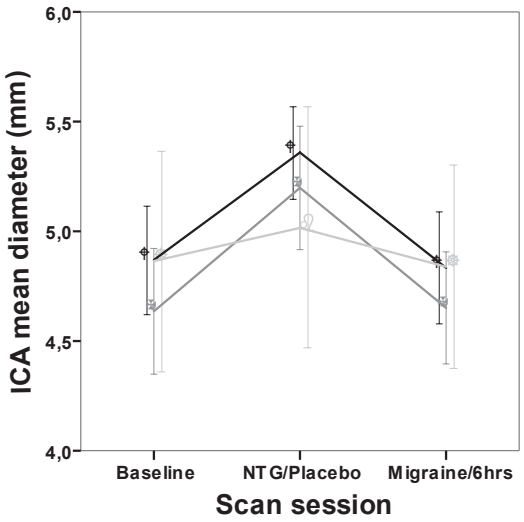

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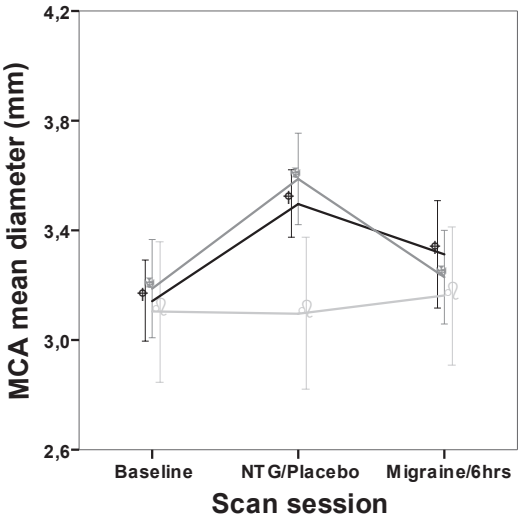

Figure 3D

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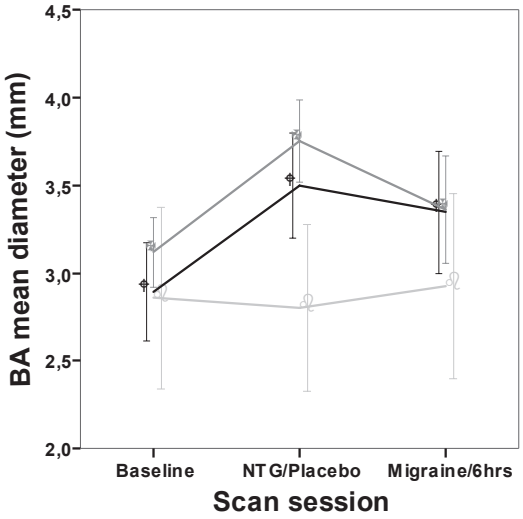

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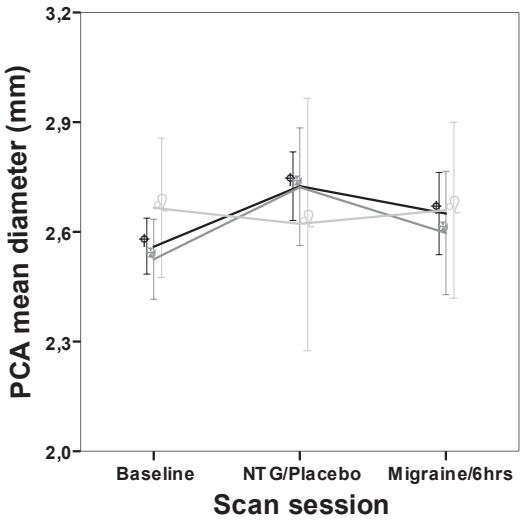

Figure 3F

Table 2 Mean blood vessel diameters (mean of right and left for bilateral blood vessels) of six selected intracranial blood vessel at baseline and during an NTG-provoked migraine attack or, if no attack had occurred, at 6 hours after infusion in 32 migraine patients.

Blood vessel	Inter-vention	Migraine attack	N	A) Baseline	B) During migraine or at 6 hours	Change (B vs A)
				mm (SD)	mm (SD)	mm (% from A)
MMA	NTG	Yes	20	1.66 (0.19)	1.65 (0.17)	-0.01 (-0.6)
	NTG	No	7	1.61 (0.12)	1.66 (0.08)	0.05 (3.1)
	Placebo	No	5	1.67 (0.73)	1.82 (0.14)	0.16 (9.6)
ECA	NTG	Yes	20	3.53 (0.42)	3.38 (0.36)	-0.12 (-3.4)
	NTG	No	7	3.29 (0.16)	3.22 (0.19)	-0.07 (-2.1)
	Placebo	No	5	3.51 (0.27)	3.36 (0.32)	-0.15 (-4.3)
ICA	NTG	Yes	20	4,87 (0.53)	4,83(0.53)	-0.04 (-0.8)
	NTG	No	7	4,64 (0.31)	4,65(0.28)	0.01 (0.2)
	Placebo	No	5	4,86 (0.41)	4,84(0.37)	-0.02 (-0.4)
MCA	NTG	Yes	20	3,14 (0.32)	3,31 (0.41)	0.17 (5.4)
	NTG	No	7	3,19 (0.19)	3,23 (0.19)	0.04 (1.3)
	Placebo	No	5	3,10 (0.20)	3,16 (0.20)	0.06 (1.9)
BA	NTG	Yes	20	2,89 (0.60)	3,35 (0.72)	0.48 (16.6)
	NTG	No	7	3,12 (0.21)	3,36 (0.33)	0.24 (7.7)
	Placebo	No	5	2,86 (0.42)	2,93 (0.42)	0.07 (2.5)
PCA	NTG	Yes	20	2.56 (0.16)	2.65 (0.23)	0.09 (3.5)
	NTG	No	7	2.52 (0.12)	2.60 (0.18)	0.07 (2.8)
	Placebo	No	5	2.67 (0.15)	2.66 (0.19)	0.01 (0.4)

NTG denotes nitroglycerin, MMA middle meningeal artery, ECA external carotid artery, ICA internal carotid artery, MCA middle cerebral artery, BA basilar artery and PCA posterior cerebral artery. There were no significant changes in diameter during the migraine attack.

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Tabel 3 Blood flow in the basilary (BA) and internal carotid artery (ICA) (mean of left and right) in migraine patients at baseline and during a migraine attack or, if no attack had occurred, at 6 hours after infusion of NTG or placebo.

Blood vessel	Inter-vention	Migraine Attack	N	A) Blood flow Baseline	B) Blood flow During Migraine or at 6 hours	Difference (B vs A)
				ml/min (SD)	ml/min (SD)	ml/min
BA	NTG	Yes	20	173.7 (69.4)	128.5 (40.1)	-46.2
	NTG	No	7	177.2 (71.9)	189.7 (26.3)	12.5
	Placebo	No	5	170.5 (39.4)	176.9 (63.6)	6.4
ICA	NTG	Yes	20	589.6 (128.5)	542.0 (166.8)	-57.7
	NTG	No	7	542.9 (101.2)	468.6 (151.2)	-74.3
	Placebo	No	5	542.0 (211.1)	522.8 (276.7)	-19.2
Total cerebral blood flow	NTG	Yes	20	763.3 (124.1)	670.5 (166.6)	-92.8
	NTG	No	7	720.1 (97.7)	658.3 (153.4)	-61.8
	Placebo	No	5	712.6 (202.4)	699.7 (253.9)	-12.8

NTG denotes nitroglycerin, BA basilary artery and ICA internal carotid artery. Difference between patients with an attack compared to patients without an attack after NTG were not significant.

Diameter and blood flow changes during migraine attacks

Compared to baseline, there were no significant (p>0.05) diameter changes during attacks for any of the blood vessels (Table 2 and Figures 3A to F). This was also true when controlling for the headache side in the 18 patients with an unilateral headache; the changes on the headache side were no different compared to those on the non-headache side (Supplemental Table s4). Similarly, there were no significant (p>0.05) differences when comparing the mean diameter changes (baseline vs. attack) in the 20 patients who developed a migraine attack after NTG with the changes (baseline vs. 6 hours post infusion) in the 7 patients who did not develop an attack and were measured 6 hours after infusion. The attack vs. no-attack change-differences were for the MMA = 0.06 mm (95% CI: -0.8; 0.21), for the ECA = 0.05 mm (95% CI: -0.14; 0.24), for the ICA = 0.06 mm (95% CI: -0.19; 0.31), for the MCA = -0.13 (95% CI: -0.41; 0.14), for the BA = -0.24 (95% CI: -0.59; 0.11), and for the PCA = -0.02 (95% CI: -0.22; 0.18). There were also no significant (p>0.05) changes in total-, BA-, or ICA-blood flow during a migraine

attack when compared to baseline, nor were there significant (p>0.05) differences in the changes observed during attacks when compared to the changes in the patients who did not develop an attack and were measured 6 hours after infusion (Supplemental Table 4).

Figure 4A -B Mean blood flow in ICA (mean of left and right) and BA at baseline, during infusion of nitroglycerin (NTG) or placebo, and during an NTG-provoked migraine or, if no attack had occurred, at 6 hours after infusion. (● Migraine patients (NTG) with a provoked attack, ▲Migraine patients (NTG) without an attack, **X** Migraine patients (placebo) without an attack)

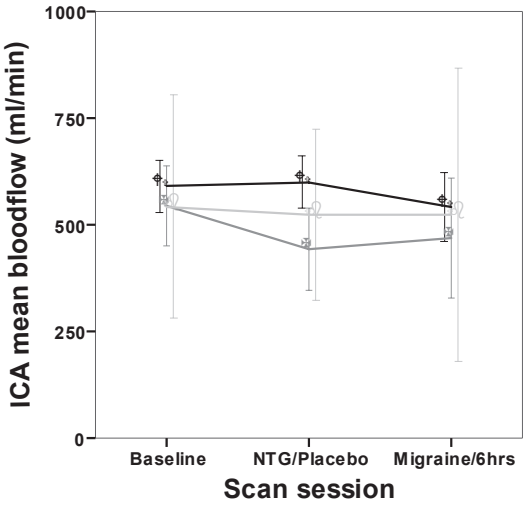

Figure 4A

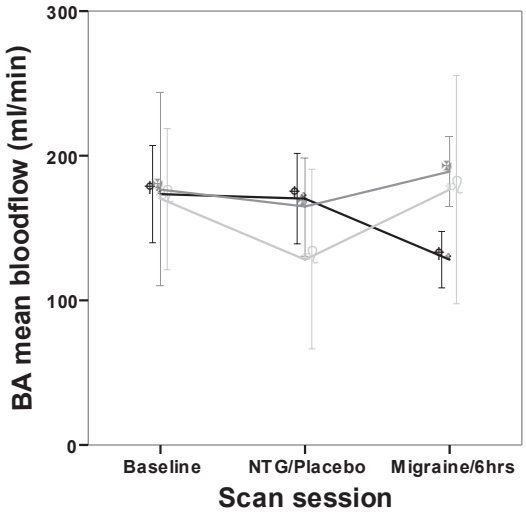

Figure 4B

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Discussion

We used a well established NTG provocation model to induce faithfully migraine attacks and a highly sensitive, non-invasive 3T MRA technique to visualise and measure even small intra-individual diameter changes of cerebral and meningeal blood vessels. Contrary to longstanding and widespread belief, we failed to detect any evidence for a clinically relevant vasodilatation of major cerebral or meningeal blood vessels during migraine attacks. This finding has important implications for the understanding of the pathophysiology of the migraine headache and the development of future antimigraine agents. Novel antimigraine treatments may not require vasoconstrictior activity as predicted earlier [61].

In our provocation experiments, we infused NTG over a 20 min period and observed a vessel-dependent 7 – 30% vasodilatation at 10 minutes after beginning of the infusion. The vasodilatatory effect is believed to be due to a direct local effect of NO on vascular smooth muscle cells [240] or through the release of vasoactive peptides such as CGRP [114,241]. Our findings on the early vascular effect of NTG are in accordance with those of [109]. Using 1.5T MRA they found a peak vasodilatation at 10 - 15 minutes after beginning of the NTG infusion and a normalisation of the vascular diameters back to baseline at 45 minutes after stopping of the infusion. For logistic reasons, we did not scan at 45 min after the infusion to confirm normalisation of the blood vessel diameter. However, in view of the well known short duration of action of NTG [242] and the observed time course of the early vascular responses by [109], we feel confident that blood vessel diameters had returned to baseline by one hour after the second (infusion) scan. It therefore seems justified to compare measurements during attacks with those obtained at baseline, before infusion.

The most important finding of the present study is that migraine headache was not associated with a clinically relevant vasodilatation of major cerebral or meningeal blood vessels, not even when controlled for headache side. We feel confident that this was not due to too low a sensitivity of the detection method. The very fact that we were able to detect an early transient vasodilatation in response to NTG of as low as 7% shows that the method we used is sufficiently sensitive to measure even small diameter changes. The clinical relevance of smaller changes is doubtful as during NTG infusion we observed an up to 30% increase in blood vessel diameter without associated migraine headache. Our results are also in agreement with at least some older TCD studies failing to show blood velocity changes indicative for vasodilatation during migraine attacks. [243-246]

Finally, BA and ICA blood flow did also not change during migraine attacks. Cerebral blood flow is dependent on cardiac output, arterial caliber, and vasomotor tone in small resistance vessels.[247] As blood pressure (as a measure for cardiac output; data not shown) and the BA and ICA diameters had not changed, It seems likely that there were also no changes in the intracranial resistance microvasculature during migraine attacks. In conclusion, our data seem to refute an important role of cerebral or meningeal vasodilatation in causing migraine headache. This would certainly be in accordance with observations that non-vascular mechanisms, such as exposure to sildenafil, [58] are capable of inducing migraine attacks.

Potential limitations of our study include that we didn't measure just before or at the onset of the migraine headache. We could thus have missed a brief transient vasodilatation at the very beginning of the migraine headache. Although unlikely, we cannot exclude this possibility. Another important question is whether and to what extent NTG-provoked migraine attacks are similar to spontaneous attacks. There are strong clinical and pathophsyiological arguments in favour of this notion. The clinical symptoms and features, including the occurrence of premonitory symptoms several hours before the headache[56] and the response to anti-migraine drugs[239], are strikingly similar between spontaneous and NTG-induced attacks. Likewise, in both there is an increase of CGRP in jugular venous blood [61,124] and activation of the dorsal rostral brainstem on positron emission tomography.[82,202] The fact that NTG provokes migraine aura's only rarely, even in patients with migraine with aura [162 248], seems to point at a trigger site of action beyond the aura triggering mechanism. We thus feel confident that our findings in NTG-provoked attacks can be extrapolated to spontaneous migraine headaches.

In this study, we did not observe significant changes in blood vessel diameter or blood flow during the headache phase of provoked migraine attacks. However, there were some (non-significant) changes in the posterior circulation that need to be discussed. First, the diameter of the BA did not return to baseline levels, unlike the other blood vessels. This was, however, true for both patients who had developed a delayed migraine headache and for those who had not. Secondly, the blood flow in the BA was decreased (although not significantly) from 174 ml/min at baseline to 129 ml/min in patients who had developed a migraine headache after GTN, whilst there was no such change in patients who had not developed a migraine headache. Whether these findings are clinically relevant, needs to be explored. A tentative correlation, for instance, could be made with previous findings of In previous studies our group has shown our group demonstrating increased prevalence of pontine hyperintensities and cerebellar infarcts in migraineurs from the general population [249 250].

We conclude that, contrary to a longstanding and widespread belief, cerebral and meningeal diameter changes in migraine attacks, if at all happening, appear not to be of primary importance to the pathophysiology of the migraine headache.

Supplemental Tables

Table s1 (only for publication on the web)
Characteristics of the NTG provoked migraine attack per subject

Subject	Sex	Age	Attack freq (per month	Characteristics of provoked attack								Time to attack (hours)
				HS	UH	PH	AH	N	V	PT	PN	
1	M	40	2	2			+			+	+	4.5
2	M	35	3	2	+	+		+	+			2.5
3	F	42	2	2	+	+	+	+				4.5
4	M	54	4	2	+	+	+	+				4
5	F	49	4	2		+	+	+		+	+	4.5
6	F	55	2	2	+		+	+		+	+	3
7	F	48	1	2	+	+	+			+	+	3
8	F	37	2	2	+	+		+	+	+	+	2.5
9	F	33	2	2	+		+	+	+	+	+	4
10	F	32	2	2	+	+	+	+			+	5
11	F	51	3	2	+	+	+	+		+	+	3
12	F	28	3	2	+		+			+	+	3
13	F	55	4	2	+	+	+	+		+		5.5
14	F	55	4	2	+	+	+			+	+	2
15	F	53	2	2	+		+			+	+	5
16	M	46	3	2	+		+	+	+	+		2
17	M	51	4	2	+		+	+	+	+		4
18	F	49	0.5	2	+		+			+	+	4
19	F	50	4	2	+		+	+	+	+	+	3.5
20	F	31	1	2	+	+	+	+	+	+	+	1.5

F denotes female, M male, HS headache severity (2=moderate), UH unilateral headache (+ indicates yes, empty box no), PH pulsating headache, AH aggravation of headache during physical activity, N nausea, V vomiting, PT photophobia, PN phonophobia

Table s2 (only for publication on the web)
Mean blood vessel diameters (mean of right and left for bilateral blood vessels) of six selected intracranial blood vessel at baseline and during infusion of nitroglycerin or placebo in 32 migraine patients.

Blood vessel	Inter-vention	N	A) Baseline	B) During NTG or placebo	Change (B vs A)
			mm (SD)	mm (SD)	mm (% from A)
MMA	NTG	27	1.65 (0.18)	1.93 (0.24)	0.27 (16.4)*
	Placebo	5	1.67 (0.07)	1.64 (0.12)	-0.02 (-1.2)
ECA	NTG	27	3.46 (0.38)	4.50 (0.38)	1.05 (30.3)*
	Placebo	5	3.51 (0.27)	3.56 (0.39)	0.05 (1.4)
ICA	NTG	27	4.81 (0.49)	5.32 (0.42)	0.51 (10.6)*
	Placebo	5	4,86 (0.41)	5,02 (0.44)	0.15 (3.1)
MCA	NTG	27	3.16 (0.29)	3.52 (0.24)	0.37 (11.7)*
	Placebo	5	3,10 (0.20)	3,10 (0.22)	-0.01 (-0.3)
BA	NTG	27	2.95 (0.53)	3.56 (0.57)	0.61 (20.7)*
	Placebo	5	2,86 (0.42)	2,80 (0.38)	-0.06 (-2.1)
PCA	NTG	27	2.55 (0.15)	2.72 (0.19)	0.17 (6.7)*
	Placebo	5	2.67 (0.15)	2.62 (0.28)	-0.04 (-1.5)

NTG denotes nitroglycerin, MMA middle meningeal artery, ECA external carotid artery, ICA internal carotid artery, MCA middle cerebral artery, BA basilar artery, PCA posterior cerebral artery. * NTG effect on diameter was significant in all six blood vessels ($p<0.01$).

Table s3 (only for publication on the web)
Blood flow in BA, ICA and total cerebral blood flow in migraine patients at baseline and during infusion of nitroglycerin or placebo.

Blood vessel	Inter-vention	N	A) Baseline	B) During NTG or placebo	Change (B vs A)
			ml/min (SD)	ml/min (SD)	ml/min (%)
BA	NTG	27	174.6 (68.7)	169.3 (57.9)	-5.4 (-3.1)
	Placebo	5	170.5 (39.4)	128.6 (49.9)	-41.9 (-24.6)
ICA	NTG	27	577.1 (121.6)	557.5 (139.4)	-19.5 (-3.4)
	Placebo	5	542.0 (211.1)	523.2 (161.9)	-18.8 (-3.5)
TCBF	NTG	27	751.7 (117.3)	726.8 (149.5)	-24.9 (-3.3)
	Placebo	5	712.6 (202.4)	651.8 (198.8)	-60.7 (-8.5)

NTG nitroglycerin, ICA internal carotid artery, BA basilar artery, tCBF total cerebral blood flow.

Table s4 (only for publication on the web)
Blood vessel diameter of five bilateral intracranial blood vessels at baseline and during an NTG-provoked migraine attack in 18 migraine patients with unilateral headache.

Blood vessel	Side	A) Baseline	B) During migraine	Change (B vs A)
		mm (SD)	mm (SD)	mm (% from A)
MMA	Headache	1.69 (0.22)	1.67 (0.21)	-0.03 (-1.78)
	Non-headache	1.60 (0.18)	1.58 (0.17)	-0.03 (-1.88)
ECA	Headache	3.51 (0.39)	3.34 (0.38)	-0.18 (-5.13)
	Non-headache	3.43 (0.46)	3.33 (0.39)	-0.04 (-1.17)
ICA	Headache	4.87 (0.59)	4.79 (0.64)	-0.09 (-1.85)
	Non-headache	4.89 (0.55)	4.87 (0.55)	-0.02 (-0.41)
MCA	Headache	3.19 (0.34)	3.32 (0.44)	0.13 (4.08)
	Non-headache	3.15 (0.34)	3.36 (0.45)	0.24 (7.62)
PCA	Headache	2.58 (0.19)	2.72 (0.30)	0.13 (5.0)
	Non-headache	2.58 (0.21)	2.66 (0.23)	0.08 (3.1)

MMA denotes middle meningeal artery, ECA external carotid artery, ICA internal carotid artery, MCA middle cerebral artery and PCA posterior cerebral artery. There were no significant differences in diameter change between headache side and non-headache side.