SCHOOL OF POPULATION HEALTH

INVESTIGATING THE EFFICACY OF L-ARGININE AND AGED GARLIC EXTRACT IN A RANDOMISED PLACEBO-CONTROLLED CLINICAL TRIAL: A VASODILATORY NUTRACEUTICAL STRATEGY FOR MIGRAINE PREVENTION

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THIS THESIS IS PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DECLARATION

To the best of my knowledge and belief, this thesis titled, 'Investigating the Efficacy of L-Arginine and Aged Garlic Extract in a Randomised Placebo-Controlled Clinical Trial: a Vasodilatory Nutraceutical Strategy for Migraine Prevention', contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material that has been accepted for the award of any other degree or diploma in any university.

The clinical research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007). The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number HRE2020-0466.

Devahuti ("Rhia") Chaliha

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When King Lear asked each of his daughters how they loved him, it was Cordelia whose "love's more ponderous than [her] tongue", and so to describe the gratitude I have to my father for so patiently and calmly bringing me through life, I could only say, "Nothing, my lord". The same goes to my sister for being the twin half I could indulge my silly side with – you are the best thing that ever happened to me! And also a word of thanks to my mother to whom I will always be her precious baby; thanks for pampering me with so much yummy food and cups of chai every day.

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LIST OF PRIMARY PUBLICATIONS

This PhD includes first-author peer-reviewed publications to address my candidacy objectives. The following articles have been published in each of the journals listed below. Author contributions for each publication are detailed at the end of their respective chapter.

1. **Chaliha DR,** Vaccarezza M, Takechi R, Lam V, Visser E, Drummond P, Mamo JCL. A Paradoxical Vasodilatory Nutraceutical Intervention for Prevention and Attenuation of Migraine—A Hypothetical Review. Nutrients. 2020; 12(8):2487.

   Impact Factor: 6.706 (2021)

2. **Chaliha DR,** Vaccarezza M, Lam V, Takechi R, Mamo JC. Attenuation of chronic tension headache frequency and severity with daily L-arginine and aged garlic extract dietary supplementation. Eur J Clin Nutr. 2022 Feb;76(2):317-319.

   Impact Factor: 4.016 (2021)

3. **Chaliha DR,** Vaccarezza M, Corti E et al. L-arginine and aged garlic extract for the prevention of migraine: a study protocol for a randomised, double-blind, placebo-controlled, phase-II trial (LARGE trial). BMC Neurol 23, 122 (2023).

   Impact Factor: 2.72 (2022)

4. **Chaliha DR,** Vaccarezza M, Charng J, Chen FK, Lim A, Drummond P, et al. Using optical coherence tomography and optical coherence tomography angiography to delineate neurovascular homeostasis in migraine: a review. Frontiers in Neuroscience. 2024;18.

   Impact Factor: 4.3 (2024)

LIST OF SECONDARY PUBLICATIONS

The following publications are not directly linked to my candidacy objectives, but further demonstrate research acumen, productivity and engagement during the course of my candidacy.

1. **Chaliha DR,** Albrecht M, Vaccarezza M, Takechi R, Lam V, Al-Salami H, Mamo J: A Systematic Review of the Valproic-Acid-Induced Rodent Model of Autism. Dev Neurosci 2020;42:12-48. doi: 10.1159/000509109.

   Impact Factor: 3.421 (2021)

2. **Chaliha DR,** Mamo JC, Albrecht M, Lam V, Takechi R, Vaccarezza M. A Systematic Review of the MDMA Model to Address Social Impairment in Autism. Curr Neuropharmacol. 2021;19(7):1101-1154. doi: 10.2174/1570159X19666210101130258. PMID: 33388021; PMCID: PMC8686313.

   Impact Factor: 7.363 (2021)

3. Hill M, Takechi R, **Chaliha DR,** Albrecht MA, Wright J, James AP, Clark K, Dhaliwal SS, Lam V, Mamo JCL. Dietary saturated fats and apolipoprotein B48 levels are similarly associated with cognitive decline in healthy older aged Australians. Asia Pac J Clin Nutr. 2020;29(3):537-544. doi: 10.6133/apjcn.202009_29(3).0012. PMID: 32990613.

   Impact Factor: 1.662 (2021)

4. Swanepoel I, Roberts A, Brauns C, **et al.** Trimethylamine N-oxide (TMAO): a new attractive target to decrease cardiovascular risk. Postgraduate Medical Journal 2022;98:723-727.

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COPYRIGHT STATEMENT

ABBREVIATIONS

ADMA	Asymmetric dimethylarginine
AGE	Aged garlic extract; Kyolic
ANS	Australasian Neuroscience Society
ET-1	Endothelin-1
L-arg	L-arginine
L-VISS	Leiden Visual Sensitivity Scale
MSQ	Migraine-Specific Quality-of-Life Questionnaire
NOx	Nitrites and nitrates
OCT	Optical coherence tomography
OCTA	Optical coherence tomography angiography
SDMA	Symmetric dimethylarginine
SF-MPQ	Short-Form McGill Pain Questionnaire
SWAN	Symposium of Western Australian Neuroscience
VLSQ-8	Visual Light-Sensitivity Questionnaire 8

CONTENTS

GENERAL ABSTRACT

Not many diseases can boast the sheer amount of human suffering caused by migraine. Its Arabic word, “shaqueequah”, beautifully conveys the sharp shock it can have on a person’s health and wellbeing. It is the most common and one of the costliest neurological disorders to treat (1, 2). It affects women more than men, and causes massive losses in lifestyle and work productivity to the point of suicidal ideation. Episodic migraine has higher costs than chronic, due to its higher prevalence, and we thereby lean towards exploring the former more than the latter form of migraine in this study. The current conventional medications prescribed for migraine present challenges such as exceeding economic cost, inefficacy and adverse effects, risk of adding to the list of comorbidities that migraine already has, addictiveness with the more potent analgesics, as well as contraindications for large cohorts such as those with vascular insufficiencies.

While studies imply that it is cerebral vasodilation that triggers migraine pain onset, it has been shown that decreasing oxygen tension of inspired air provides immediate relief of migraine (3, 4). Since carbon dioxide is a potent cerebral vasodilator, this seems at odds with interpretation of those studies. In addition, psychobiological studies demonstrate that stress modulates endothelial function (5) and is associated with frequency and severity of migraines (6), with exercise-induced migraines being common (7). Endothelial function is negatively correlated with patient age (8, 9), and age is positively associated with migraine prevalence (10). The mechanism for this is related to the NO-arginine pathway which is responsible for systemic vascular dilation.

Therefore, we provide an alternate hypothesis for the pathogenesis of migraines, in that we suggest that the carbon-dioxide-inducing vasodilation rescues migraine phenotype by restoring central capillary perfusion. This would inhibit local nociceptor activation from compromised blood supply, thereby precluding headache onset. We therefore looked at systemic vasodilators which could be used as migraine-preventive treatment, as we were aware that triptans and nonsteroidal anti-inflammatory drug overuse is frequently reported (11, 12), and that currently prescribed drugs have many adverse effects and even cardiovascular contraindications (13). We found, in the literature, that L-arginine and aged garlic extract (AGE) are regularly clinically used to increase vascular tone in patients. L-arginine directly induces vasodilation via acting as a precursor to nitric oxide (NO) synthesised in the endothelium, which travels up to the smooth muscle cell layer and sequesters calcium from the cytosols, inducing vascular muscle relaxation; and AGE inhibits capillary inflammation especially from its active component, S-allyl-cysteine, and also includes compounds with antioxidant and antithrombotic effects. AGE also can promote vasodilation by increasing vasodilatory prostaglandins

or imitating the molecule (14). We searched both animal and human studies for evidence of both L-arginine and AGE use in vasodilation via measures such as blood pressure, vascular resistance, vessel elasticity, temperature rebound and systemic vasodilation biomarker molecules. The overarching implication of this is that L-arginine and AGE may induce chronic microvascular dilation, in turn preventing migraine frequency/severity due to increased oxygen demand and decreased oxygen supply to the brain parenchyma. Hence, randomised controlled trials are needed to test this theory.

Once we obtained ethical approval for this project, we launched the trial and demonstrated the case of a 74-year-old Maltese female with a 20-year history of tension headaches, who improved with our proposed treatment. Her headaches were regular, mostly severe, lasted days and were exacerbated at times of increased psychological stress. They significantly impacted her day-to-day functioning, and nausea and vertigo were common concurrent symptoms. Her headaches started at menopause, indicating a vascular origin. Normally, the onset of migraine tends to increase at menarche, peaking in the late 30s and declining sharply after menopause (15). However, ongoing migraine frequency and burden tends to get worse midlife, believed to be caused by hormonal fluctuations surrounding menopause (15). Sudden oestrogen falls could increase migraine frequency during menstruation and at menopause (15). The usual abortives she resorted to were paracetamol, aspirin or ibuprofen, but these had negligible effect. Two years ago, she decided to try L-arginine and AGE as dietary supplementation with meals. Within 6 weeks of starting daily oral 1.5 g L-arginine and 1.2 g AGE, she found decreased headache severity and duration. After 2 years on treatment, headache frequency also lessened by ~70%, and intensity reduced to half the pre-treatment subjective score. She thus gained confidence to add anxiety- and stress-reducing strategies to this regime, and altogether she found that her lifestyle improved dramatically, including socially. This was significant, as socially lonelier patients are less likely to be satisfied with their own symptom management and ability to avoid migraine triggers (16). Importantly, the patient experienced no adverse symptoms from the treatment, including changes in wound-healing (given AGE's antithrombotic properties).

To address the aforementioned need for a randomised controlled trial to test the migraine-preventive effects of L-arginine and AGE, we followed the guidelines for controlled drug trials in migraine as outlined by the International Headache Society Clinical Trials Subcommittee (17) and guidelines for reporting as outlined by the Recommendations for Interventional Trials Statement (18). We conducted a 12-week, phase-II, single-site, randomised, double-blind, parallel-group-design, placebo-controlled trial in 18-80-year-old male and female adults with chronic frequent episodic migraines. We block-randomised participants into 4 treatment arms: placebo, 1.5 g L-arginine, 1 g AGE, and 1.5 g L-arginine + 1 g AGE (combination). The participants took these daily as oral supplements, and outcome measures included frequency of migraine attacks, intensity of headache pain, changes in

photosensitivity as the most common bothersome concurrent symptom of migraine, retinal vascular diameter, blood biomarkers of vascular tone, and interictal subjective measures of daily migraine-caused disability. After filtering for eligible participants through stringent criteria, we took them through 2 weeks of single-blind placebo and then 12 weeks of double-blind verum treatments. The first visit involved taking anthropometric and demographic measurements, baseline migraine-related characteristics, and instructing the participant on how to fill in hardcopy diaries for the next 2 weeks alongside taking capsules every day (at their chosen time, to increase adherence). The second visit involved change of capsules into one of the 4 treatment arms, providing identical migraine diaries for the next 12 weeks with sufficient capsules for the duration. The second and third visits involved a photosensitivity test, phlebotomy for later vascular-biomarker analysis, and optical coherence tomography angiography to assess retinal capillary tone. The third visit included the migraine-characteristic long-term questionnaires given on the first visit. Therefore, each duplicate measure allowed us to compare migraine parameters before and after treatment.

For the sake of curiosity, we further explored previous migraineur studies which had used the OCT/OCTA technology to visualise the retinal microvasculature as an extension of the cerebral. These showed that migraineurs tend to have thinner layers of the posterior eye, corresponding with decreased microvascular tone. The individual extents of these effects also positively correlated with the individual migraine severity/duration/history/impact recorded for these patients. These effects seem to be further exacerbated in chronic migraineurs and those with aura. We realised that OCT and OCTA technologies can therefore be used as a diagnostic and therapeutic biomarker in migraineurs. Further research needs to be done in this area to distinguish between migraine and other systemic disorders, as well as between different migraine types.

The results of our clinical trial showed noteworthy results. L-arginine treatment showed particularly positive effects on alleviating migraine symptoms (subjective measures) and signs (objective measures), with AGE having a marginally better effect compared to placebo. The combination treatment seems to have been compromised in effect. Whether these are real effects or the result of an insufficiently powered study is uncertain; therefore, future studies are required to determine the real effects and the mechanisms of those.

STRUCTURE OF THE THESIS

This thesis comprises 5 papers addressing the use of two systemically vasodilatory nutraceuticals for the prophylactic treatment of migraine. The first paper expounds on the overarching hypothesis of a vasodilatory aetiology of migraine that the treatment is based on, in the form of a literature review to date. The second paper is a case study supporting the investigation of these nutraceuticals in a subsequent clinical trial to alleviate different migraine features in different individuals. The third paper is a protocol of the following clinical trial I conducted, investigating the same as well as physiological indicators of the expected changes in vascular tone. The fourth paper is a literature review of the uses and findings of our retinal vascular-tone measurement technique (optical coherence tomography angiography) in migraineurs in previous studies. The fifth paper summarises the results of our clinical trial. What follows is a general discussion of various aspects of the project which did not fit into any of the above papers alone. This thesis demonstrates, for the first time, that a regularly taken vasodilatory nutraceutical oral treatment may be effective to prevent migraines in a safer, cheaper and more effective way than previously discovered.

CHAPTER 1: REVIEW OF THE LITERATURE

The content of this chapter is covered by published Paper 1:

Chaliha DR, Vaccarezza M, Takechi R, Lam V, Visser E, Drummond P, Mamo JCL. A Paradoxical Vasodilatory Nutraceutical Intervention for Prevention and Attenuation of Migraine—A Hypothetical Review. Nutrients. 2020; 12(8):2487.

PAPER 1

Paper 1 provides a comprehensive literature review of an alternate hypothesis for the aetiology of migraine and opportunities for prevention.

A PARADOXICAL VASODILATORY NUTRACEUTICAL INTERVENTION FOR PREVENTION AND ATTENUATION OF MIGRAINE: A HYPOTHETICAL REVIEW

ABSTRACT

Studies suggest that migraine pain has a vascular component. The prevailing dogma is that peripheral vasoconstriction activates baroreceptors in central large arteries. Dilation of central vessels stimulates nociceptors and induces cortical spreading depression. Studies investigating nitric oxide (NO) donors support the indicated hypothesis that pain is amplified when acutely administered. In this review, we provide an alternate hypothesis which, if substantiated, may provide therapeutic opportunities for attenuating migraine frequency and severity. We suggest that in migraines, heightened sympathetic tone results in progressive central microvascular constriction. Suboptimal parenchymal blood flow, we suggest, activates nociceptors and triggers headache pain onset. Administration of NO donors could paradoxically promote constriction of the microvasculature as a consequence of larger upstream central artery vasodilation. Inhibitors of NO production are reported to alleviate migraine pain. We describe how constriction of larger upstream arteries, induced by NO synthesis inhibitors, may result in a compensatory dilatory response of the microvasculature. The restoration of central capillary blood flow may be the primary mechanism for pain relief. Attenuating the propensity for central capillary constriction and promoting a more dilatory phenotype may reduce frequency and severity of migraines. Here, we propose consideration of two dietary nutraceuticals for reducing migraine risk: L-arginine and aged garlic extract.

Keywords: aged garlic extract; calcium; L-arginine; migraine; nitric oxide; vasodilation; vasoconstriction

INTRODUCTION

Migraine is the most common and one of the costliest neurological disorders to treat (1, 2). Migraines are described to occur through a stress-induction pathway and may, in some individuals, represent a continuum of tension headaches (19). As a global health burden, migraines have an estimated worldwide prevalence of 1.04 billion with a staggering disability-adjusted life burden of approximately 45.1 million years per annum (20). The aetiology of migraines is not entirely clear; however, there is a body of opinion which suggests that pain in migraine is associated with vasodilation of large cerebral arteries (21). This hypothesis is paradoxical, as dilation of large cerebral arteries is not suggested by studies examining net change in cerebral blood flow in migraine sufferers. Rather, the latter would suggest that, along with dilation of large arteries, there is downstream vasoconstriction of the cerebral microcirculation, raising an alternate consideration of causation (22).

Studies implicate the trigeminovascular system in the aetiology of migraines (23), with cerebral vasodilation being suggested to exacerbate pain by causing depolarisation of perivascular nociceptive nerve terminals and promoting vascular inflammation (24). The primary evidence for this hypothesis comes from clinical studies investigating the acute effects of intravenous administration of NO donors such as glyceryl trinitrate (GTN). Acute administration of GTN markedly accentuates pain intensity in migraine sufferers. However, conflicting with those studies, there is accumulating evidence that decreasing oxygen tension of inspired air (via enrichment with carbon dioxide), can provide immediate relief of migraine (3, 4) via an autoregulatory mechanism (25). Carbon dioxide is a potent cerebral vasodilator, so these findings seem at odds with interpretation of the studies, considering the mechanism of pain amplification afforded by acute administration of NO donors such as GTN.

Age and sex are factors positively associated with the prevalence of migraines and, in addition, changes in cerebrovascular “tone”. Studies have shown that endothelial function changes with ageing (8), generally described as becoming less responsive to regulating factors and less patent (increased arterial stiffness) (9). Women are also three times more likely to experience migraines than are men (26), which manifest around menarche and become increasingly common in their 30s and 40s. The monthly frequency of migraine also worsens during perimenopausal changes. The greater risk of migraine in women is of particular interest in the context of potential mechanisms triggering migraine. Oestrogen strongly stimulates synthesis of the potent vasodilator (NO) in arterial vascular endothelial cells, whilst concomitantly suppressing secretion of vasoconstrictive endothelin (27). The net effect of oestrogen loss during menorrhoea would therefore notionally result in cerebrovasoconstriction, an association that is inconsistent with the prevailing hypothesis that cerebral vasodilation underlies headache onset during attacks of migraine. Several explanations are possible for these contradictory

observations, including direct regulatory effects of oestrogen on the trigeminovascular system independent of vascular tone and/or differential triggers for migraine pain.

A paradoxical vascular axis for risk of migraine is, in part, also suggested by psychobiological studies which demonstrate that stress modulates endothelial function (5) and is associated with frequency and severity of migraines (6). Psychosocial stress causes heightened sympathetic nervous system (SNS) and suppressed parasympathetic nervous system (PNS) responses (5), and triggers vasoconstriction via sympathetic efferent fibres that is generally proportional to the level of stress realised (5). Indeed, stress-induced vascular constriction can be sufficient to significantly heighten blood pressure (28). Psychological stress is also reported to be associated with vascular inflammation which can amplify vasoconstriction (29). For example, stimulation of trigeminal nerve fibres causes plasma protein extravasation, mast cell activation, and degranulation in the dura mater (30). The neurogenic inflammation realised is proposed to exacerbate migraines by activating trigeminal sensory fibres in the meninges (31). It is on this basis that behavioural and cognitive therapies have been used to manage stress levels, with the intent to reduce frequency and severity of migraines (32).

THE ROLE OF NITRIC OXIDE IN MODULATING CEREBRAL VASCULAR TONE

There is a positive association of migraine prevalence with ageing, associated with a phenotypic shift towards decreased NO production that results in impaired vasodilatory function (10). NO is the primary endogenous vasodilator synthesised by vascular endothelial cells (33), which stimulates arterial smooth muscle cells and capillary pericytes to relax by regulating cellular ionised calcium (Ca^{2+}) homeostasis. For vasoconstriction to occur, Ca^{2+} enters smooth muscle cells or pericytes through calcium ion channels and, once released from the sarcoplasmic reticulum, binds with calmodulin, resulting in myosin phosphorylation and cell constriction (34). Endothelial-derived NO promotes vasodilation by promoting calcium ion efflux (34) or reducing the cell contractile apparatus to calcium ions (35). NO causes vascular relaxation via 2 pathways: a non-cGMP-dependent (cyclic guanosine monophosphate (cGMP)) stimulation of sarcoendoplasmic-reticulum calcium ATPase (SERCA) to reuptake Ca^{2+} from acting in the cytosol, and/or through soluble guanylyl cyclase (sGC)-stimulated cyclic guanosine monophosphate (cGMP) which desensitises the contractile apparatus to Ca^{2+} via L-type calcium ion channels (34). The combined effects result in a decrease of smooth muscle cell/pericyte cytosolic Ca^{2+} ions which are recycled back into the sarcoplasmic reticulum for sequestration/storage (34). This antagonistic process is shown in Fig. 1.

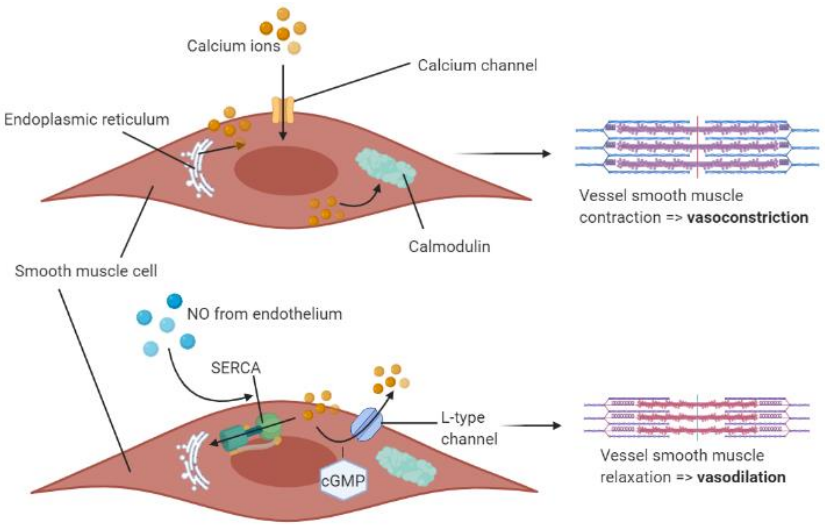

Fig. 1. *Antagonistic mechanism of NO-mediated vasodilation and Ca^{2+}-mediated vasoconstriction in the smooth muscle cell layer of blood vessels. Calcium ions bind to calmodulin in the cell cytosol, which triggers constriction of the smooth muscle cell lining the vessel. The endothelium secretes NO which prevents this pathway via two different mechanisms in the cell cytosol: SERCA-mediated and cGMP-mediated pathways. NO: nitric oxide; SERCA: sarcoendoplasmic-reticulum calcium ATPase; cGMP: cyclic guanosine monophosphate. Created on BioRender.com (BioRender).*

A REVISED HYPOTHESIS FOR THE PATHOPHYSIOLOGY OF VASCULAR-BASED MIGRAINES

Normal physiological vascular tone modulation in a healthy human brain is depicted as follows (Fig. 2A). The regional microvasculature is ordinarily highly responsive to parenchymal energy requirements. With increasing energy demand, the microvascular endothelium synthesises vasodilators (NO) and releases them into the bloodstream, causing precapillary sphincters, small arterial smooth muscle cells and capillary pericytes to vasodilate. Functional magnetic resonance imaging elegantly demonstrates marked changes in microvascular blood flow (36) with acute mental challenges. Depending on the microvascular demand, regional upstream larger vessels may also dilate in order to support changes in flow and, critically, in response to intravascular pressure. The regional intrinsic factors, which regulate microvascular tone, may be direct innervations from neuronal cells and astrocytes to endothelial cells, arteriolar smooth muscle cells and capillary pericytes. The innervations to endothelial cells will modulate NO biosynthesis by causing intracellular distributional changes in the cytoplasmic pools of ionised calcium (Fig. 1).

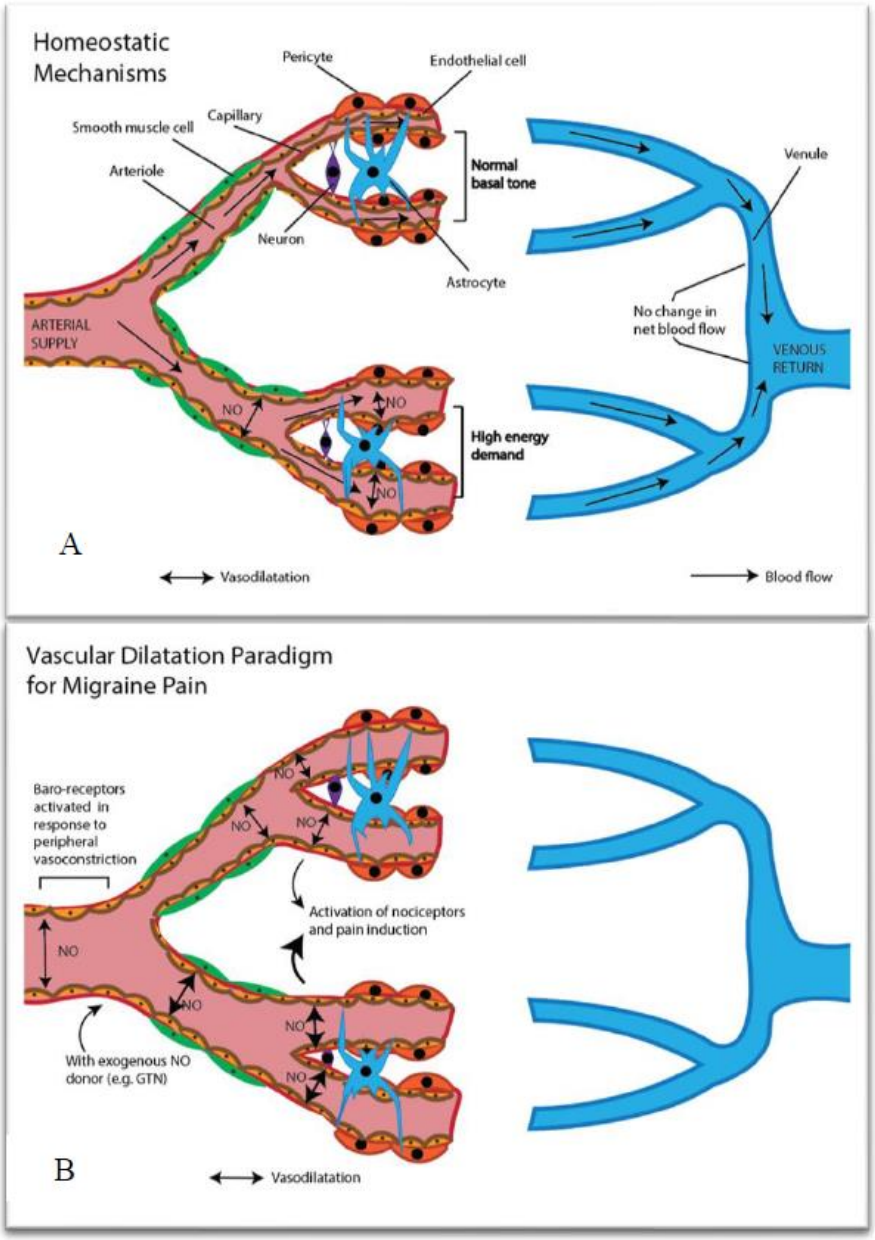

Fig. 2. *Homeostatic microvascular tone modulation and the prevailing microvascular dogma in the migraine literature**.** (A) Focal brain parenchymal changes in energy demand triggers microvascular vasodilation, lowering resistance and increasing capillary blood flow (provision of energy substrate). Capillary dilation is mediated through intrinsic pathway(s), including direct astrocytic modulation of pericytes, and astrocytic and neuronal innervation of endothelial cells to stimulate endothelial nitric-oxide synthase (eNOS), resulting in upregulation of synthesis of the potent vasodilator NO. Parenchymal changes in microvascular flow may be several-fold in response to regional energy demand; however, net cerebral blood flow remains essentially unchanged. "Regional" demand may result in upstream vasodilation of the arterioles, in order to*

adequately supply larger regions of tissue during periods of heightened energy demand, and to maintain hydrostatic arterial pressure. (B) Exaggerated sympathetic tone results in peripheral vasoconstriction—for example, in the head and neck muscles. Vasomotor sympathetic-induced peripheral vasoconstriction activates baroreceptors in central large arteries, by activating eNOS and stimulating production of NO. Exaggerated NO stimulates nociceptors and induces cortical spreading depression via K^+ release, causing migraine aura. NOS are purported to alleviate headache pain by inhibiting attenuation of vasodilation. GTN: glyceryl trinitrate.

The prevailing dogma of vascular tone in the aetiology of migraine is illustrated in Fig. 2B. Endocrine/stress pathways are thought to progressively induce peripheral vasoconstriction. Migraine patients have been reported to have exaggerated muscle-nerve sympathetic response, and to show heightened vasoconstriction during muscle contractions (37). Peripheral microvascular vasoconstriction activates baroreceptors in central large arteries, activating endothelial nitric-oxide synthase (eNOS) and stimulating production of NO. The heightened release of NO and/or vasodilation, *per se*, stimulates nociceptors and induces cortical spreading depression via K^+ release, causing migraine aura in many subjects. Consistent with the indicated hypothesis, NOS inhibitors are found to alleviate headache pain, presumably as a consequence of inhibiting vascular dilation.

We present an alternate hypothesis with respect to purported cerebrovascular changes in the aetiology of migraines. The hypothesis reconciles the vascular paradoxes described. We propose that as a consequence of stress-induced heightened sympathetic tone, or as a consequence of endocrine mediators (such as depletion of plasma oestrogen during menorrhoea), microvascular blood flow is compromised, not only in peripheral vascular beds but also within the central microvasculature (Fig. 3). Suboptimal central parenchymal blood flow, or indeed pseudo-hypoxia, may activate cranial nociceptors and cause headache. The acute intravenous provision of exogenous NO donors, such as GTN, will promote upstream central larger artery dilation (Fig. 3). To respond to upstream vasodilation and critically maintain parenchymal perfusion pressure within the central microvasculature, capillaries must further constrict. The microvascular response of constriction would exacerbate the pre-existing suboptimal parenchymal flow in regional areas of high energy demand, effectively amplifying nociceptor activation and pain intensity (38). The acute provision of NOS inhibitors to treat migraine will result in central large-artery constriction, concomitant with increased heart rate. In response, downstream parenchymal microvessels would need to vasodilate in order to maintain perfusion pressure within an optimal range. The latter would restore microvascular blood flow. The restoration of flow provides nutrients and oxygen, and there is alleviation of pain.

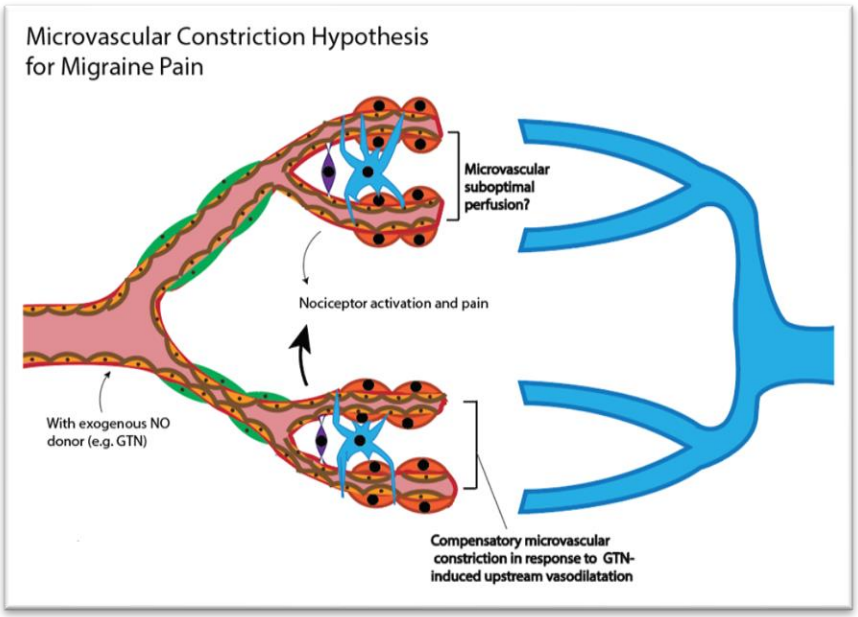

Fig. 3. *Alternate hypothesis for vascular changes in migraine. Stress-induced heightened sympathetic tone results in a chronically constrictive microvascular phenotype, both centrally and within the periphery. Suboptimal central parenchymal blood flow (potential hypoxia) could activate nociceptors and cause headache pain onset. Traditional NO donors (such as GTN) principally modulate upstream larger central arteries, and would only exacerbate downstream vasoconstriction. Provision of eNOS inhibitors will reduce blood flow to the brain through larger arteries, requiring compensatory downstream dilation of capillaries. The latter restores the provision of nutrients and oxygen.*

In contrast to NO donors such as GTN, it is established that lowering blood oxygen saturation, by inhaling air enriched in carbon dioxide, will immediately result in global vasodilation of both the peripheral and the central microvasculature (Fig. 3). Our contention is that restoring central capillary perfusion, via carbon-dioxide treatment protocols, would attenuate activation of nociceptors caused by insufficient perfusion of blood.

A NUTRACEUTICAL APPROACH TO PROMOTE MICROVASCULAR DILATION AND ATTENUATE RISK FOR MIGRAINE ONSET AND SEVERITY

In contemporary times, with greater accessibility and affordability of non-prescription and prescription medicines, potential overuse of triptans and nonsteroidal anti-inflammatory drugs (NSAIDs) is regrettably increasingly reported (11, 12). The potential for significant contraindications and/or adverse effects of drugs used to treat migraines is therefore a significant driver to consider therapeutic opportunities with fewer potential adverse side effects. In the context of a cerebrovascular axis

approach to treat migraine, there is an accumulating body of evidence that the nutraceutical agents L-arginine (2-amino-5-guanidinopentanoic acid, arginine) and aged garlic extract (AGE) may be particularly worthy of consideration.

L-arginine is a precursor for the vasodilator NO (39), and studies have shown an association between vascular tone and plasma concentrations of L-arginine (40). In physiological conditions, the L-arginine molecule consists of a protonated α-amino group, a deprotonated α-carboxylic acid group, a 3-carbon aliphatic straight side chain, and ends with a protonated guanidino group (33). NO production by vascular endothelial cells is enhanced by L-arginine, as NO is formed from the terminal guanidino-N atoms in L-arginine (33). This involves two steps: mobilisation of L-arginine and conversion to NO—both of which are activated by bradykinin and the calcium ionophore, A23187 (33). In particular, it is the extracellular L-arginine which induces endothelial NO synthesis, not intracellular abundance.

Aged garlic extract (AGE, Kyolic, *Allium sativum*) is a capillary inflammation inhibitor (41) with the relevant active component, S-allyl-cysteine ((R)-2-amino-3-prop-2-enylsulfanylpropanoic acid; S-2-propenyl-L-cysteine; S-allyl-laevo-cysteine; S-allylcysteine) (42), which comprises the amino acid, cysteine, with an allyl group attached to its sulphur atom. AGE is prepared by extracting sliced raw garlic and storing it in 15–20% ethanol at room temperature for 20 months, increasing antioxidant activity (42). There are ~1000 micrograms per gram of S-allyl-cysteine in AGE, but only 20 micrograms per gram of S-allyl-cysteine in raw garlic (43). AGE enhances NO synthesis (44), promoting vasodilation (45); however, indirect positive effects on vessel diameter have been proposed because of its potent antioxidant, antithrombotic and antiatherosclerotic effects (46). Despite AGE demonstrating antiplatelet effects, there has been no increased bleeding risk in patients concurrently taking blood-thinning medication (47).

VASODILATORY EFFECTS OF L-ARGININE

Animal studies were reviewed to ascertain reported vascular effects of L-arginine. We present studies from 2009 to 2019 in Appendix 1. To determine whether these effects extended to humans, we then conducted a search of completed clinical studies from 2009 to 2019 using L-arginine (Appendix 2). Similar effects were noted. Moreover, only mild gastrointestinal effects were reported in humans, and those participants dropped out in only two studies (48, 49).

L-arginine directly induces vasodilation. Of note, Kharazmi et al. (2015) reported that a 0.01 M L-arginine infusion directly into a rat mesenteric vessel increased local vasodilation (50). Closer to the cerebrum, microdosing 30 µL of 1 mM L-arginine into the vitreous humour of a minipig increased arteriolar diameter (51). In humans who took 1.5 g thrice daily, flow-mediated dilation decreased at a slower rate where L-arginine was replenished (52). With 7 g, flow-mediated dilation and forearm blood flow increased (53, 54).

We used increased blood flow as another measure of vasodilation in humans. Decreased blood flow to brain areas led to strokes, also associated with migraines (55), so it is not surprising that vasodilatory L-arginine also improved stroke-like episodes (56). Some studies measured reactive hyperaemia, which measures vascular dilatory response following occlusion of blood via an inflatable cuff. The surge of blood and acute increase in pressure after a brief period of vessel occlusion activates the vascular endothelium via sheer stress. Reactive hyperaemia increased in 2 studies, after giving 6.4 or 7 g of L-arginine to humans (54, 57). In NO-deficient humans, blood flow or endothelium-mediated dilation could be re-established with 7 g thrice or 8 g twice *per os* (p.o.) L-arginine daily (58, 59).

We expect vasodilation to decrease blood pressure or cerebral microvascular resistance, as there is a greater flow or force of blood passing through a wider area by vessel diameter. Therefore, we looked at the effects of L-arginine on blood pressure and vascular resistance. Giving L-arginine decreased systolic and arterial blood pressures (60-62), and decreased vascular and precapillary resistance in rats (62). In humans, L-arginine lowered both diastolic and systolic blood pressures (63). In fact, there is an inverse correlation between diastolic blood pressure and blood levels of L-arginine (64). In clinical trials, L-arginine improved hypertension (65) via increased NO formation in patients causing vasodilation (39). In terms of dosing, taking 3–3.3 g of L-arginine once or twice per day resulted in fewer pre-eclampsia cases later on in gestation (66, 67). Taking 4 g of L-arginine twice/thrice a day decreased blood pressure (49, 68), and fewer mildly hypertensive women had to be given antihypertensives during pregnancy with this supplementation daily (69).

Studies suggest that measuring blood levels of NO, eNOS and/or cyclic guanosine monophosphate (cGMP) may serve as surrogate markers of systemic vascular dilation (70). Consistent with an exogenous induction of NO, dietary supplementary L-arginine p.o. in rats and rabbits significantly increased plasma NO and cGMP (71), as well as endothelial nitric-oxide synthase (eNOS) (72). A 500 mg/kg intraperitoneal injection (i.p.) of L-arginine increased eNOS expression/phosphorylation and NO production in rats (73). Giving L-arginine intravenously (i.v.) or p.o. to humans increased NO concentration in the bloodstream (74).

Migraine-without-aura headaches increase with exercise (7), suggesting that greater oxygen demand drives nociception. Vasodilation increases oxygen delivery to tissues. Therefore, we would expect a vasodilatory agent, such as L-arginine, to inhibit muscle fatigue. In healthy humans, Pahlavani et al. (2017) found that taking 2 g of L-arginine increased maximal oxygen uptake (48). Taking 0.1 g per kg body weight or 4 g of L-arginine decreased ischaemic pain and treadmill exercise duration, respectively (49, 75). One of these studies safely used L-arginine to alleviate painful vaso-occlusive episodes in sickle-cell disease (75). Indeed, sickle-cell disease itself is thought to derive from limited L-arginine bioavailability inducing NO-resistance (76), reflected by low L-arginine and subsequently NO serum

levels during vaso-occlusion (77). Chen et al. (2010) found that taking 5.2 g of L-arginine increased the anaerobic threshold in men aged 50–73 years old (78). Taking 8 g of L-arginine twice a day also improved pain-free walking distance as well as endothelial vasodilation (40).

We also used vessel elasticity as a proxy for vessel dilation, as the amount a vessel is able to stretch perpendicularly can often determine how much it can dilate vertically. We found that peripheral vascular elasticity was increased when rats were given 300 mg/kg L-arginine (61). However, it seemed that this vasodilation-elasticity association is not always the case. In humans, taking 7 g of L-arginine decreased brachial artery stiffness but increased central aortic stiffness (54), as measured by decreased carotid-femoral pulse-wave velocity (53). Fahs et al. (2009) attributed this finding to increased central arterial load due to exercise, thereby requiring stiffer collagen fibres in the central arterial walls and more elastic elastin in peripheral artery walls (54). Hence, L-arginine still causes dilation of both central and peripheral arteries but, physiologically, central dilation results in decreased vessel elasticity and peripheral dilation results in increased vessel elasticity (54).

In summary, L-arginine is worth investigating as a prophylactic migraine medication, due to its potential role in the NO vasodilatory pathway (79). Doses of 3–8 g per day appear safe in humans (39). However, individual patient response also depends on endogenous asymmetric dimethylarginine (ADMA) levels, as it competes with (and therefore inhibits) NO synthase which synthesises NO from L-arginine (74). In patients with high ADMA, L-arginine seems to restore endothelial function to a vasodilating normal by increasing NO synthesis via NO synthase (74). A study showed a linear correlation between L-arginine:ADMA ratio and pain-free walking distance (40). Upon the addition of L-arginine, the L-arginine:ADMA ratio is normalised (58). As with most disease conditions, earlier treatment has been recommended for better results, measured by changes in vascular function (39). According to our proposed alternate hypothesis, dietary L-arginine should reduce blood pressure and promote tissue perfusion by promoting microvascular dilation via enhanced biosynthesis of endothelial NO. By extension, provision of dietary L-arginine might be an effective strategy for attenuating frequency/severity of vascular-based migraines.

VASODILATORY EFFECTS OF AGED GARLIC EXTRACT

We found no studies that reported on the effects of AGE in the context of migraine. Nonetheless, we first looked at animal studies to ascertain the vascular effects of AGE, and we present studies from the last 11 years (2009–2019) in Appendix 3. To see if these effects extended to humans, we then conducted a search of completed clinical studies during the same period (Appendix 4). We found the following effects.

AGE can promote vasodilation by increasing vasodilatory prostaglandins or imitating the molecule (14). AGE has been hence shown to improve vascular function (increase vasodilation and decrease vasoconstriction) both in humans and rats (80-82). Furthermore, in rats, giving 1.2 mL/kg AGE i.p. decreased the area of a cerebral arterial infarction that would have occurred at 2 h post-infarction induction (83, 84).

Temperature rebound (the temperature increase following arterial occlusion) is another measure of vascular dilatory capacity (85, 86). This is because temperature rebound occurs quicker in non-occluded arteries after arm cuff inflation obstructs blood flow temporarily (85, 86). Temperature rebound was increased in patients who took 0.25 g of AGE once daily, indicating more dilated vessels (80, 87). Larijani et al. (2013) found that asymptomatic men who took 0.3 g of AGE with 30 mg of Coenzyme Q10 every 3 months also had improved temperature rebound after 1 year (88).

As stated above, we expect vasodilation to decrease blood pressure or cerebral microvascular resistance. AGE produced intra-abdominally lowered blood pressure and vascular resistance in rats (82), while daily 0.48–0.96 g of AGE p.o. reduced systolic blood pressure in humans (89). In humans, central blood pressure, central pulse pressure, mean arterial pressure and augmentation pressure improved with 1.2 g of AGE p.o. once daily (47, 90). Ried et al. (2013) found that AGE inhibited hypertension dose-dependently in humans (91).

Again, we used biochemical markers in the bloodstream to reflect smooth muscle cell vasodilatory action. In rats, AGE increased heart NO, citrulline (an eNOS by-product) and NOx (nitrite and nitrate) (82). In humans, AGE can cause vasodilatory effects directly, as it dose-dependently inhibits vasoconstrictive endothelin-1, also controlled by NO levels (92). Homocysteine is known to cause endothelial dysfunction directly by stimulating endothelial inflammation and by inhibiting vasodilatory NO synthesis (93). AGE has also been shown to inhibit homocysteine (87).

As before, we used vessel elasticity as a proxy for vessel dilation. Breithaupt-Grogler et al. (1997) found that chronic intake of garlic powder slowed age-related aortic stiffness, suggesting that garlic *per se* protects vessel wall elasticity (94). This can be measured via carotid-radial pulse-wave velocity (distance of pulse wave travelled from carotid to radial artery divided by time taken for that travel) (88). Other studies showed that pulse-wave velocity and arterial stiffness decreased in people who took 0.3–1.2 g of AGE daily, measured by aortic pulse-wave velocity, calculated from the brachial and radial blood pressures (47, 88, 90). Since the pulse wave travelled the same distance in more time, this result suggested greater vessel elasticity. So slower pulse-wave velocity corresponds with lower blood pressure and increased vascular elasticity.

In summary, the body of evidence suggests that AGE could be a suitable candidate for vascular-induced migraine therapy; however, this would need to be assessed, preferably through randomised controlled

trials. As seen here, garlic extract has been investigated widely as potential treatments for cardiovascular diseases (95). Moreover, garlic-derived therapeutics have minimal adverse effects, with the major ones being limited to halitosis and bromhidrosis (96). Halitosis applies when the raw form of garlic is used (97), whereas AGE is odourless (42). Potential side effects of AGE include nausea and vomiting (97). Due to its fibrinolytic property, bleeding events can potentially occur (98), so its dose has to be monitored (99). Only 3 studies mentioned adverse side effects of AGE (47, 89, 91). However, these gastrointestinal symptoms were minor, short-lived and readily managed by the subjects (47, 89-91). One study even reported improved digestion (47). According to our proposed alternate hypothesis, dietary AGE should reduce blood pressure and promote tissue perfusion by promoting microvascular dilation by inhibiting vasoconstrictive factors. By extension, provision of dietary AGE might be an effective strategy for attenuating frequency/severity of migraines.

PHARMACOLOGICAL TREATMENT OF MIGRAINES BY MODIFYING CEREBROVASCULAR TONE

The reinterpretation of literature and presented hypothesis suggest that preventing or attenuating chronic microvascular constriction may reduce the frequency or severity of frequent episodic or chronic migraines. We are not suggesting this approach in the context of acute treatment. Of the currently available pharmacotherapies, no agents are generated on the basis of the alternate hypothesis provided. However, some do have vasomodulatory effects. Pharmacotherapies used to treat migraines are reviewed elsewhere (100), but we have included a synoptic table of currently used common approaches to treat migraines, shown in Appendix 5.

Of the agents routinely used to treat migraines, and in some instances tension headaches, the mechanisms of action are not fully established (101, 102). Nonetheless, there are nine classes of agents with vasodilatory properties, and three drug classes reporting both vasodilatory and vasoconstrictive effects. In addition, five classes of drugs serve as vasoconstrictors. It is beyond the scope of this hypothesis-generating review to consider each individual agent; suffice to say that there are no robust studies available that explore microvascular perfusion, central to the hypothesis proposition presented.

Collectively, the primary mechanism by which preventative migraine pharmacotherapies elicit vasomodulating effects is principally by regulating serotonin or noradrenaline pathways, calcitonin gene-related peptide (CGRP), calcium-channel blockers, anticonvulsants and membrane stabilisers. Serotoninergic receptors tend to predominate in the cerebrovasculature and noradrenergic receptors in the peripheral vasculature, suggesting ordinarily a central physiological predisposition/sensitivity to a vasodilatory phenotype (103).

In some instances, medication overuse—particularly of the nonsteroidal anti-inflammatory drugs (NSAIDs) and triptans (11, 12)—can paradoxically exacerbate migraine frequency and severity.

Thorlund et al. (2016) hypothesised that repeated medication use increases neuronal plasticity, in turn causing a lower threshold to migraine headache progression (11). In addition, vasoconstrictors such as triptans cannot be used for patients with disorders involving restricted blood supply to tissues, such as cardiovascular disorders (104). These include ischaemic heart disease, stroke, hypertension and hemiplegic/basilar migraine (105). Collectively, there are many potential adverse effects of these commonly prescribed synthetic drugs to treat migraines (13), and the cardiovascular contraindications may be a significant shortcoming.

CONCLUSIONS

Given the positive action on vasodilation exerted by L-arginine and AGE in the above cardiovascular studies, we surmise that these agents should be investigated in future randomised controlled trials for prevention of migraines induced by cerebral microvascular constriction. To assess and confirm regional vasoconstriction of cerebral arterioles and capillaries, we recommend imaging of the cerebral microvasculature directly using functional imaging techniques (36). L-arginine is also present as a minor component in AGE (106). Although new treatments and preventions are being explored for migraine treatment (107), L-arginine and AGE have not been clinically considered. One limitation is that presently, no studies investigated the effects of L-arginine and AGE in the context of gender or ageing. These variables are established risk factors for headaches, concomitant with decreased endogenous biosynthesis of NO. In this review, we did not address the effects of L-arginine and AGE on inflammation or oxidative damage, lipids or thrombotic activity, which can also affect vessel diameter. However, all studies showed an alleviating effect of both L-arginine and AGE in all 5 aspects: decreased inflammation and oxidative damage markers, decreased triglycerides and low-density lipoproteins, increased high-density lipoproteins, decreased atherosclerosis and decreased thrombotic events. The fact that neither L-arginine nor AGE have been tried in randomised controlled trials, specifically for the prevention of migraine, is a current shortcoming. Reported well-tolerated doses of L-arginine and AGE, considered for cardiovascular disease risk, serve as a useful guide to explore the putative efficacy of L-arginine and AGE for prevention/treatment of vascular-associated migraine.

AUTHOR CONTRIBUTIONS

Conceptualisation, JCLM; methodology, DRC; investigation, DRC; writing—original draft preparation, DRC; writing—review and editing, JCLM and MV; visualisation, JCLM and DRC; supervision, JCLM, MV, RT, VL, EV and PD; project administration, JCLM. All authors have read and agreed to the published version of the manuscript.

CHAPTER 2: CASE STUDY

The content of this chapter is covered by published Paper 2:

Chaliha DR, Vaccarezza M, Lam V, Takechi R, Mamo JC. Attenuation of chronic tension headache frequency and severity with daily L-arginine and aged garlic extract dietary supplementation. Eur J Clin Nutr. 2022 Feb;76(2):317-319.

PAPER 2

The novel hypothesis of a vasoconstrictive hypothesis for the aetiology of migraine and potential therapeutic potential of L-arginine + AGE was tested in a case study, herein presented as published Paper 2.

ATTENUATION OF CHRONIC TENSION HEADACHE FREQUENCY AND SEVERITY WITH DAILY L-ARGININE AND AGED GARLIC EXTRACT DIETARY SUPPLEMENTATION

ABSTRACT

A 74-year-old female subject with suboptimal management of episodic tension headache was treated with a daily dose of 1.5 g L-arginine and 1.2 g aged garlic extract (AGE). The aim of the intervention was to promote vasodilation of parenchymal cerebral blood vessels. Within 6 weeks of commencing treatment, her self-reported symptoms improved markedly and were sustained at 2 years following commencement. We propose that the putative beneficial effect of L-arginine and AGE in this patient is because of the well-established systemic vasodilatory effects of L-arginine and aged garlic extract. On the hypothesis that migraine is precipitated by cerebral microvascular constriction, we recommend a double-blind randomised controlled trial to clinically test this hypothesis in migraine patients.

BACKGROUND

Herein we describe a 74-year-old female patient, with modest hypercholesterolemia and adult-onset anxiety, who presented with a 20-year history of a tension headache syndrome. We report that a sustained attenuation in frequency and severity occurred, following commencement of dietary supplementation with L-arginine and aged garlic extract.

PATIENT

The patient is of a Maltese background, with a BMI of 26.4, height 164.6 cm and weight 72 kg. She was diagnosed with hyperthyroidism (Graves' disease) at 54 years of age, which was well-controlled with carbimazole.

PATHOPHYSIOLOGY

The commencement of chronic tension headaches started at onset of menopause, which was 20 years ago at 56 years old. The patient described that the post-menopausal headaches were rapid-onset and exacerbated during periods of acute stress. The patient described the experience akin to “being hit hard behind the head”, with spasmodic episodes of “severe and immediate pain around the eye and within the head, the latter particularly behind the eyes.” During onset, the headaches would initially feature a transient sensation of pressure around the forehead, described as “having a tight broad band positioned around the head”. The patient also described that when she became even modestly anxious, she “tensed up” and thereafter, headaches rapidly ensued. This cyclical headache syndrome occurred almost daily, with varying severity and with a self-reported average pain score of 8 on a scale of 1–10, with 10 associated with the most painful headache.

Once a headache was established, the patient described concomitant muscular tightness associated specifically with regions of most pain at forehead and neck, and radiating to shoulders. A headache would typically last for several days. During a headache event, the patient described that she was “able to continue with modest light domestic activities”; however, she explained that the headaches “made it particularly difficult to think, function or to make decisions”. During severe headache events, the patient would sometimes feel nauseous, and she would occasionally also experience vertigo. However, the patient did not describe ever experiencing auras.

IMPACT

Collectively, the patient experienced significant morbidity due to the severity and chronic nature of her headache pain. She found ordinary daily tasks difficult to complete, and consequently, she substantially reduced her participation in social engagement activities. The patient described her headaches as “crippling”, with the overall impact as “spoiling life”.

POSSIBLE AETIOLOGY

The patient has no known family history of migraines. Hyperthyroidism can cause hypertension (108) which can be associated with headache (109); however, this was unlikely in this patient, whose medical records consistently show that she was normotensive. Hyperthyroidism is also known to be associated with increased anxiety (110), and the patient self-reported that anxiety has been a major amplifier for headaches.

HISTORIC TREATMENT REGIMEN

The patient would routinely take non-prescription medications limited to paracetamol, aspirin or ibuprofen at ordinary recommended dosages. The patient said these agents had little or no effect on

headache pain intensity or duration. After experiencing tension headaches for some 16 years, the patient commenced 4 years ago with the anxiolytic, venlafaxine (37.5 mg/day). She reported feeling calmer following commencement of venlafaxine; however, headache frequency was not substantially alleviated. The patient estimated a potential reduction in frequency of ~10% (several episodes persisting, per month). The patient also reported no significant reduction in duration, or intensity of pain when headaches occurred with venlafaxine treatment. Patient headache frequency and severity were essentially unchanged. The patient has no other known metabolic or chronic disorders, and takes no other prescription medications, non-prescribed drugs or supplements on a regular basis.

RATIONALE FOR INTERVENTION

The intervention was based on the hypothesis that tension headaches and migraines may be a consequence of microvascular (capillary) constriction downstream to dilation of larger cerebral arteries (111). A microvascular aetiology for headache pain in this patient was supported by the observation that frequency markedly increased post-menopause. Oestrogen is a potent vasodilator (27), and low levels of oestrogen are associated with headache pain during menorrhea (112). In addition, we found that rapid postural repositioning in our patient could exacerbate headache pain acutely. We also found that the inhalation of expired air, to reduce blood oxygen saturation and induce vasodilation, would also provide transient headache pain relief in our patient. In addition, evidence of photosensitivity, a surrogate marker of cerebral microvascular constriction, was also described by the patient.

The intention of this intervention was to capture the potential microvascular dilation properties of L-arginine as a precursor substrate for synthesis of capillary endothelial nitric-oxide synthase. Nitric oxide is a potent vasodilator of capillary vasculature. Aged garlic extract, a stable homogeneous extract rich in S-allyl-cysteine, may promote microvascular dilation as a consequence of increasing or imitating vasodilatory prostaglandins (113). Several studies suggest it has potent anti-inflammatory effects on the microvasculature (114).

INTERVENTION

The patient commenced 2 years ago with daily dietary supplement intervention of L-arginine (1500 mg/day) (2 × 500 mg capsules (iHerb™) morning and 1 × 500 mg evening) and aged garlic extract (Kyolic™, 600 mg B.I.D.), both taken with meals.

PUTATIVE EFFECT OF INTERVENTION

Following commencement of supplementation with L-arginine and aged garlic extract, the patient self-reported a progressive attenuation of headache severity and duration, approximately 6 weeks

following commencement of intervention. The headaches became less frequent, and after 2 years of intervention, the patient reported a reduction of ~70% compared to pre-treatment frequency. The patient also reported that the average pain intensity score was also significantly reduced to a headache pain of score 4–5, compared to 8 prior to treatment.

OUTCOME FOR PATIENT

The marked improvement experienced by the patient provided the patient with increasing confidence to explore additional allied health strategies to reduce tension and anxiety. There were no other markedly different changes in the patient's living environment following commencement of the intervention with L-arginine and aged garlic extract, that she could identify, which might explain the change in headache experience. The patient described how she had been able to resume gardening, housework and personal hobbies. Overall, she feels the intervention was associated with "more than a 70% improvement" in her lifestyle.

SAFETY AND TOLERABILITY

There are no significant adverse events indicated for L-arginine, with some studies exploring efficacy of up to 8 g/day on blood flow parameters on both healthy subjects and those with vascular disorders (58). However, L-arginine at higher doses can lower systolic and diastolic blood pressure, which needs to be considered, particularly in ageing subjects prone to hypotension. Aged garlic extract is also ordinarily exceedingly well-tolerated with few adverse events reported. However, AGE is an antithrombotic (115), and whilst this was not specifically investigated in this patient, it would need further consideration. The patient reported no changes in wound-healing.

CONCLUSION

The case study report described is consistent with the hypothesis that the proven systemic dilatory effects of L-arginine and aged garlic extract could potentially serve as a prophylactic to attenuate tension-headache frequency and severity. However, the observational case hypothesis detailed here needs to be appropriately tested through a blinded randomised controlled trial.

AUTHOR CONTRIBUTIONS

DC wrote the primary manuscript; JM facilitated the interview and edited the manuscript; MV oversaw the development of, and edited, the manuscript; and RT and VL co-supervised the PhD candidacy and edited the manuscript.

CHAPTER 3: PROTOCOL

The content of this chapter is covered by published Paper 3:

Chaliha DR, Vaccarezza M, Corti E et al. L-arginine and aged garlic extract for the prevention of migraine: a study protocol for a randomised, double-blind, placebo-controlled, phase-II trial (LARGE trial). BMC Neurol 23, 122 (2023).

PAPER 3

The hypothesis that L-arginine in combination with AGE would be effective in reducing frequency and severity of headache pain in chronic migraines was tested in a double-blind randomised controlled trial (ACTRN12621001476820).

L-ARGININE AND AGED GARLIC EXTRACT FOR THE PREVENTION OF MIGRAINE: A STUDY PROTOCOL FOR A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE-II TRIAL (LARGE TRIAL)

ABSTRACT

Background: Migraine is a common and distressing neurological condition characterised by recurrent throbbing headaches, nausea and heightened sensitivity to light and sound. Accumulating evidence suggests that cerebral arteries dilate during migraine, causing distal microvessels to constrict, which could activate nociceptors and cause onset of headache pain. If so, preventing or attenuating chronic microvascular constriction, and promoting a dilatory phenotype, may reduce frequency and/or severity of migraines. The primary aim of the L-Arginine and Aged Garlic Extract (LARGE) trial is to investigate whether oral treatment with dietary nutraceuticals, L-arginine and aged garlic extract (AGE), both systemic vasodilatory agents, will alleviate migraine frequency, duration and severity in adults with chronic frequent episodic migraines.

Methods: The study is a randomised double-blind placebo-controlled phase-II single-site clinical trial conducted in Perth, Australia. The target sample is to recruit 240 participants diagnosed with chronic frequent episodic migraines between 18 and 80 years of age. Participants will be randomised to one of four treatment groups for 14 weeks (placebo induction for 2 weeks, followed by 12 weeks on one of the respective treatment arms): placebo, L-arginine, AGE, or a combination of L-arginine and AGE. The doses of L-arginine and AGE are 1.5 g and 1 g daily, respectively. The primary outcome is to assess migraine response using change in migraine frequency and intensity between baseline and 12 weeks. Secondary outcomes include the impact of L-arginine and/or AGE on photosensitivity, retinal vessel changes and blood biomarker concentrations of vascular tone, following a 12-week intervention.

Discussion: The protocol describes the oral administration of 2 nutraceutical-based interventions as possible prophylactic treatments for chronic frequent episodic migraines, with potential for direct clinical translation of outcomes. Potential limitations of the study include the fixed-dose design of each treatment arm and that *in vivo* neuroimaging methods, such as magnetic resonance imaging (MRI), will not be conducted to determine putative cerebrovasodilatory changes to coincide with the outcome measures. Dose-response studies may be indicated.

Trial registration: The trial was retrospectively registered with the Australia and New Zealand Clinical Trials Registry ACTRN12621001476820 (Universal Trial Number: U1111-1268-1117) on 04/08/2021. This is protocol version 1, submitted on 25/11/2022.

Keywords: aged garlic extract; L-arginine; chronic frequent episodic migraine; photosensitivity; randomised controlled trial; retinal imaging; vascular tone

BACKGROUND

Migraine is one of the most common and disabling neurovascular disorders, affecting more than one billion people worldwide (116). Considered the third most costly neurological disorder to treat, the socio-economic impacts of migraines are substantial (1, 2). The understanding of the pathophysiology and aetiology of migraines has increased substantially over the past few decades, with emergence of new therapeutic approaches. However, appropriate access and use of current therapeutic options are scant, as migraine treatments are often related to adverse effects, and are unavailable to many patients due to strict prescribing guidelines (117-119).

The prevailing dogma of migraine suggests that vasodilation of large cerebral arteries contributes to pain associated with migraines. We provide an alternate hypothesis for the pathophysiology of vascular-based migraines, whereby stress-induced heightened sympathetic tone results in a chronically constrictive cerebral microvascular phenotype (111). Reduced central parenchymal blood flow could then activate nociceptors and cause headache onset. Thus, we put forward the hypothesis that prevention or attenuation of chronic microvascular constriction reduces the frequency or severity of vascular-mediated headaches. There are some limitations with current migraine medications, including medication overuse that can paradoxically induce headache (11, 12), a vasoconstrictive effect which can be a contraindication for patients with cardiovascular complications (104, 120, 121), subcutaneous administration which may feel invasive to some individuals, high costs and regulatory restrictions (120, 122). Accordingly, benign vasodilating alternatives to prevent and treat patients with vascular-induced headaches need to be urgently considered.

L-arginine (2-amino-5-guanidinopentanoic acid, arginine) and aged garlic extract (AGE) are two oral nutraceutical agents that have been used to improve several cardiovascular disease risk factors via vasodilatory mechanisms (46, 113, 123-128). However, neither have been investigated as a prophylactic option to treat chronic frequent episodic migraines. Briefly, through enhanced biosynthesis of endothelial nitric oxide (L-arginine) or increasing systemic vasodilatory prostaglandins

(AGE) amongst other anti-inflammatory and antioxidant properties, L-arginine and AGE are likely to be efficacious in attenuating migraine severity/frequency induced by cerebral microvascular constriction. A significant advantage of these two nutraceuticals over current conventional migraine drug treatments is that the safety profile of both agents has been widely documented and well-tolerated in the context of cardiovascular indications with minimal side effects. McNeal et al. looked at the safety and effectiveness of L-arginine in adults, concluding that up to ~20 g/day should be safe in most adults including in pregnancy (129). Much ado has been made on AGE's antiplatelet effects, but in fact, Macan et al. used oral warfarin therapy to assess this, and found no evidence of increased haemorrhage in patients on 5 ml twice a day for 12 weeks of AGE – nor were there any adverse-effect differences between the placebo and AGE groups in their study (99). L-arginine has been clinically indicated for various pathological diseases (130), and AGE's antioxidant and immunomodulatory effects have been noted for various disorders like dementia, hypertension and even cancer (42, 131, 132).

Here, we detail the study protocol for the 'L-Arginine and Aged Garlic Extract (LARGE) for the Prevention of Migraine' trial. The study is a 12-week, phase-II, single-site, randomised, double-blind, parallel-group-design, placebo-controlled trial, investigating the efficacy of oral L-arginine, AGE or a combination of these nutraceuticals in adults with chronic frequent episodic migraines. Key outcome measures include frequency of migraines attacks and intensity of headache pain. Additional exploratory outcome measures include changes in photosensitivity, retinal vascular diameter and blood biomarkers of vascular tone.

OBJECTIVES

We are trying L-arginine and AGE for the first time for headaches, based on our proposed underlying mechanism and these nutraceuticals' vascular applications in the past. The rationale for this study is that migraine may be caused by sympathetically induced and/or compensatory-to-vasodilation capillary constriction, leading to insufficient blood flow in the brain relative to increased energy demands in times of stress, and the triggering of trigeminal nerve endings via large-vessel dilation. We are investigating whether the known capillary-dilating agents, L-arginine and/or aged garlic extract, prevent the capillary constriction we propose underlies the headache. Therefore, our primary measure will be alleviation in migraine frequency and/or severity via self-reported subjective questionnaires; and our secondary measures will determine expansion of brain capillaries via blood biomarkers of systemic vascular tone and via retinal optical coherence tomography angiography. An additional secondary measure will be a photosensitive test using a brightening light source, to determine difference in this major migraine symptom, also before and after treatment.

The primary objective of this study is to evaluate the impact of oral 1.5 g/day L-arginine, 1 g/day AGE, or a combination of the two nutraceutical doses, on reducing migraine frequency and severity, compared with placebo, in adults with chronic frequent episodic migraines, over a 12-week

intervention period. The secondary objectives are to measure the independent and combined effects of each intervention on physiological vasodilation in the retinal vessels, onset of photosensitivity, blood biochemical markers of vascular tone, and to evaluate the impact on quality of life, in adults with chronic frequent episodic migraines.

METHODS/DESIGN

The methods reporting of this study follow the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials Statement (18).

TRIAL DESIGN

This is a single-site, randomised, double-blind, parallel-group-design, placebo-controlled, phase-II clinical trial on the efficacy, safety and tolerability of oral L-arginine and/or AGE in 18-80-year-old individuals with chronic frequent episodic migraines, based in Perth, Western Australia (WA). Study participants and members of the research team will be blinded to randomisation of the intervention treatments.

The maximum study duration is 14 weeks with a 2-week placebo run-in followed by a treatment period of 12 weeks. Eligible participants will be randomised to one of four parallel treatment groups in a 1:1:1:1 ratio, using permuted block randomisation: placebo, L-arginine, AGE, and combined L-arginine and AGE. Factoring for up to 20% drop-out rate, we aim to recruit 240 participants with up to 60 individuals randomised to each of the four treatment groups. Fig. 1 shows an overview of the study design for the LARGE trial. The trial is registered through the Australia and New Zealand Clinical Trials Registry (ACTRN12621001476820).

STUDY SETTING AND RECRUITMENT

The single-site trial will be based in Perth (WA). Study visits will be completed at the Sarich Neuroscience Research Institute (SNRI), Nedlands (WA), with retinal imaging conducted at the Lions Eye Institute (LEI), Nedlands (WA) (adjacent to SNRI). To reach the targeted sample size, potential participants will be recruited through advertising and editorials on social media, print, radio and/or television; correspondence and promotions via not-for-profit organisations and research partners; and website content.

Prospective participants will be informed that participation is voluntary, and that withdrawal of consent is possible at any time without having to provide justification. All relevant information will be disclosed during the consent procedure with the opportunity to clarify questions with the investigator team. Enrolment is only possible after written informed consent is obtained (see Appendix 8 for Participant Information Sheet).

ELIGIBILITY CRITERIA

The eligibility criteria for the participants in this study are listed in Appendix 6 below. The inclusion

criteria must be fulfilled, and the exclusion criteria must not apply. Eligibility will be verified by delegated research personnel at the inclusion/consent visit.

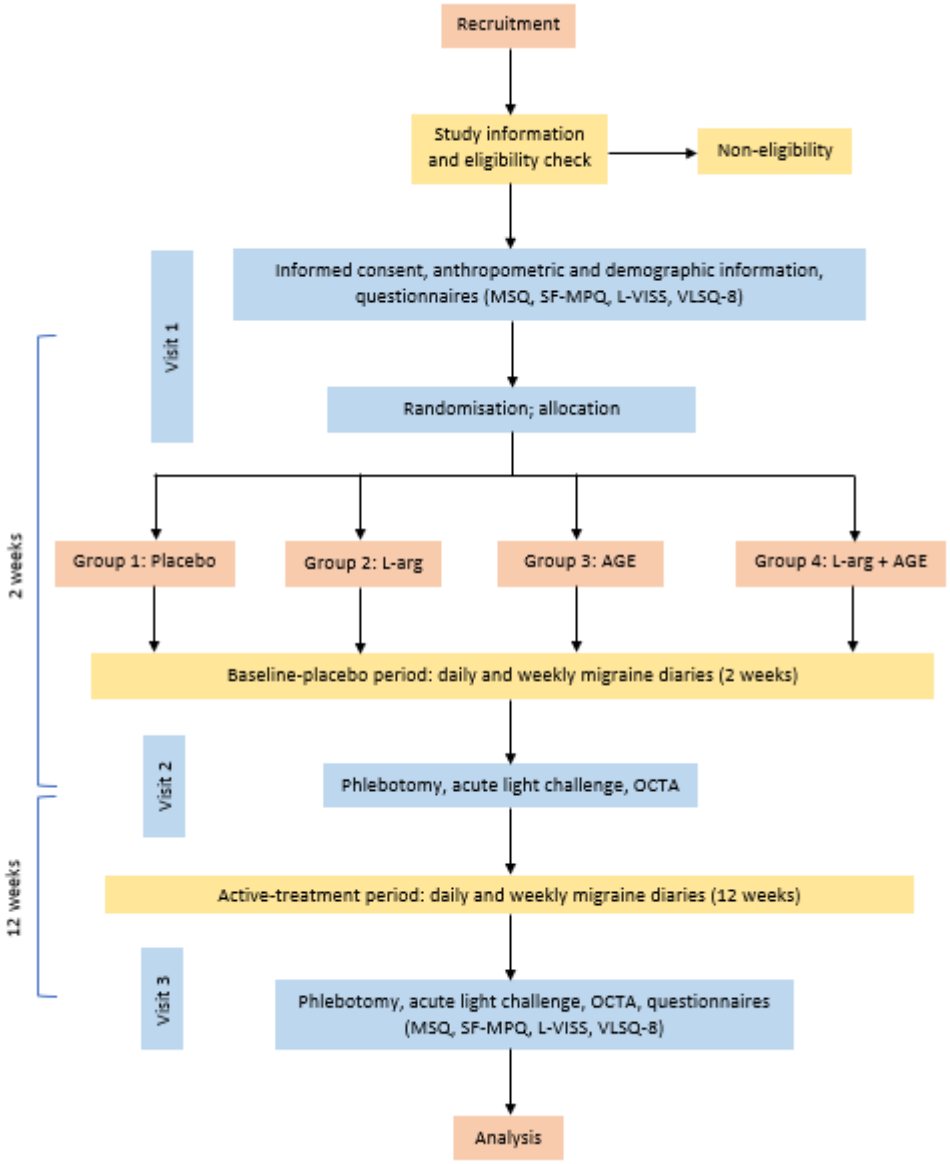

Fig. 1. *Overview of flow of participants. L-arg: L-arginine; AGE: aged garlic extract; OCTA: optical coherence tomography angiography. Participant self-report long-term questionnaires consist of the Migraine-Specific Quality-of-Life Questionnaire (MSQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Leiden Visual Sensitivity Scale (L-VISS) and Visual Light-Sensitivity Questionnaire – 8 (VLSQ-8). Migraine diaries are completed daily and weekly over the 2 and 12 weeks between visits.*

STUDY INTERVENTION

INTERVENTION DESCRIPTION AND ADHERENCE

This study consists of 2 phases, including: (a) a 2-week placebo run-in phase (week -2 to 0); and (b) a 12-week treatment phase (day 0 to day 84). The Diener and Tassorelli guidelines suggest that a 4-8-

week screening phase is ideal to ensure diagnostic migraine criteria are met for both episodic and chronic migraines (133, 134). However, since participants could benefit from 3 months' treatment without a high attrition rate, 4-8 weeks' placebo would already take up half the investigation time for each participant. We are relying on participant numbers to compensate to power the study, and we have already ensured diagnostic criteria are met, as all participants must have confirmation from a physician or neurologist that they have migraine. We have included the extensive list of eligibility criteria, including frequency of migraines participants must have to be included in the trial. Participants are asked to report any adverse effects that arise from the treatment, at absolutely any point during their time in the trial. They are contacted one week after each of Visits 1 and 2 to reinforce this and to check treatment compliance. Leftover capsules are counted at the end of each participant's 2 and 12 weeks, to check placebo and verum treatment compliance, respectively.

Once individuals are deemed eligible according to the inclusion and exclusion criteria listed in Appendix 7, participants will be asked to provide written consent and will be enrolled into the trial. At the consent visit (Visit 1), participants will be asked to provide basic demographic information, complete medical and migraine history (i.e. duration of migraine, age of onset, monthly migraine frequency), current medication, menstrual status (if applicable) and medical history. Baseline demographic and anthropometric measures were adapted from the National Institute of Neurological Disorders and Stroke Common Data Elements (www.commondataelements.ninds.nih.gov) and the PhenX toolkit (https://www.phenxtoolkit.org/), respectively. During this visit, participants will be instructed on how to fill out the daily and weekly migraine diaries. All participants are required to undergo a 2-week placebo run-in and are required to take oral placebo capsules for 2 weeks (5 capsules per day). Thereafter, at Visit 2 (baseline), a 12-week supply of the respective randomised treatment will be dispensed to each participant; 5 capsules a day for 12 weeks (placebo; L-arginine; AGE; combination of L-arginine and AGE). Participants are contacted one week following commencement of treatment, to discuss any potential adverse events and treatment compliance.

Each participant will be provided with a paper-based diary to improve adherence of all groups (see Appendices 9 and 10). Treatment efficacy in terms of headache pain, severity and frequency will be evaluated by a standardised daily migraine diary (Appendix 9) and a summarising weekly migraine diary to track clearer long-term progress (Appendix 10). A capsule information sheet is dispensed with the intervention capsules to provide details instructions on missed doses where relevant. Similar to the placebo run-in, participants will be required to return capsule bottles at the end of the 12-week intervention to determine adherence to treatment. Poorly compliant participants will be excluded from subsequent analyses.

Visit 3 will be an exact replica of Visit 2, with the difference that no further capsules/diaries will be given, and the Visit-1 long-term questionnaires will have been filled out again to compare with the results of the Visit-1 ones. Blood biomarkers, photosensitivity tests and retinal scans will be compared between Visits 2 and 3 to test differences due to treatment over the 12 weeks.

SUPPLY OF INTERVENTION

Trial interventions (including placebo) are provided as identical capsules bottled in participant-ID-labelled containers by a GMP-certified compounding pharmacy, Oxford Compounding (North Perth, Australia). The placebo capsules are comprised of microcrystalline cellulose with no active ingredients. No dose modification of the trial intervention is permitted. All capsules are lightly scented with garlic to avoid potential bias.

DISCONTINUING OR MODIFYING ALLOCATED INTERVENTIONS

If a participant decides to withdraw from the project, participants are asked to notify a member of the research team. If a participant withdraws consent during the research project, relevant project team members will not collect additional personal information from the participant. However, personal information already collected will be retained to ensure that the results of the research project can be assessed and to comply with the law. Participants will be made aware that data collected by the investigator team up to the time the participant withdraws will form part of the research project results.

RELEVANT CONCOMITANT CARE DURING THE TRIAL

Apart from the medications in the exclusion criteria above, participants will be able to continue their regular medically prescribed regimen. Participants will be encouraged to inform their general practitioners (GPs) that they are participating in this study. Participants should not partake in other clinical trials while participating in this study.

STUDY PROCEDURES

Appendix 7, schedule of assessments, details the overall schedule of the trial.

PRIMARY AND SECONDARY OUTCOMES

PRIMARY OUTCOMES

The primary objective of the LARGE trial is to evaluate the effectiveness of oral supplementation of L-arginine, AGE or the combined treatment of oral L-arginine and AGE, in reducing the severity and frequency of migraine attacks in chronic frequent episodic migraines in adults. By chronic frequent episodic migraineurs, we include both episodic and chronic, as well as uncertain: from published official guidelines, some clinical trial participants may not fit neatly into having episodic or chronic migraines, and/or into with or without aura, but they are nonetheless included (17). In accordance with the International Headache Society guidelines for RCTs in preventative treatment of episodic migraines in adults (133), the differences in the mean number of migraine episodes per month, between baseline (Visit 2) and end of study (EoS; 12 weeks treatment, Visit 3), will be determined as the primary outcome measure assessed by a standardised daily migraine diary (Appendix 9). Migraine

frequency will be measured not just within each migraine day, but the number of migraine occurrence days over the weeks. From the daily migraine diaries, treatment efficacy will be evaluated by the mean change in outcome measures between baseline and EoS within each group, and thereafter compared with changes between each intervention group. Primary outcomes collated from the diaries include: (a) mean number of migraine episodes per month (migraine frequency); (b) mean number of migraine days per month; (c) mean number of moderate-or-severe migraine days per month; (d) mean total duration of migraines per month (hours); (e) mean number of acute migraine medication doses per month; and (f) number and type of self-reported adverse events per month. We will not measure migraine episode duration, because the participant is able to use an abortive drug at any point, and sleep intervals can disrupt countability of each episode.

SECONDARY OUTCOMES

Secondary outcomes will comprise the changes in acute photosensitivity, quality of life (QoL), retinal vessel changes, and blood biomarkers of vascular tone, from baseline to EoS.

To explore the effects of each intervention arm relative to placebo on photosensitivity, a visual discomfort threshold will be determined via an acute light challenge on the second and third visits. Briefly, adapted from Woodhouse and Drummond (1993) (135), to determine the visual discomfort threshold, participants were subjected to an increased brightness of an LED panel facing them 200 cm away by 1% every 4 seconds. The percentage brightness of the Nanlite Mixpanel 150 RGBWW 150W LED Panel (emission range: 0-11,320 Lux) at which the participant expresses discomfort and requests no further increase in brightness will be recorded at both study visits and compared to determine differences if any, in visual sensitivity.

The self-reported Migraine-Specific Quality-of-Life Questionnaire (version 2.1) (MSQ) (136, 137) and Short-Form McGill Pain Questionnaire (SF-MPQ) (138) will be completed by each participant at baseline and EoS. The MSQ is utilised to assess the change, before and after treatment, in each of the treatment groups in participants' day-to-day functioning about impeding activity, preventing activity, and emotional feelings, which are usually affected by migraines. The SF-MPQ is used to evaluate weekly differences in pain intensity reported by each of the participants. The Leiden Visual Sensitivity Questionnaire (L-VISS) measures the impact of light- and pattern-sensitivity on daily functioning ("visual allodynia") (139), and the Visual Light-Sensitivity Questionnaire (VLSQ-8) measures the presence and severity of photosensitivity symptoms (140).

A number of recent studies have implicated lower vessel densities in the macula and optic nerve, as well as reduced thicknesses of the choroid and retina, in adults with migraines compared to controls without migraines (141-153). Thus, an additional exploratory outcome will comprise the changes in retinal vessel density and the foveal avascular zone area measured via optical coherence tomography angiography (OCTA), with the AngioVue Imaging System (RTVue XR Avanti, Optovue Inc., Fremont, CA,

USA), at baseline and 12-weeks post-intervention, as a surrogate measure of cerebral vascular changes.

Moreover, blood biomarkers of vascular tone will be analysed in samples collected at baseline and EoS. Systemic concentrations of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), nitrates and nitrites, and endothelin-1, will be analysed in fasted plasma samples. Among our proposed panel of biomarkers, the antagonism between vasodilatory NO and vasoconstrictive endothelin-1 allows the assessment of potential changes in vascular tone (154). Nitrates and nitrites are more stable derivatives of reactive NO in the bloodstream (155), and thus were chosen as a proxy measure of dilation of the vessels supplying the brain. ADMA and SDMA, additionally, would be valuable measures of interictal propensity to migraine, as higher levels of both vasoconstrictive molecules would reflect inhibition of vasodilatory NO production from L-arginine (154).

STATISTICAL ANALYSIS

SAMPLE SIZE AND STATISTICAL POWER

We propose an RCT with a factorial treatment structure. This design is efficient and allows the estimation of both the main effects of L-arginine and AGE, and the interaction between L-arginine and AGE. This design with two factors at 2 and 2 levels has 4 arms/groups (treatment combinations). A total of 240 individuals are required to provide 60 subjects per group. This design achieves 87% power when an F-test is used to test factor L-arginine at a 5% significance level with the effect size of 0.2; achieves 87% power when an F-test is used to test factor AGE at a 5% significance level with the effect size of 0.2; and achieves 87% power when an F-test is used to test the L-arginine*AGE interaction at a 5% significance level with the effect size of 0.2. Cohen's Effect-Size Table (156) provides the interpretation of effect sizes as: Small: 0.1; Medium: 0.25; Large: 0.40. Our research study can therefore detect between small and medium effect sizes between each of the main effects and interactions. An estimated effect size of 0.2, used in the sample size calculation for testing the main effects and interaction, was obtained from our preliminary data. Assuming a dropout rate of 20%, a total of 300 participants will be initially recruited into the study, with 75 participants in each of the 4 treatment combinations.

STATISTICAL METHODS AND EXPLORATORY ANALYSIS

We will analyse the data using IBM SPSS® Statistics for Windows (version 24.0, IBM Corp., Armonk NY, USA) and Stata (version 17, StataCorp, 4905 Lakeway Dr., College Station, TX 77845, USA). P-values less than 0.05 will be considered statistically significant. As the proposed study has 4 treatment arms, multiplicity adjustments will be utilised in the statistical analysis of data. The family-wise error rate is set to be 0.05 to safeguard against the increase in the probability of type-I error due to the number of multiple comparisons.

Continuous data will be reported as a group mean ± standard deviation (SD), and categorical data as count (percentage). Data from continuous variable outcomes will be analysed using an Analyse of Covariance procedure as a General Linear Model, with the baseline values as covariates. Post-hoc comparison of means between the 4 treatment combinations will only be conducted if the interaction between sex and treatment is statistically significant. We will take differences between treatment groups as mean differences with associated 95% confidence intervals. We will analyse categorical outcome variables using a Multivariable Logistic Regression Model, with the baseline values as covariates. Treatment effects will be expressed as odds ratios with associated 95% confidence intervals. Residuals from each of the analyses will be inspected to ensure validity of the statistical procedures utilised. Appropriate graphical displays will be used to demonstrate and communicate the main effects and interactive effects of the treatment groups.

Mixed models, which explicitly account for the correlations between repeated measures within each subject, will be utilised in the statistical analysis of the data. The mixed model will assess the extent to which the subject's trajectory is influenced both by the effect of an intervention and by baseline characteristics. Plots illustrating the outcome trajectories of individual study subjects over time will be used to determine whether the observed data patterns appear consistent with model assumptions.

ALLOCATION

Randomisation occurs on Visit 1, in that the coded capsule bottle is picked up randomly and allocated to this next participant scheduled. One week after this first visit, they are texted to check progress on the capsules. We are using block randomisation: eligible subjects will be computer-randomised in blocks of 8, to one of the 4 treatment groups (placebo control, L-arginine, aged garlic extract (AGE), and L-arginine + AGE). Each participant will be randomised by participant ID, as this should preclude group allocation by order of study entry, as per the IHS guidelines (17, 134). We will do this as the participants enter the trial, whereby the statistician will then have access to the list on the Research Data Capture (REDCap) tool where we will be entering all participant details and responses.

IMPLEMENTATION

The randomisation schedule is sent to an independent pharmacist (Oxford Pharmaceuticals) who will then dispense trial intervention in blank packaging with only the participant ID displayed. The investigators will receive this by post and will supply each participant with the treatment pack on Visits 1 and 2 (placebo for 2 weeks, then randomised treatment for 12 weeks).

BLINDING

Although participants will be blinded to treatment allocation, we will inform them of the general nature and composition of the treatment prior to their participating. The investigator team will be blinded as to which treatment we are giving which participant, during the 12 intervention weeks. Only the statistician and pharmacist will not be blinded as to which participant receives which treatment,

as they will be handling the randomisation schedules linking identification numbers to participant names. In case of emergency (an adverse event sustained by the participant, which requires medical treatment to know which study treatment has been given), the investigator will break the seal and be unblinded as to which treatment the participant has received. This will immediately be reported back to the investigator team and the HREC. The participant always has the option of dropping out of the trial.

DATA COLLECTION AND MANAGEMENT

Data will be collected by delegated personnel from the research team. We will compile de-identified data into monthly data sets, using Microsoft Excel® (version 15.0, Microsoft Corporation, Redmond WA, USA). For OCTA, photosensitivity and plasma biomarkers, the computed results will be copied via a password-protected USB stick to the university research drive. Identifiable data will be sorted by date in separate folders according to each questionnaire and stored in a locked filing cabinet with access restricted to the investigator team. All other data will be de-identified to ensure confidentiality of participant data. Data will be stored on a password-protected web-enabled clinical-trial-data electronic management system (REDCap) located in an ISO27001-compliant facility at Curtin University. Participants' study information will not be released outside of the study without the written permission of the participant. All data will be securely archived as per the Institute's data policy for a minimum of 15 years. A formal data-monitoring committee was not required for ethics approval. The principal investigator will terminate the trial if appropriate, for example due to unforeseen adverse events associated with the intervention.

DISCUSSION

Our trial proposal stems from the positive action on vasodilation exerted by L-arginine and AGE in the cardiovascular setting (111, 124, 157). Although new treatments and preventions are being explored for migraine treatment (158, 159), L-arginine and AGE have not been clinically considered.

L-arginine is an amino acid that is involved in the production of nitric oxide which helps to relax blood vessels and improve blood flow. Aged garlic extract is a supplement derived from garlic that is aged for up to 20 months (42, 160). It is believed to have antioxidant and anti-inflammatory properties, and from some studies, we hope that, similar to L-arginine, it may also be effective in reducing the frequency of migraines (161, 162). However, more research is needed to confirm these findings and understand how aged garlic extract may work to prevent migraines.

We surmise that these agents should be investigated in future larger randomised controlled trials for prevention of migraines induced by cerebral microvascular constriction. To assess and confirm regional vasoconstriction of cerebral arterioles and capillaries, we recommend imaging of the cerebral microvasculature directly using functional imaging techniques (163, 164).

It is important to note that while these supplements may have potential benefits, in principle, they should not be used as a replacement for conventional migraine treatments. Additionally, it is also worth noting that both L-arginine and aged garlic extract can interact with certain medications, so it is important to talk to one's medical doctor before taking them if one is currently on any prescription medications.

One important limitation is that currently, no studies have investigated the effects of L-arginine and AGE in the context of gender or ageing. These variables are established risk factors for headaches, concomitant with decreased endogenous biosynthesis of NO (10, 27, 165). All previous studies show an alleviating effect of both L-arginine and AGE in all 3 aspects: decreased inflammation and oxidative damage markers, decreased triglycerides and low-density lipoproteins, increased high-density lipoproteins, decreased atherosclerosis and decreased thrombotic events (111). The fact that neither L-arginine nor AGE have been tried in randomised controlled trials, specifically for the prevention of migraine, is a current shortcoming. Our study is amending this issue, and it is of potential clinical value, even considering the study population (appropriate numbers, subject intake not limited by age in principle) that we are going to assess.

The value of our proposal is also based on the fact that accessibility, optimal usage and costs are still unresolved issues for new drugs against migraine (158, 166), and the molecules that we are testing are affordable and already used in humans, with minimal side effects. Other strengths of our proposal include the randomised placebo-controlled trial structure, the use of both males and females, and a wider age range to include above-65-year-olds – which allows better assessment of potential safety issues in more vulnerable (until age 80) populations. Also, >60-year-olds are different in symptom onset (167), so including this subgroup would make the results more generalisable. Given the stringent eligibility criteria, we realised that there was no reason not to include individuals older than 65 as specified in Diener and Tassorelli (133, 134), especially as a significant safety issue was not present. Older than 80 years old may present comorbidity-related confounders.

Since older subjects also tend to use polypharmacy, any unforeseen interaction effects (despite having checked for potential contraindicated drug interactions in the eligibility criteria) should come to light. Our treatment is an oral preventive which is non-invasive, and would hopefully preclude the need for combination therapy in treatment-refractive populations with a vasoconstrictive migraine aetiology. Combination therapy generally increases risk of adverse effects, and it is difficult to ascertain which drug each effect may come from and may need to be withheld (158). We use both subjective (diaries and questionnaires) and objective measures (vascular tone, photosensitivity and blood markers) to track migraine progress longitudinally in each participant, with a placebo wash-in to further increase reliability of results. The nutraceutical agents are mildly vasodilatory, hence are not contraindicated for patients with pre-existing vasoconstrictive issues, nor should they pose a danger to patients with hypotension (we also measure blood pressure at each visit). The 2x2 group schedule allows a placebo

control, as well as checking for interaction effects when both nutraceuticals are simultaneously given. Reported well-tolerated doses of L-arginine and AGE, considered for cardiovascular disease risk, serve as a useful guide to explore the putative efficacy of L-arginine and AGE for prevention/treatment of vascular-associated migraine. We have chosen the minimally effective doses consistent across previous human studies using L-arginine and aged garlic extract, to compromise between precluding adverse effects of its first use in migraine patients, and providing ample therapeutic agent to reliably assess efficacy.

RESEARCH ETHICS APPROVAL AND RESULTS DISSEMINATION

The LARGE trial has been approved by the Curtin University Human Research Ethics Committee (HREC) (Approval Number: HRE2020-0466; version 4; 16th August 2021). The trial is registered with the WHO Trial Registration Data Set. The Universal Trial Number is U1111-1268-1117. Written consent will be obtained from all participants prior to commencing their participation in the trial. The consent form is included in Appendix 8. Any protocol amendments will be submitted straight to ethics for approval before implementation, and the trial registry accordingly updated. The results of the study will be disseminated in peer-reviewed publications and presented at key national and international conferences and local stakeholder events.

ABBREVIATIONS

L-arg: L-arginine

AGE: aged garlic extract

CHIRI: Curtin Health Innovation Research Institute

GP: general practitioner

HREC: Human Research Ethics Committee

HIS: International Headache Society

LED: light-emitting diode

LEI: Lions Eye Institute

L-VISS: Leiden Visual Sensitivity Scale

MSQ: Migraine-Specific Quality-of-Life Questionnaire

OCTA: optical coherence tomography angiography

PGE-5: phosphodiesterase type 5

REDCap: Research Data Capture

SF-MPQ: Short-Form McGill Pain Questionnaire

SNRI: Sarich Neuroscience Research Institute

DECLARATIONS

Ethics approval and consent to participate

The Curtin University Human Research Ethics Committee (HREC) has approved this study (Approval Number: HRE2020-0466; version 4; 16th August 2021). Written informed consent will be obtained from all participants prior to commencing their participation in the trial. All methods will be carried out in accordance with the relevant guidelines and regulations as well as the Declaration of Helsinki.

Authors' contributions

DC and VL wrote the manuscript and led the study design and protocol development. MV supervised and edited the final manuscript. EC trained and aided DC in the clinical trial coordination. PD and EV provided advice on study design and methodologies. VL and RT provided advice on study design, recruitment, data acquisition and editing of the manuscript. SD was the lead statistician and advised on study design and power calculations. JM conceived the study concept and study design, and edited the final manuscript. All authors have read and approved the final manuscript.

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The authors wish to thank Dr. Fred Chen and Dr. Jason Charng for providing training for the OCTA.

CHAPTER 4: OCTA-MIGRAINE REVIEW

The content of this chapter is covered by published Paper 4:

Chaliha DR, Vaccarezza M, Charng J, Chen FK, Lim A, Drummond P, et al. Using optical coherence tomography and optical coherence tomography angiography to delineate neurovascular homeostasis in migraine: a review. Frontiers in Neuroscience. 2024;18.

PAPER 4

Paper 4 provides a comprehensive literature review of the use and findings of OCT and OCTA to assess vascular tone in the posterior eye of migraineurs.

USING OPTICAL COHERENCE TOMOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY TO DELINEATE NEUROVASCULAR HOMEOSTASIS IN MIGRAINE: A REVIEW

ABSTRACT

Migraine is one of the world's most debilitating disorders and it has recently been shown that changes in the retina can be a potential biomarker for the disease. These changes can be detected by optical coherence tomography (OCT) which measures retinal thickness, and optical coherence tomography angiography (OCTA) which measures vessel density. We searched the databases, Google Scholar, ProQuest, Scopus and Web of Science, for studies in English using OCT and OCTA in migraineurs, using the search terms, "optical coherence tomography", "OCT", "optical coherence tomography angiography", "OCTA" and "migraine". We found 73 primary studies, 11 reviews and 8 meta-analyses pertaining to OCT and OCTA findings in migraineurs. They showed that migraineurs had reduced retinal thickness (via OCT), retinal vessel density and greater foveal avascular zone area (via OCTA), than controls. OCTA changes reflect a perfusion compromise occurring in migraineurs as opposed to in healthy controls. OCT and OCTA deficits were worse in migraine-with-aura and chronic migraine than in migraine-without-aura and episodic migraine. Certain areas of the eye, such as the fovea, may be more vulnerable to these perfusion changes than other parts. Direct comparison between study findings is difficult because of the hetereogeneity between the studies in terms of both methodology and analysis. Moreover, as almost all case-control studies were cross-sectional, more longitudinal cohort studies are needed to determine cause and effect between migraine pathophysiology and OCT/OCTA findings. Current evidence suggests both OCT and OCTA may serve as a retinal marker for migraineurs, and further research in this field will hopefully enable us to better understand the vascular changes associated with migraine, perhaps also providing a new diagnostic and therapeutic biomarker.

INTRODUCTION

OCT measures retinal thickness, employing infrared wavelength for image acquisition and has an 8-10μm axial resolution which may be improved to 3μm in some devices (144, 168). Spectral-domain OCT (142, 143, 169, 170) provides higher resolution, faster acquisition speed and fewer artefacts (171). However, limitations include a small field of view, lack of vessel leakage detection, dependency of resolution on coherence length of light source, artefacts from minute patient movements and inability to detect slower blood flow (172, 173). Retinal thickness data in OCT images can be partitioned into 4 (superior, inferior; nasal, temporal) or 6 (nasal, superior nasal, inferior nasal; temporal, superior temporal, inferior temporal) regions and one meta-analysis showed that the 4-region partition method detected superior and inferior quadrant retinal nerve fibre layer (RNFL) thickness differences better than the 6-region method for delineating migraine findings (174). The RNFL is the layer formed by the retinal ganglion cell axons which collect visual impulses from the rods and cones of the retina.

Optical coherence tomography angiography (OCTA) is a non-invasive retinal vascular imaging technique generating a 3D image of the layers of retinal vasculature via motion contrast of blood flow (172, 175-180). This fast continuous repeated longitudinal scanning method allows blood cells inside the vessel lumen to be discriminated from surrounding tissue and hence blood flow to be tracked scan by scan (172, 180). This technique has several advantages over invasive retinal imaging methods such as fluorescence angiography and indocyanine green angiography which have adverse effects and contraindications associated with the dye and its injection, as well as superimposed imaging of all layers in the retina owing to lack of depth resolution and being time-consuming and expensive (172, 177). The compromise between signal bandwidth and detection sensitivity affects the maximum acquisition rate in OCT (173), but OCTA may overcome this with greater imaging speeds (172). It also outperforms fundus photography which only provides a 2D visualisation of blood vessels with low resolution (141).

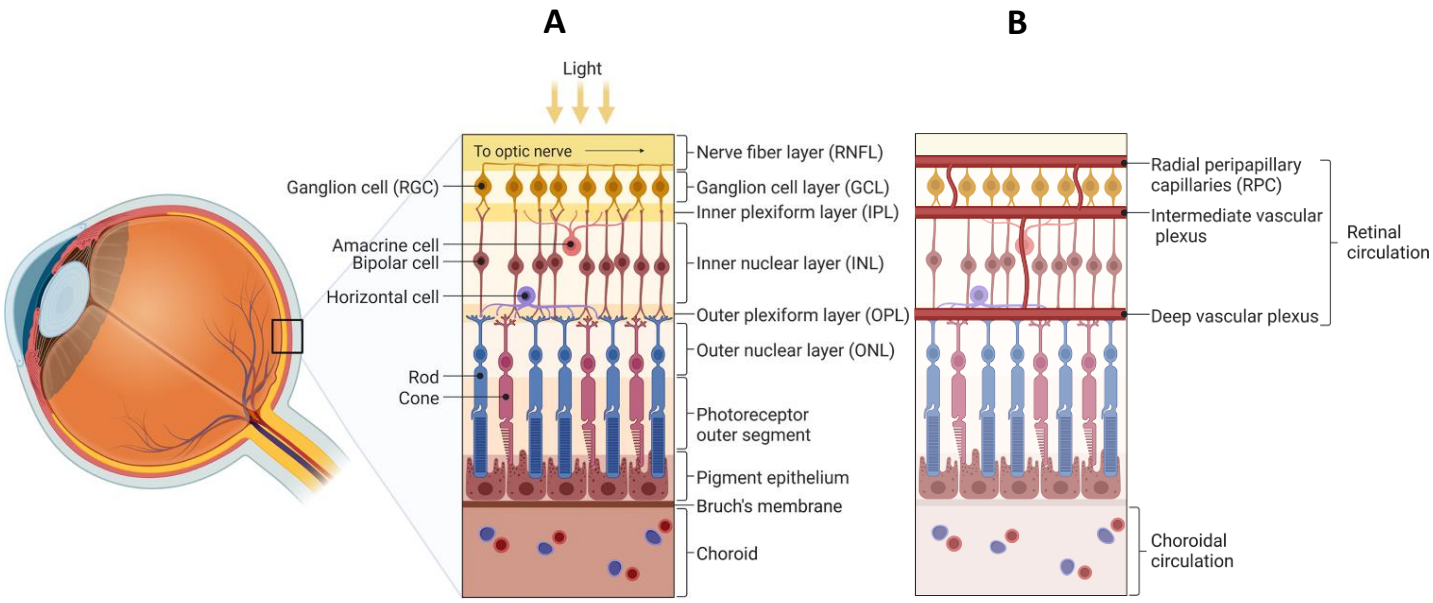
Tomography involves taking cross-sectional images of 3D objects, and in 1991, Huang, Swanson and Fujimoto used these coherence properties of light waves to apply the technique to a human eye *in vitro* (173). This can now be used via ophthalmoscope and camera *in vivo*, whereas previous eye-imaging methods could only take place on fixed tissue (180). Since the first publication of this innovative technique in 1991 (173), optical coherence tomography (OCT) has been used in several medical fields with an increasing recognition that stems from its properties and potential clinical applications (176, 180, 181).

OCTA, on the other hand, provides robust assessment of retinal vasculature and it can also discriminate between superficial and deep capillary plexus networks (182). OCTA can precisely show capillaries undergoing ischaemia (183). OCTA is superior to traditional methods in imaging radial pericapillary and deep capillary networks based on flow characteristics (172, 177) and non-perfusion can therefore be quantified accurately (175) with a high data-acquisition rate (173). In fact, projection-resolved (PR) OCTA improves on conventional OCT by addressing the problem of the superficial vessels projecting flow artefacts detected from the deeper layers, therefore enhancing depth resolution (175).

Nowadays, OCTA is widely used in ophthalmology and cardiovascular disease as a powerful tool as an "optical biopsy" (176, 184, 185). The retina is the only vasculature that can be visualised non-invasively *in vivo* (183). We consider the eye as an extension of the brain, because the optic nerve, retina and brain derive from the anterior neural tube during embryonic development (186). The retina and cortex have similar angiogenesis patterns in development (187), so brain and retina have close and similar blood regulations (188).

One of the vascular-derived disorders that can be assessed via OCT/OCTA is migraine. Migraine incidence worldwide was 87.6 million in 2019 (189). Episodic migraine (<15 migraine days per month) is believed to originate from neuronal hyperexcitability in the trigeminal vascular system (190), and can transform to chronic (15 or more days/month) through increased attack frequencies, perhaps due to functional and structural brain changes, central sensitisation and neuroinflammation (191). Migraine can occur with or without aura, where aura occurs in 15% of migraineurs from the cortical spreading depression preceding a migraine attack. Aura symptoms are sensory disturbances, with 90% being visual, associated with more severe ischaemic risk (192).

It is believed that migraine involves chronic systemic vasoconstriction, with (141) and without aura (193). We previously hypothesised that heightened sympathetic tone results in progressive central microvascular constriction. Suboptimal parenchymal blood flow may activate nociceptors and trigger the migraine (111). This may be able to be seen in the fundus as reduced vessel density. Vessel density can be measured as blood vessel length divided by scan area (141), or the percentage of vascularised tissue within the area (145). From this, several areas of the posterior eye can be analysed using OCTA. A schematic of the eye, showing vascular and neural anatomy, is shown in Fig. 1 (194).



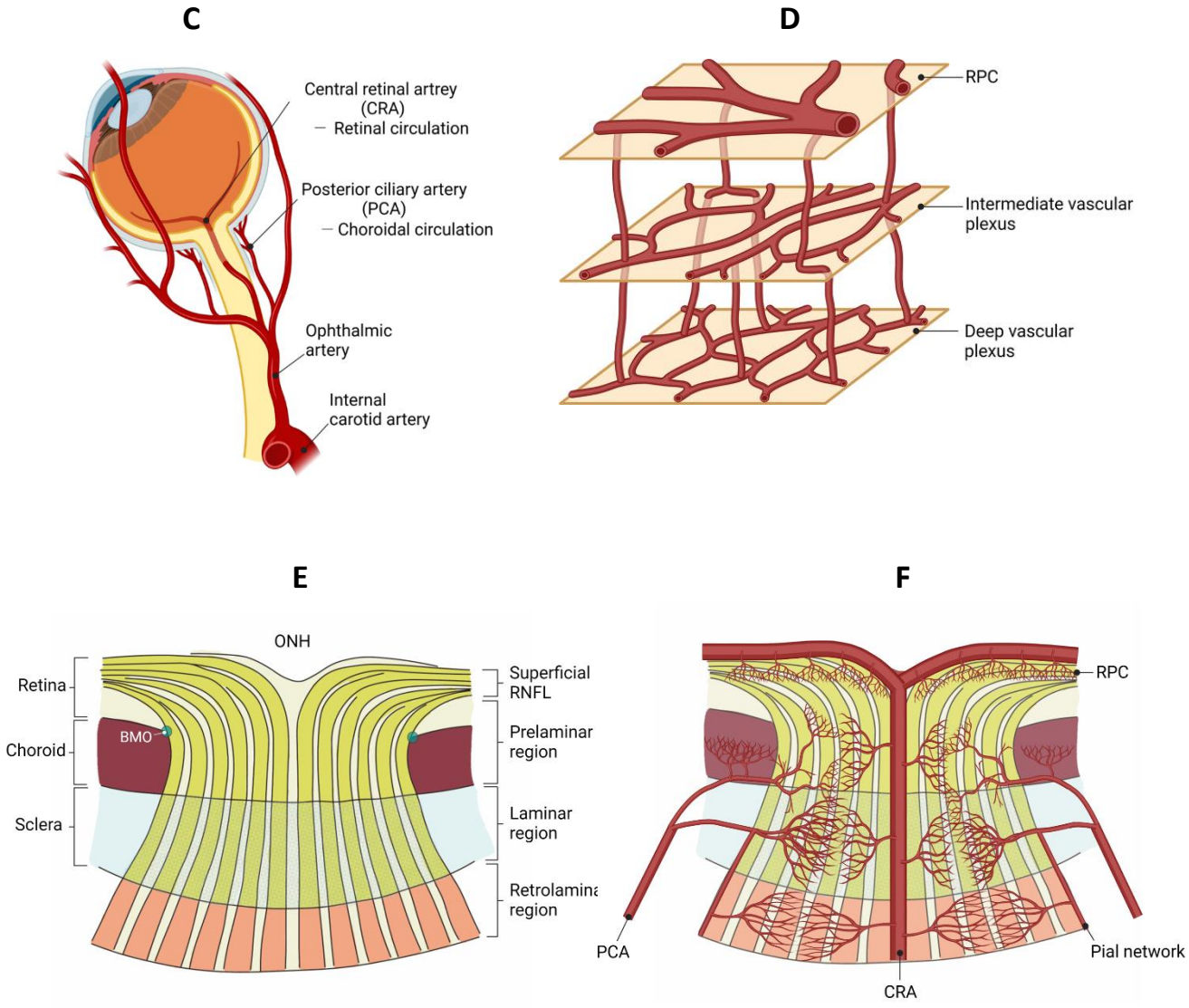


Fig. 1. *Vascular and neural anatomy of the eye (194). (A) Neuronal layers of the retina. (B) Vascular layers of the retina. (C) Retinal (supplying the inner retina) vs. choroidal (supplying the outer retina) circulations. (D) The three interconnected and anastomosing vascular plexuses of the retina. (E) Neuronal layers of the eye converging into the optic nerve (ONH: optic nerve head). (F) Vascular layers of the eye branching out from the central retinal artery (at the ONH). Adapted from Shiga et al. (2023; permission obtained from authors).*

AIM/BACKGROUND

The current literature regarding cerebral and retinal vascular perfusion suggests that retinal vascular changes can indicate cerebral vascular disease, even proportionally, due to the common embryological origin and resultant homology between the retinal and cerebral microvasculatures (195, 196). For example, retinopathy signs such as retinal artery occlusion or greater retinal vein calibre may indicate cerebrovascular compromise (195). In recent studies, lower retinal perfusion was associated with MRI

biomarkers of cerebral small-vessel disease (197, 198). Previous studies examining migraine patients using OCT and OCTA show several differences between migraineurs (also with vs. without aura) and healthy controls in vascular tone in the retinal macular and retinal optic nerve areas, as measured via vessel density as well as choroidal and retinal thicknesses. This particular review highlights the relevance such OCT/OCTA signs have for the severity, classification and prediction of migraine episodes. The purpose of this study was to collate the information from various OCTA studies pertaining to migraine, and sorting them into ocular structural categories as has not been done before to our knowledge. In the anterior eye, there seemed to be no differences in axial length, corneal curvature radius, anterior chamber depth, central corneal thickness and pupil size between migraineurs and controls, ictally or interictally (199). Hence, we will focus only on the retinal and choroidal layers of the eye. For this review, we found different studies using OCT and OCTA to investigate migraineurs, using the search terms, “optical coherence tomography”, “OCT”, “optical coherence tomography angiography”, “OCTA” and “migraine”, present in the abstracts and sorted by relevance, on the databases, Google Scholar, ProQuest, Scopus and Web of Science via institutional access, as well as the reference lists of all the OCT/OCTA-migraine systematic reviews and meta-analyses we found. Only articles in English or with available English translations have been included. Our results are presented in Appendix 11.

RETINA

Vessel density (VD) is taken as the length/area of flowing vessels as a percentage of total area scanned (183), or the percentage area of vessels with active (OCTA-detectable) blood flow (200). The deep capillary network comprises multi-capillary units converging towards a central vortex of capillaries draining to superficial venules, which OCTA is able to detect (201). Both our search and Pang’s 9-study meta-analysis show that migraineurs had lower superficial plexus, deep plexus, macular, peripapillary (area around the retinal papillary region) and foveal VD, than did controls (183, 202). Often these reductions were present in those with aura only, although there was a similar tendency in non-aura migraineurs.

Aura and chronic migraineurs seem to have greater reductions than non-aura and episodic migraineurs. Aura migraineurs especially tended to have decreased superficial, deep and parafoveal deep capillary plexus VD than did controls (200). Participants with a history of migraines with aura show lower retinal arteriole calibre compared to controls with no migraine history (203). In chronic but not in episodic migraineurs, retinal arteries were bulkier ipsilaterally to the headache compared to controls (147), but this did not correspond with retinal vein diameters which were similar (147). This suggests greater increased energy demand at the retina in chronic migraineurs. Unsurprisingly, migraineurs, especially those with aura, seem to have retinal capillary damage (204).

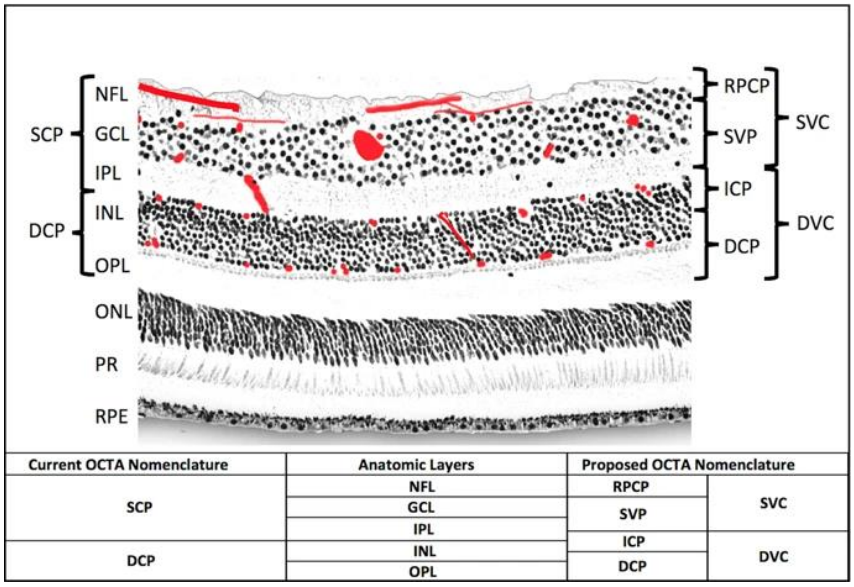
During migraine attacks and/or auras, transient vasospasm can compromise perfusion in both the eye and head (204). This can change retinal/neuronal perfusion, in turn causing hypoxic/ischaemic injury,

ultimately damaging the retinal nerve (204) and structures. Lower deep retinal VD is correlated with lower signal strength index, longer axial length and higher creatinine (where creatinine acts as an energy buffer for retinal cells (205)) (206). OCTA seems to be able to detect these changes, but there may be confounders. Lower superficial retinal VD is correlated with lower signal strength index and participant sex being male (206). This may underscore the importance keeping male/female proportions similar between groups.

On OCT, retinal thickening may indicate oedema, whereas retinal thinning may indicate atrophy (180). It can be split into looking at the retinal nerve fibre layer (RNFL) and the ganglion cell layer (GCL). The OCT signal originates from two plexuses in the inner retina (SCP and DCP), both of which seem affected by migraine pathophysiology.

RETINAL NERVE FIBRE LAYER (RNFL)

Retinal thickness can also perhaps be examined to indicate perfusion related to migraines, and OCT allows high-resolution RNFL, GCL and choroid thickness determination *in vivo* (144). RNFL thinning is actually associated with brain atrophy in general, with direct correlations with the central cingulate and pericalcarine cortices especially in certain neurodegenerative diseases (207, 208). OCT can be used to measure the thickness of the RNFL, usually in the peripapillary (optic nerve head) region and macula (144). In controls, the inner retinal layers' thickness correlate with retinal microvascular perfusion, when using OCTA to visualise macular and peripapillary areas (169). On OCTA, vessel density in the RNFL is another measure of perfusion and predictably, in some studies, investigators found that aura migraineurs had lower VD there than controls and that the higher the migraine frequency/disability/history, the lower the VD (209, 210). Fig. 2 shows an histology section showing the two capillary plexuses from which OCTA signals are derived (175). Out of the superficial vascular complex (SVC), it should be noted that the radial peripapillary capillary plexus (RPCP) is found predominantly adjacent to the optic nerve and the superficial vascular plexus (SVP) is found predominantly in the macula. The SVP density and GCL thickness decrease away from the optic nerve (175).



Current OCTA Nomenclature	Anatomic Layers	Proposed OCTA Nomenclature	
SCP	NFL	RPCP	SVC
	GCL	SVP	
	IPL		
DCP	INL	ICP	DVC
	OPL	DCP	

Fig. 2. *An histological cross-section of the retina (175). The red blots represent vascular plexuses. DCP: deep capillary plexus; DVC: deep vascular complex; GCL: ganglion cell layer; ICP: intermediate capillary plexus; INL: inner nuclear layer; IPL: inner plexiform layer; NFL: nerve fibre layer; ONL: outer nuclear layer; OPL: outer plexiform layer plus Henle's fibre layer; PR: photoreceptor layers; RPCP: radial peripapillary capillary plexus; RPE: retinal pigment epithelium; SCP: superficial capillary plexus; SVC: superficial vascular complex; SVP: superficial vascular plexus. Adapted from Campbell et al. (2017; permission obtained from authors).*

We found 35 studies examining specifically the RNFL in migraineurs and find that studies have inconsistently shown that migraineurs have thinner RNFL than controls (142, 149, 150, 206, 211-232), generally ipsilateral to the usual side of headache (151, 153, 233). Lin et al. examined 26 studies and also found thinner mean RNFL in migraineurs than in controls, especially in the superior and inferior eye quadrants (174). Feng et al. meta-analysed 6 studies whose investigators found migraineurs having much thinner average RNFL than controls, significant in all 4 retinal regions (superior, inferior, nasal, temporal) (214). Notably, they did not detect publication bias (214), although this may have been difficult to identify as they tested fewer than 10 studies.

RNFL thinning indicates axon loss (153). It could serve as a marker for hypoxic damage to ganglion cell and retinal nerve fibres/axons (234, 235), especially given an association between macular RNFL thinning and the antioxidant molecular marker, catalase (236). The longer the migraine history/attack/aura or the higher the migraine frequency/disability, the thinner the RNFL (149, 153, 218, 223, 225, 227, 237-240). In children, there may not have been time for this effect to develop (240). Migraineurs have transient retinal vasospasm ictally, leading to visual aura postdromally (241). These recurrent retinal vasospasms may chronically lead to permanent vascular changes in the eye, detectable via OCT (241). The recurring transient retinal and ciliary artery constrictions may cause

hypoxic damage to the optic nerve, retina and choroid – thereby, the RNFL and GCL may undergo thinning with general retinal capillary decrement (242).

Migraine-related disability and average RNFL thinning may be strongly correlated through the Migraine Disability Assessment (MIDAS) (153, 220) but not through Visual Analogue Scale (VAS) (225) scoring systems. Sample heterogeneities may have also played a role in this discrepancy. For example, both eyes, a randomised eye, or only the right or left eye may be selected for study. A systematic review showed that those choosing only the left eye found no such RNFL thinning, whereas the other studies' investigators did find RNFL thinning in migraineurs compared to controls (174). The random- and right-eye selection methods yielded medium effects, whereas both-eye selection yielded small effects (174). We wonder if most of the migraineurs had right-sided migraines most of the time. In addition, physiological confounders such as visual-aura could have exacerbated RNFL thinning in some studies (174).

Aura migraineurs show more severe changes than do non-aura migraineurs. The effect size and amount of thinning were greater in migraineurs with than without aura (174). Aura migraineurs had thinner RNFL in many different ocular areas compared to controls and often non-aura migraineurs (174, 215, 229, 230, 239, 243, 244), whereas non-aura migraineurs sometimes did (215) and sometimes did not (238, 239) have significant changes relative to controls. Still others did not find such differences between aura and non-aura migraineurs (220, 222, 245, 246), especially at the macula (210, 247-249). Aura migraineurs were likelier to have RNFL thinning than non-aura migraineurs, while most auras were visual (174, 250). Indeed, visual-aura migraineurs had thinner RNFL than non-visual aura migraineurs in another study (251). Aura migraineurs had RNFL thickening interictally but remaining thinner than controls (252). Ictally, the posterior hemisphere shows hypoperfusion with ipsilateral aura (253) and migraineurs with aura may show greater RNFL changes than those without (144).

Chronic migraineurs had thinner RNFL than episodic migraineurs (149) and controls (235, 243, 247, 254). Studies show that mainly chronic migraineurs (as opposed to episodic) have thinner retinae and choroids compared to controls, with the RNFL and fovea more affected in those with aura than those without (144). An acute attack may not affect macular or peripapillary perfusion (202). However, RNFL thinning does not seem to be related to migraine duration or history, so perhaps it is not cumulative, but rather, its likelihood of acute occurrence may increase with each acute attack (241). Participants with a migraine history less than 15 years had thinner temporal RNFL than controls, but those with more than 15 years' migraine history had thinner average, superior, inferior and temporal RNFL than controls (214). On the other hand, age, sex, migraine history and frequency and intraocular pressure do not seem to correlate with RNFL thickness (174) and migraineurs with and without white-matter hyperintensities (WMH) have similar thicknesses (216). WMH are considered a marker of focal hypoperfusion and are associated with aura (255). They are believed to arise from microvascular

damage, are made of myelin and gliosis (255) and are correspondingly associated with ischaemia (142).

In migraineurs with aura, average RNFL, superior hemisphere and superior layer are decreased (142). In migraineurs without aura, those with WMH had thinner RNF and superior hemisphere and superior layers, than those without (142). Additional non-perfusion-related factors may also contribute to WMH formation (213).

There are potential confounders to interpretation. Whilst OCT angiography also detects depth-resolved motion contrast, reconstructing vessel perfusion at different layers of the retina, projection artefacts can get in the way of examining the layers underneath the topmost RNFL (241). Between studies, the retinal depths segmented for OCTA output varied, making comparison difficult (241) and the different layers of the eye are vulnerable to different extents to confounding by the same artefact (241).

GANGLION CELL LAYER (GCL)

Nineteen studies showed ganglion cell complex thinning in migraineurs (202). Compared to controls, both aura and non-aura migraineurs had thinner GCL (149, 211, 213, 229, 231), where chronicity and severity yielded greater GCL reduction (211). The presence of WMH did not influence this effect (213). Yet again, aura and chronic migraineurs seem most affected. Aura migraineurs had thinner GCL than non-aura migraineurs (144, 243) and controls (229, 230). The corresponding reduced perfusion could explain this, especially given that migraineurs without aura do not seem to have interictal choroidal hypoperfusion. Chronic migraineurs had thinner ganglion cell layer (GCL) and complex (GCC) than controls (234, 235, 256) and episodic migraineurs (149). Migraineurs with more than four attacks a month had thinner GCL than controls (215). The recurrent perfusion fluctuations and transneuronal retrograde degenerations in the primary visual cortex may cause chronic retinal damage in migraineurs, leading to ganglion cell loss (153). GCL thinning could be a more accurate biomarker of axonal damage than RNFL (144). If the GCL is thinner but the RNFL is not, this may indicate fewer and/or shorter migraines (234).

OPTIC NERVE

OCTA has also been able to detect optic nerve area differences between migraineurs and controls. RNFL thickness measurement has been used to monitor optic nerve diseases as an indirect measure of retinal ganglion cell loss and is not specific to migraine (144, 214). Fig. 3 shows an example of the optic nerve area imaged via OCTA (141).

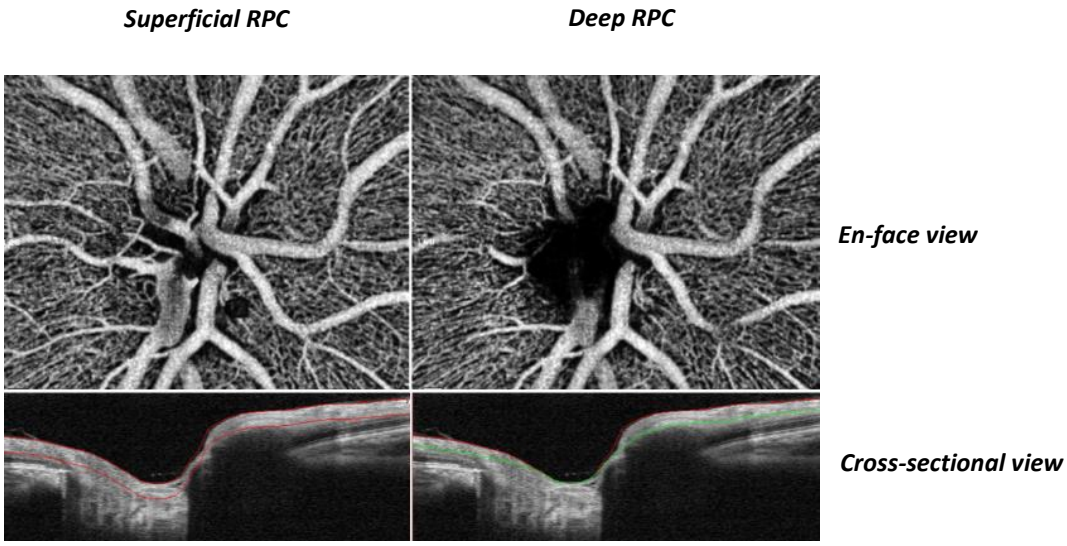


Fig. 3. *OCTA imaging of the optic nerve area, taken at the superficial RPC (left) and deep RPC (right) (141). RPC: radial peripapillary capillary. Adapted from Chang et al. (2017; permission obtained from authors).*

Migraineurs, especially those with aura, tend to have optic nerve head damage (204). Migraineurs had smaller optic and neuroretinal rim disc area and lower cup-disc ratio at the optic nerve head, greater disc area and larger cup volumes, than controls (232, 233). Both aura (more so those with WMH than those without) and non-aura migraineurs had reduced whole optic disc VD than controls (142). Different peripapillary areas of left and right eyes may have RNFL thinning in migraineurs, compared to controls (213). In some studies, investigators found no differences between aura and non-aura migraineurs (213, 230, 231).

Some areas of the RNFL may be more vulnerable than others, seen via both depth and *en-face* imaging. For example, migraineurs have less superior peripapillary vessel density and marginally thinner superior RNFL than controls, suggesting that the superior retinal region may be more vulnerable to hypoperfusion (227, 257). The peripapillary RNFL has many unmyelinated ganglion cell axons which require more energy to maintain, so is more vulnerable to hypoxic damage (258). Liu et al. examined 16 studies on episodic migraineurs in their systemic review and meta-analysis, finding that aura migraineurs had exceedingly thinned peripapillary RNFL in most areas (204). Some studies reported mean peripapillary RNFL thinning in migraineurs, whereas others only in a specific quadrant (144). Feng's analysed studies showed lower mean peripapillary RNFL in migraineurs than controls (214). Peripapillary VD is lower in migraineurs and chronic hypoperfusion there leads eventually to RNFL thinning/atrophy (183). In a meta-analysis, average peripapillary RNFL thickness was lower in migraineurs with and without aura than in controls possibly due to vascular dysregulation and focal cerebral ischaemia (204). There seems to be peripapillary RNFL thinning in all quadrants, with superior and inferior being more susceptible (which may be associated with higher ganglion axon

vulnerabilities) than temporal and nasal quadrants (204), corresponding with fundus hypoperfusion occurring mostly there (151). Migraineurs with WMH had thinner RNFL than controls (245), whereas those without WMH did not (152) or had thicker RNFL than controls interictally (245). RNFL is more susceptible to damage due to greater retinal axon vulnerability than other optic areas (151). Ictal vasospasm involving retrobulbar (ophthalmic, posterior ciliary, central retinal) arteries, may culminate in optic nerve head hypoperfusion and thus necrosis of retinal ganglion cells (214, 247).

Again, aura migraineurs seem to have a more exaggerated differences than non-aura migraineurs or controls. At the optic nerve head, aura migraineurs had reduced VD (OCTA) (141, 259) and RNFL thickness (OCT) (244) and larger optic disc rim (OCTA) (210), than non-aura migraineurs and controls, with some studies finding no difference between non-aura migraineurs and controls (OCTA) (141) and some finding there was a difference between the latter two groups (also OCTA) (202). The lower the VD was, the higher the migraine frequency and severity (260). Decreased superior peripapillary VD and tendency of decreased superior peripapillary RNFL thickness in migraineurs with aura (141) suggest peripapillary hypoperfusion and that the optic nerve head may be more susceptible to damage in local hypoperfusion (241). Decreased VD at the optic nerve could lead to peripapillary hypoperfusion (241). Optic nerve injury may be caused by vascular disturbances and focal ischaemia (225), which is supported by Kara et al.'s finding of reduced perfusion in the central retinal and posterior ciliary arteries of migraineurs compared to controls (261). If the retina or optic nerve head perfusion is compromised, ganglion cell damage may ensue (153). Migraine chronicity is characterised by ictal recurrent vasospasms and focal ischaemia, which may explain optic nerve damage and peripapillary RNFL thinning (149).

MACULA

Migraineurs had reduced macular retinal vessel and perfusion densities and thinner maculae, than controls (142, 145, 212, 226, 231), especially those with more than four attacks a month (215) – the more this effect was, the higher the migraine frequency and severity (260). However, in some studies, investigators found similar macular thicknesses between all groups (150, 210, 246, 247). These contradictory findings could be a result of the former group of studies having slightly higher participant numbers and/or the latter group including a study with children where we do not yet expect such differential results due to shorter migraine history. Fig. 4 shows an example of the macular vessel densities imaged via OCTA.

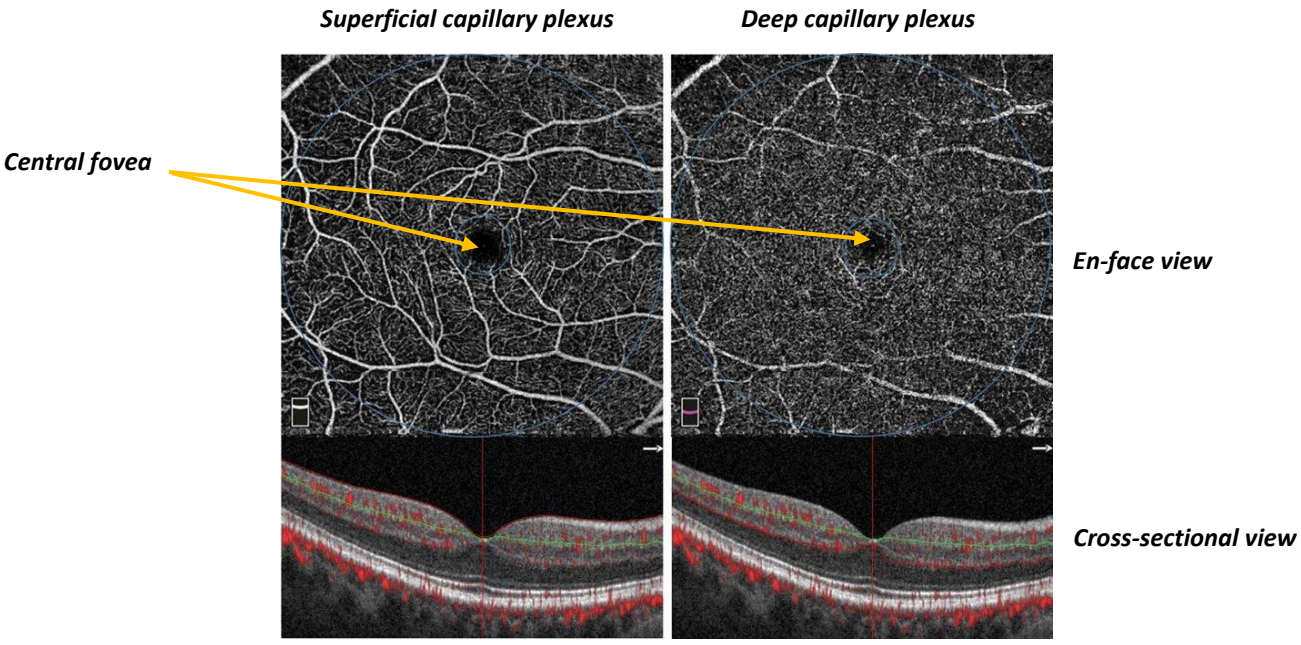


Fig. 4. *OCTA imaging of macular vessel densities (141). Adapted from Chang et al. (2017; permission obtained from authors).*

Retinal ganglion cells predominate in the macula, so macular thinning is indicative of ganglion cell loss (comprised of cell bodies) and RNFL (comprised of axons) loss (234, 262, 263). In one study, investigators found no difference between those with and without WMH (145). Although deep foveal VD tended to be lower in migraineurs than controls, this was not significant – but this may be because of projection artefacts (175) and the small number of studies analysed (183). Chronic migraineurs had thinner macular RNFL and macular thickness than controls (235).

The changes in aura migraineurs were more severe. Aura migraineurs had thinner maculae and lower VD than non-aura migraineurs and controls (141, 142, 204, 238, 259, 264). In the deep capillary plexus, the VD in aura migraineurs was lower in the parafovea than in controls (200), but the measurement of the parafoveal ring differs from study to study (200). Aura migraineurs had decreased deep foveal VD ipsilateral to headache during prodromal aura, improving three hours post-aura (265). In one study, investigators found that aura migraineurs had right-eye macular hypoperfusion during right aura but left hemicranial pain, resolving within a week after resolution of pain and aura (266). Aura migraineurs had thinner maculae after 6 months post-attack (219). Interestingly, the macular deep capillary density may be lower in all regions *but* the fovea in aura migraineurs (possibly due to retinal ischaemia) (204).

Aura migraineurs with brain WMH had lower deeper foveal VD, thinner maculae and superior hemisphere densities and increased FAZ area, than those without WMH (142, 215). Hence, retinal VD may also be related to brain WMH (142) which have been visualised in migraineurs using MRI (267). In migraineurs without aura, those with WMH had lower deeper foveal and superior hemisphere VD (142). However, in another study, non-aura migraineurs had lower superficial and deep macular vessel

densities than controls, whether they had accompanying WMH or not (145), suggesting that neither WMH nor aura are sensitive markers of retinal hypoxic threat.

FOVEAL AVASCULAR ZONE (FAZ)

The foveal avascular zone is the centre of the macula. The lack of blood vessels here serves to reduce light scattering to provide maximal optical quality at the point of ocular fixation on an object. Fig. 5 shows an example of the central FAZ imaged via OCTA (268).

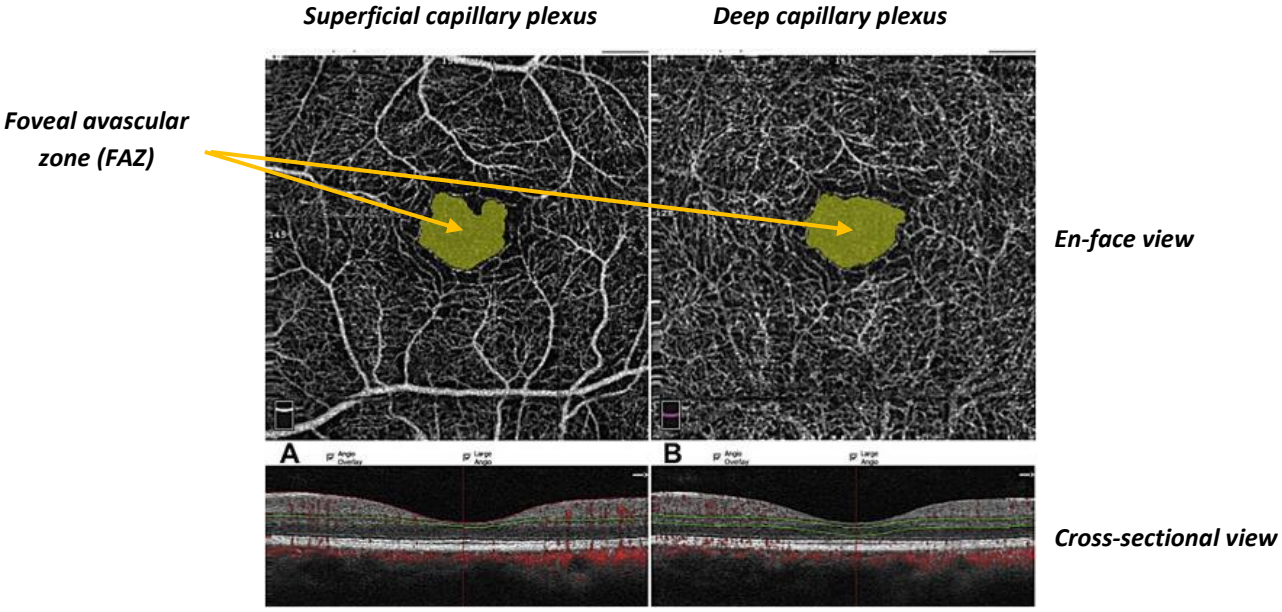


Fig. 5. *OCTA imaging of the FAZ (268). Red dots: blood flow signals. Adapted from Ghassemi et al. (2017; permission obtained from authors).*

Pang's meta-analysis of nine studies shows that migraineurs had a larger FAZ area than controls (183). Ke et al. (2022) examined nine studies, where they found that the FAZ area of aura migraineurs was greater than controls after correcting for publication bias (200). We found seven studies that looked at FAZ parameters (area and/or perimeter/circumference). They showed that FAZ area and its perimeter were larger in migraineurs compared to controls (141, 145, 204, 206) and most studies' investigators found that aura migraineurs had increased FAZ area and perimeters compared to and non-aura migraineurs and controls (141, 200, 206, 209, 210, 269). One study's investigators also found that most of their aura migraineurs had deep-plexus FAZ enlargement (259).

These observations may indicate an association between aura and ischaemia (200). The larger FAZ may indicate permanent changes due to the recurrent retinal capillary ischaemia causing chronic capillary ischaemia or remodelling (183, 241). FAZ area and circumference/perimeter may be greater in both aura and non-aura migraineurs, to which retinal ischaemia from vasospasms may contribute (204). Recurrent attacks are associated with intracranial and intraocular vessel spasms, which result in both acute and chronic capillary and blood flow changes in the retina and trigger the FAZ

enlargement via vessel death (204). In one study checking for ocular alterations and clinical differences between non-aura migraineurs and controls, investigators found no difference in FAZ size or VD whether they had accompanying WMH or not (145). This suggests that neither WMH nor aura are necessarily sensitive markers of hypoxic threat. The FAZ size increases with subjective headache pain and disability scores, just as superficial and deep macular VD decrease with age and these subjective scores (145, 270). Women may already have larger superficial and deep FAZ than men, as seen in non-migraine volunteers (268). Therefore, investigators ought to control for similar age and sex proportions between groups evaluated.

CHOROID

OCT can also be used to measure the width of the choroid (144). Fig. 6 shows an example of choroidal thickness measured via OCT (271).

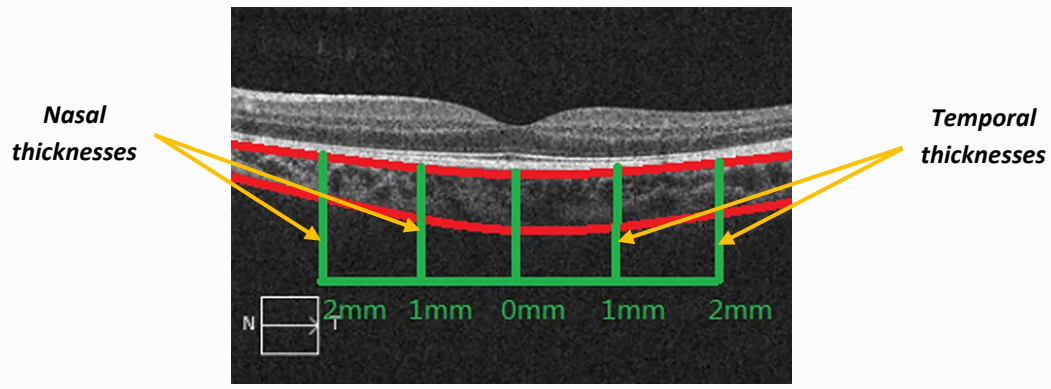


Fig. 6. *OCT cross-sectional imaging of the choroid (271). The choroid is outlined in red; the green vertical lines represent its nasal and temporal thicknesses; the green horizontal lines represent the measured distances (from the fovea), to the right and the left. N: nasal; T: temporal. Adapted from Li et al. (2016; permission requested from authors).*

Choroidal thickness could be another indicator of hypoperfusion associated with migraine. This is because the choroid receives 95% of oxygenated ocular blood flow, so changes in choroidal structure are likely to reflect choroidal and ocular blood flow (143, 272, 273). As up to 90% of the ophthalmic artery blood supply goes to the choroid which then perfuses the outer retina and regulates foveal heat retention, blood supply disturbances here can cause ganglion cell necrosis (274). Indeed, in a previous study, investigators showed that increased blood flow into the choroid leads to its increased thickness (275). Choroidal hypoperfusion can lead to focal ischaemic damage in the optic disc (276). Ocular pulse amplitude is an indirect indicator of choroidal perfusion, reflecting intraocular pressure fluctuation between from systole to diastole which appears to be similar in migraineurs without aura and controls (212). The greater the migraine duration and frequency, the greater the increase in subfoveal choroidal thickness in migraineurs compared to controls (277). Migraineurs may have thinner choroids because of reduced perfusion of the posterior ciliary artery and central retinal vessels (278). Reduced

choroidal perfusion (with subsequent thinning) may cause ischemic damage even in the retina, such that retinal pigment epithelium, photoreceptor and ganglion cells malfunction and die – more so in migraineurs with aura than those without (144).

We found 26 studies assessing the choroid in migraineurs. In general, migraineurs have (often asymmetrically between eyes (233)) thinner choroids than controls (148-150, 224, 233, 279), especially those with aura (148, 229, 230, 238, 251, 277). This may be more pronounced in the non-central choroid (211). We wonder whether a self-regulatory mechanism protects the central choroid whose function it is to supply blood to the outer retina. Gouravani et al. cross-sectionally checked macular choroids and found that migraineurs, both with and without aura, had thinner subfoveal choroids than controls, with aura migraineurs having them even thinner than non-aura migraineurs, although they only assessed 10 studies. Thinner choroids in aura, compared to non-aura, migraineurs might be due to greater hypoxic damage in those with aura (277). During attack-free periods (seven continuous days without an attack), investigators find that choroidal thickness is decreased due to hypoperfusion. For example, in studies using OCT, investigators found that headache-side choroids were thinner in migraineurs with ≥5 migraine attacks per month, especially during attack-free periods; however, this was assessed at the same time of day to avoid diurnal variation between measurements as opposed to the same phase of the migraine cycle (143, 147, 148).

These results were especially associated with migraine history (211, 213, 270), which supports the hypothesis that recurrent transient vasospasms of migraine attacks (especially with aura and trigeminovascular system stimulation) may result in choroidal and retinal thinning (143, 148, 241, 277, 279) through posterior ciliary artery hypoperfusion (241). Chronic migraineurs (especially those with aura) had thinner choroids ipsilaterally to headache than controls (147, 257). In fact, where there is monocular transient loss of vision, the choroidal circulation is implicated (280). Interictally, there may also be predominating vasoconstriction in the peripheral circulation in migraine patients (detected through fingertip photoplethysmography) (193).

Choroidal thickness appears to increase during a migraine attack but decreases between attacks (143, 220, 234). In one study, forty-six ictal migraineurs had thicker central and peripheral choroids than controls, perhaps due to rebound vasodilation (143). Another study found that migraineurs ictally had thicker choroids in both eyes than controls, also possibly from rebound vasodilation (213). Using OCT, investigators found that during a migraine attack, choroidal thickness was greater on the headache side with unilateral headaches (146). With bilateral headaches, foveal choroids increased more in the left than right eyes during migraines (146). The investigators attributed this phenomenon possibly to neurogenic inflammation triggered by cortical spreading depression, reflex autonomic activity due to trigeminovascular activation (which is known to occur in migraines), or indeed altered ocular circulation (146). McKendrick et al. proposed that it may be due to acute vascular variations in the

aftermath of the migraine attack (241). Considering that migraine is associated with decreased perfusion (281), one would expect choroidal thickness to decrease during attacks. Instead, given that the choroid provides blood supply to the outer retina and sometimes thickens during an attack, it appears that the overall ocular vascular perfusion is increased intraictally. We hypothesise that this acute thickening of the choroid during a migraine attack could be a cause or effect of retinal thinning. As a cause, if the brain is underperfused, the choroid thickening may be induced autonomically to compensate, but that may result in an adverse effect on the vascular tone in the retina, leading to retinal thinning. As an effect, the choroid may attempt to compensate retinal thinning by increasing its vascular tone to supply the retina. Similar to within the brain, this may instead cause further constriction of the retinal capillaries (to maintain blood perfusion gradient within the eye), exacerbating the problem. Counterintuitively, in three studies, investigators found choroids thinner ictally than interictally (279) and migraineur choroids thicker than control choroids interictally (213, 228, 282, 283). In one study, investigators found chronic migraineurs have thicker choroids than controls (tested both interictally and ictally) especially during migraines (234). These inconsistencies could be due to different severities and perfusion effects (144) or we think perhaps compensatory vascular changes to reduced focal blood flows surrounding attacks. Inferences were hard to make, because studies rarely examined migraineurs ictally, understandably due to the difficulty in managing patients in pain.

These conflicting results may not be surprising, as the choroid is the most vascular layer of the eye and thus most sensitive to any blood circulation changes (225). These include acute, chronic, internal and external changes, such as caffeine, smoking, medication, age, systemic disease, nicotine, systemic circulation issues, light exposure, diurnal rhythm and sex (165, 277, 284, 285), so all these factors need to be controlled in future studies. For example, menstrual/hormonal changes affect choroidal vascularity (286). Whilst non-aura migraineurs have thinner choroids than controls, this difference becomes smaller with increasing age (277). Choroidal thinning especially occurs in those with chronic migraine, but some studies may be compromised in their ability to detect posterior choroidal changes due to device limitations (149, 229), with those using Enhanced-Depth Imaging (EDI) OCT possibly being more reliable due to better distinguishing of posterior structures of the eye. EDI OCT allows screening of 10 retinal layers and the choroid at 2µm resolution using spectral-domain technology (277). Also, a study showed that at distances of 500, 1000 and 1500 µm from the fovea, there were no differences in choroidal thicknesses between migraineurs and controls interictally (277), whilst others showed no difference in thickness or vascularity index between chronic migraineurs and controls (202, 230, 246, 287) (although investigators often did not specify whether they assessed interictally or ictally. For instance, some investigators found migraineurs have thicker choroids than controls, but they admit their migraineurs may have had attacks during imaging (282)).

DISCUSSION

Migraine is the world's leading cause of disability in terms of productivity loss, given its prevalence in young working people (288). Therefore, it is imperative for affordable, non-invasive and efficient instruments to be developed to monitor this widespread disorder. OCT could not only be used as a diagnostic biomarker for migraine, but also to track its therapy progress (144). We searched several databases and found papers using this technology to examine the differences between migraineurs and controls. Out of 28 studies with episodic migraineurs, 21 studies included those with aura and 25 included those without. Out of 19 studies with chronic migraineurs, 14 studies included those with aura and 14 included those without. Out of the 21 studies with episodic migraineurs with aura, 19 were cross-sectional and 2 were longitudinal. Out of the 25 studies with episodic migraineurs without aura, all 25 were cross-sectional. Out of the 14 studies with chronic migraineurs with aura, all 14 were cross-sectional. Out of the 14 studies with chronic migraineurs without aura, all 14 were cross-sectional. Therefore, it would be interesting to see more longitudinal OCT/OCTA studies with migraineurs, such as in instances where a vasoactive therapy is investigated, and its effects measured before vs. after treatment (Appendix 12).

RNFL, macular and choroidal changes have been examined as a biomarker signs for the symptom of migraine in the last few years (144). Within the macula, the FAZ was paid particular attention. We found that studies generally show that migraineurs have thinner RNFL, GCL and maculae, reduced optic nerve and VD parameters and larger FAZ and their perimeters, than controls. For example, in the retina, a study showed that migraineurs had lower superficial and deep macular, superficial foveal, deep parafoveal and peripapillary VD than controls (183, 202). These findings can indicate retrograde trans-synaptic neuronal degeneration of the retinal ganglion cells from the resultant ischaemia in the posterior visual pathway, which can be seen as a sign in OCT scans (144). It may be worth investigating whether there are different hypoperfusion patterns across the cerebral cortex for the distinct types of vascular headache. The RNFL thinning could mostly be due to many migraine attacks chronically causing optic nerve hypoperfusion (227). As the macular deep capillary density appears to have lower perfusion density in all regions but the fovea in aura migraineurs (204), we wonder whether a self-regulatory mechanism protects the fovea (as a centre of retinal function) from the worst of the hypoperfusion. This calls for further investigation.

Throughout this study, there was the recurring theme of aura and chronic migraineurs having more severe versions of the same results in the various OCT and OCTA parameters than non-aura and episodic migraineurs (Appendix 13). The migraine severity/duration/history/impact was also positively correlated with the severity of these findings. Interestingly, the lower superficial foveal VD and larger FAZ were detected only in females (141), corresponding with the observation that women have a higher ischaemic risk (289). OCT and OCTA can be used to detect significant differences between patients with and without aura, potentially helping with diagnosis and therapy. Migraineurs with aura could have higher cerebrovascular risk (290), corresponding with higher ischaemic risk in the retina

and choroid than controls and those without aura (230), perhaps due to endothelial/smooth-muscle dysfunction and hypercoagulability (291). The posterior cerebral hemispheres are similarly less perfused ictally in aura migraineurs than non-aura (269). Repetitive migraine attacks involving transient vasoconstrictions could ultimately result in permanent retinal and general cerebral damage (214), especially through hypoperfusion of the optic nerve, retina and choroid through cerebral and retrobulbar vessels and retinal and ciliary arteries repetitively constricting over time in chronic migraineurs (144). In fact, a literature review of chronic migraine and OCT imaging admitted that brain hypoperfusion may be implicated in the pathophysiology of migraine, indicating that vascular changes detected may therefore be used as a biomarker for migraine in general (144).

What happens during a single migraine attack? It has been shown that cranial vasodilation is not a prerequisite, nor enough, for migraine onset (292); in fact, it may even be a result of the headache instead (293). Calcitonin gene-related peptide drives meningeal vasodilation inducing transient hyperperfusion (cortical spreading depression), then a longer hypoperfusion or spreading ischaemia; coupled with increased energy demand, the ischaemia intensifies (183). Since ischaemia can be detected in between attacks (141), this suggests a chronic vascular indicator (systemic vasoconstriction) which we can use to diagnose migraineurs (especially those with aura) even when they are not having attacks (141, 193). Ictal changes in optic nerve and RNFL perfusion could lead to hypoxic injury and death of retinal ganglion cells (294), possibly explaining aura and retinal vascular disease in migraineurs (174). Cortical spreading depression is said to initiate aura and can induce hyperperfusion to hypoperfusion transiently in the cortex (204). Cortical spreading depression is associated with hypoxaemic injury, inflammatory activation and a neurovascular mismatch in energy supply and demand (295). Decreased VD which resolves after three hours post-aura (265) could be from transient vasospasm (241). The reduced occipital blood flow reported in some studies (296, 297), coupled with the increased neurological activity in the area (298-300) may explain visual auras which comprise 98-99% of migraine-related auras (301, 302). This is thought to be attributed to transient cerebral vasospasm occurring around the onset of pain, which is also considered a risk factor for optic ischaemic neuropathy (144). Visual auras from occipital cortex hypoperfusion are more common than from retinal/choroidal hypoperfusion (144). Migraineurs are more prone to retinal microvascular disorders (decreased VD and increased FAZ) (183).

Certain retinal layers or regions may be more vulnerable than others to the effects of hypoperfusion. This is particularly evident in the RNFL results above, perhaps due to the studies more extensively covering that layer of the eye. In addition, one paper mentioned that the central macula may be more vulnerable to inflammation and hypoxic/ischaemic insult than the optic nerve head (303). Several studies suggest that perfusion is reduced through the central retinal and posterior ciliary arteries, both ictally and interictally (214, 247, 261, 277), culminating in optic nerve head hypoperfusion, ganglionic retinal necrosis and choroidal vascular insufficiency (241, 278). Reduced choroidal perfusion (with subsequent thinning) may cause ischemic damage in the outer retina (since the inner retina is supplied

by the central retinal artery), such that the retinal pigment epithelium and photoreceptor cells malfunction and die – more so in migraineurs with aura than those without (144).

Other brain areas can also be affected. In previous studies, investigators have found that during a migraine attack even without aura, there is regional hypoperfusion in the occipital areas of the brain (297, 304). Where there is unilateral headache (with or without aura), the hypoperfusion appears to occur only on the affected (with or without aura) side (253, 305). During aura, cortical hypoperfusion could also occur in a more generalised pattern (306), although in migraine attacks themselves, hypoperfusion of the optic nerve and retina has been implicated as discussed above (149, 153). It is during those with aura that there is a greater ischaemic risk (290). Cortical thickness is related to retinal damage and higher vessel resistance (277). Ictally, vasospasm and compromised blood flow usually occurs in one hemisphere, although other parts may also be affected according to a case report (253). In one study, investigators found migraineurs having thicker irises than controls, which they attributed to pupillary dynamic in response to photophobia, although they did not specify how many of their migraineurs had photophobia (282). OCTA studies in migraineur children may show more ocular areas affected, because migraine may affect children more severely (more intense posterior retinal trigeminovascular events) and this may be from a stronger family migraine history (hence early onset) (232).

These changes in vascular tone can also involve the peripheral circulation, not just cerebral (241). Interictally, migraineurs have been shown to have vasoconstriction in the fingertip and dermal capillary networks, as well as abnormal circulation, arteriolar resistance and decreased vessel calibre in the retina (241). Interictally, migraineurs have poor perfusion and increased capillary resistance (183). Vasospasms may indicate generalised/systemic vascular dysregulation (241), with Flammer Syndrome (a constellation of signs and symptoms due to systemic perfusion dysregulation) being a potential differential diagnosis to migraine (307). There are other diseases with similar OCT/OCTA findings such as RNFL and GCL thinning in neurodegenerative diseases (308-311); therefore, these findings alone should not rule out other differential diagnoses. In fact, the vascular changes detected may reflect an ischaemic mechanism underlying all of these disorders in common. An example of this is glaucoma where vascular insufficiencies are known to be involved (241). Here, optic disc ischaemia and optic nerve head ischaemia are also present, which even increases glaucoma risk (241). In addition, neovascularisation leads to obvious changes in both OCT and OCTA. In the case of a patient undergoing active retinal neovascularisation, the changes in OCT and OCTA would confound changes to both images due to systemic conditions. Hence, investigators should assess changes in OCT and OCTA due to systemic diseases after neovascularisation has been managed medically.

Other methods can be used to find additional information such as the following. Using doppler sonography, in another paper, investigators found that it is the actual vascular resistances (central retinal and posterior ciliary arteries) that are increased in migraineurs, even when they are not having

an attack (261). During those attack-free periods, migraineurs with ≥5 migraine attacks per month have greater retinal artery diameter than controls (147). We wonder whether this is a compensatory adaptation to chronically increased oxygen demand. Using magnetic resonance imaging (MRI) scanning, WMH can be superimposed on the OCTA images to show areas especially prone to hypoperfusion in any given patient, also self-evident on the retinal OCTA.

The studies reviewed in this paper were heterogeneous in various parameters and present many confounding factors that need to be addressed to reliably compare OCT and OCTA findings across studies. For instance, while the OCT and OCTA devices are named in the methodologies, their artefact-clearing abilities are not (241). Device type did not affect effect sizes calculated in Gouravani et al.'s 2013 review on OCT (277), so we did not separate studies by device type in our own analyses. But this may be worthwhile doing in the future. Investigators should also control for age and sex proportions between groups. Based on subgroup analyses, there may also be a need for a calibrating calculation between studies using different devices, to be able to make comparisons (277). We observed that the studies vary in methodology (device, scanned ocular areas including foveal diameter and statistical analyses), which makes it difficult to compare results across them. So there is also a need to standardise both measurements and analysis in future studies (183). Smaller effects, such as those from migraine history and frequency, may be camouflaged by the differences in findings arising from different methodologies alone (241). Some parameters are not reported (such as participant migraine history or attack frequency data (141)), while others' findings are compromised by small sample sizes (183, 241, 277). In particular, the studies included participants taking vasoactive medications, so this needs to be uniformly reported and controlled in the future to avoid confounding when measuring vascular tone solely in relation to migraine pathophysiology (174). Most studies were from Turkey, and we would be interested in finding whether the findings could be generalised to migraineurs across the world. Most are cross-sectional studies, precluding us from making causative inferences as to migraine and vascular pathology, such that we cannot tell whether the findings are due to local ischaemia over time or a systemic disorder which also affects the ocular vasculature (183, 241). In our study, we noticed that the vast majority of the case-control studies seem to be cross-sectional, with only case studies looking at migraineurs longitudinally. Hence, we cannot determine cause and effect in terms of vascular tone changes and migraine outcomes. Longitudinal studies could tell us whether cortical vascular damage occurs in migraineurs before or after their migraine history. Failing this, it would help if studies recorded these vascular measurements at similar timepoints in the migraine cycles (241), to be able to compare longitudinally. If evaluating measurements during migraine attacks, we would recommend that measurements be taken within 3 hours of onset. This is because in a recent case study, Kızıltunç et al. took measurements of the optic disc and fovea during a visual aura where migraine pain followed on the right side (265). The right eye showed diffuse narrowing of the retinal vessels, as well as decreased radial peripapillary capillary and superficial and deep foveal vessel densities (265). This improved three hours after the visual aura (265). Since the right eye pain and

headache occurred after the visual aura with vascular constriction, it is possible that eye pain in migraineurs might result from hypoperfusion of the eye (265).

We also looked at the specificity of these ocular changes to migraine alone. Augustin and Atorf (2022) recently evaluated using OCTA in neurological diseases. For migraine, they acknowledge that retinal findings across studies are not consistent, and that macular and/or peripapillary vessel perfusion are often not different from non-migraineous controls (311). McKendrick and Nguyen (2022) agree that while the peripapillary RNFL is usually thinner in migraineurs across studies, macular structural changes are often not seen (241). Other neurological disorders such as glaucoma can have similar focal optic disc and optic nerve head ischaemia (241). Ageing involves vascular damage over time, and so migraine-related findings in older individuals may not be diagnostic of the condition. Any underlying vascular retinal dysfunction (including those in other neurological disorders) may mimic changes seen in migraine. Structural and vascular parameters overlap between migraineurs and non-migraineurs (241), emphasising the importance of using the constellation of symptoms (with the crucial subjective pain component) to assess presence of migraine in an individual. Migraine has been associated with ophthalmic diseases such as central retinal artery and vein occlusions, anterior and posterior ischemic optic neuropathy and normal-tension glaucoma, as well as systemic diseases involving malperfusion such as coronary heart disease, diabetes, preeclampsia, Alzheimer's disease and kidney disease (200), so these would be expected to show similar ocular findings on OCTA. An example of this is FAZ enlargement also occurring in diabetes (312, 313).

In summary, the retina (including macula and its fovea) and optic nerve head can be observed through the relatively new OCT and OCTA technologies and migraine sufferers may benefit from its use as a biomarker for diagnosis, progression and response to therapies. OCT is useful for retinal layer thickness measurement, whereas OCTA is useful to measure vascular density at different areas of the eye. Perhaps OCT and OCTA profiles can be established in the future to determine type of migraine, according to perfusion patterns across different parts of the brain ictally and interictally. Subsequent treatment and management plans would also be influenced by this. There are many issues still to be addressed and we keenly look forward to continued research into the use of OCT and OCTA in migraine.

AUTHOR CONTRIBUTIONS

DC performed the literature search and wrote the manuscript; JC, FC, AL and PD edited the manuscript; MV, JM, RT and VL supervised the PhD candidacy; SD performed the statistical analysis for the PhD project.

CONFLICTS OF INTEREST

None to declare.

CHAPTER 5: RESULTS

The content of this chapter is not yet published.

PAPER 5

The following results paper shows the findings of the clinical trial conducted as the central component of this project.

L-ARGININE AND AGED GARLIC EXTRACT FOR PREVENTIVE TREATMENT OF MIGRAINE: A PHASE-II RANDOMISED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

SUMMARY

BACKGROUND

L-arginine and aged garlic extract (AGE) are common orally administered nutraceuticals which have been used to safely increase vascular tone in cardiovascular disorders. We aimed to compare their efficacy with placebo for preventive treatment of migraine, due to their capillary vasodilatory mechanism.

METHODS

We conducted a single-centre phase-II randomised double-blind placebo-controlled trial in 18-80-year-old migraineurs in Perth, Western Australia. Otherwise-healthy male and female migraines from the community, with at least a 1-year history of migraine, were recruited. After a 2-week placebo period, eligible participants were split using computer-randomised allocation into 2500 mg/day placebo, 1500 mg/day L-arginine, 1000 mg/day AGE, or 1500 mg/day L-arginine + 1000 mg/day AGE, taken daily for 12 weeks of double-blind treatment. We measured changes in migraine parameters from 2 weeks to 12 weeks. Participants were analysed for treatment efficacy and monitored for treatment safety. This study is registered with the Australia and New Zealand Clinical Trials Registry as ACTRN12621001476820 (Universal Trial Number: U1111-1268-1117); the trial remains open to new participants.

FINDINGS

Between 27 May 2021 and 2 February 2023, 586 participants were recruited and assessed for eligibility, of whom 127 were randomly allocated placebo, L-arginine, AGE, or L-arginine + AGE. Seventy-one were ultimately extracted for data analysis. We found that both our primary and secondary outcomes showed that L-arginine was most effective in alleviating migraine disability, pain and vasoconstriction; and that AGE had least efficacy, but which was still greater than placebo. All participants were monitored for safety and analysed for treatment efficacy. The general order of

effectiveness was L-arginine, closely followed by combination, then AGE treatment. No participants discontinued the trial due to adverse effects including death.

INTERPRETATION

Taken daily, L-arginine was most effective for preventive treatment of migraine, and all verum treatments had similar tolerability to placebo. There were no serious adverse events from treatment.

FUNDING

McCusker Charitable Foundation

INTRODUCTION

Migraine, a prevalent, debilitating and costly neurological disorder affecting over 1 billion people worldwide, is suggested to be caused by the dilation of large central arteries. This dilation stimulates local nociceptors and leads to cortical spreading depression which heralds headaches. The prevailing hypothesis suggests that this arterial dilation is the key trigger for migraine onset (314).

In contrast, our alternative hypothesis posits that increased sympathetic tone upon the onset of a migraine causes central capillaries to constrict, resulting in inadequate blood supply to the brain parenchyma. This constriction in turn, triggers local nociceptors, inducing headaches (111). Consequently, restoring brain capillary blood flow emerges as a potential strategy to inhibit headache pain. To explore this avenue, we proposed the use of systemic vasodilators – L-arginine and aged garlic extract (AGE) – as dietary nutraceuticals to prevent both the frequency and severity of migraines (111).

L-arginine and AGE have found clinical utility in alleviating cardiovascular issues by promoting vasodilation (46, 125, 128, 315). L-arginine is a precursor for the vasodilator nitric oxide (NO), and AGE (rich in S-allyl-cysteine and other compounds) offers antioxidant and anti-inflammatory effects that may also be relevant to migraine (160, 316). Nitric oxide, produced by vascular endothelial cells, plays a pivotal role as a vasodilator by regulating calcium homeostasis in smooth muscle cells located in arterioles and pericytes around capillaries (34). Despite their potential benefits and the absence of adverse effects, these compounds have not been thoroughly explored for treating migraines via a microvascular constriction axis. Our study addresses this gap by employing a daily combination of 1.5 g L-arginine + 1 g AGE, aiming to prevent migraines across a broad age range of migraineurs, building on prior case-study research findings (111). We hypothesised that combination treatment would show the greatest efficacy due to synergistic effects between the two nutraceuticals, followed by L-arginine due to its vasodilatory capacity, followed by AGE due to its anti-inflammatory effects, followed by placebo due to a potential placebo effect.

METHODS

STUDY DESIGN

At point of interim analysis, we had completed 71 participants in a phase-II clinical trial. This single-site trial followed a double-blind, placebo-controlled design with three visits at the Perron Institute for Neurological and Translational Sciences and the Lions Eye Institute in Perth, Western Australia. Ethics clearance was granted by the Curtin University Human Research Ethics Committee (Approval: HRE2020-0466; version 4; 16th August 2021).

PARTICIPANTS

We enrolled individuals aged 18 to 80 with chronic frequent episodic migraines from Perth, WA, using diverse advertising methods such as posters around campus, radio advertisements and social-media posts. Eligibility was confirmed via phone calls (see Eligibility Criteria in Appendix 6). During the initial visit, participants shared demographic data, medical and migraine history, and current medications via a questionnaire (Appendix 14). Written consent was obtained.

RANDOMISATION AND MASKING

Participants were computer-randomised into 4 treatment groups (placebo, L-arginine, aged garlic extract (AGE), and L-arginine + AGE) in blocks of 8 by DC. An independent pharmacist mailed interventions to DC with participant IDs only, ensuring blinding. During the 12-week verum phase, all investigators and participants were double-blinded. DC did not disclose the placebo status to participants during those 2 weeks. SD was unblinded to treatment to lead interim analysis once all participants in the pilot phase had completed the study. Capsules were indistinguishable and the masking was successful.

PROCEDURES

During Visit 1, we gathered demographic and long-term information including birthdate, sex, education, medical history, smoking/drinking habits and menstruation details for females (Appendix A). Age was recorded (cross-checked with birthdate) and other anthropometric data were measured in duplicates: height, weight, hip circumference, blood pressure and pulse rate. Blood pressure and pulse rate were checked again during Visits 2 and 3. After Visit 1, participants were given 2 weeks of placebo (cellulose) capsules and migraine diaries collated from validated instruments throughout the migraine pain literature. On Visit 2, completed diaries were collected and participants received a 12-week supply of diaries and verum capsules (Table 1). After 12 weeks, participants came for Visit 3, mirroring Visit 2. For these visits, we collected completed diaries, emptied capsule bottles, obtained fasting blood samples, conducted photosensitivity tests, and performed retinal eye scans using optical coherence tomography angiography (OCTA). On Visit 3, we repeated the long-term questionnaires from Visit 1.

Table 1. *Dosage schedule for 12-week (84-day) treatment (intervention) period. AGE: aged garlic extract.*

	Placebo	L-arginine	AGE
Group A	2500 mg/day	0	0
Group B	0	1500 mg/day	0
Group C	0	0	1000 mg/day
Group D	0	1500 mg/day	1000 mg/day

OUTCOMES

PRIMARY MEASURES

In our clinical trial exploring the potential efficacy of L-arginine and aged garlic extract as prophylactic treatments for migraines, we utilised a comprehensive approach by employing 3 validated migraine pain instruments: the Migraine-Specific Quality-of-Life Questionnaire (MSQ), the Short-Form McGill Pain Questionnaire (SF-MPQ), and daily diaries following International Headache Society (IHS) guidelines. This strategic combination allows for a thorough and multifaceted assessment of the headache burden, providing valuable insight into various dimensions of migraine experience, including its impact on quality of life, pain characteristics and daily patterns. Such a comprehensive evaluation is rare and enhances the robustness of our study and ensures an holistic understanding of the potential benefits of the prophylactic treatments under investigation.

LONG-TERM QUESTIONNAIRES

The long-term questionnaires we used are highly cited, subjective instruments used throughout the literature. These included the Migraine-Specific Quality-of-Life Questionnaire (MSQ) (137), Leiden Visual Sensitivity Scale (L-VISS) (139) and Visual Light-Sensitivity Questionnaire 8 (VLSQ-8) (140). The MSQ comprises 14 items divided into 3 domains assessing the impact of migraine on daily activities and emotions over the previous 4 weeks. The L-VISS evaluates the effect of light- and pattern-sensitivity on daily functioning using 9 questions for within and between migraines, each scored on a Likert scale from 0 to 4. The VLSQ-8 measures the presence and severity of photosensitivity symptoms using 8 items, each scored on a Likert scale of 1-5. The last 2 questionnaires measure the present daily affliction from photosensitivity.

WEEKLY DIARIES

The weekly summaries included the gold-standard validated SF-MPQ (138) which assessed the intensity of different pain characteristics. It comprised a sensory domain of 7 items and an affective domain of 4 items, each with Likert scores ranging from 0 to 3. An average-migraine-pain visual analogue scale (VAS) was also used, scored from 0 to 10. Additionally, participants indicated

concurrent headache pain while completing the questionnaire on a Likert scale of 0-5. The SF-MPQ for the last week of the trial for each participant served as the Visit-3 long-term questionnaire for comparison with their Visit-1 SF-MPQ long-term questionnaire.

DAILY DIARIES

The daily diaries followed International Headache Society guidelines (17, 134) and were collated by the investigatory team from various validated instruments cited widely in other migraine studies. They included: date, migraine occurrence (yes/no), number of migraines, average pain severity (VAS), whether pain relief was used, medication list, prodromal symptoms checklist and prodromal/dromal sensations checklist (see Appendix 9).

SECONDARY MEASURES

OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

Optical coherence tomography angiography shows promise as a surrogate marker for central (brain) capillary tone by non-invasively visualising and quantifying retinal microvascular changes (317). Since the retina shares a vascular supply with the brain, alterations in central capillary tone may be reflected in the retinal microvasculature. OCTA, providing high-resolution retinal blood flow imaging, facilitates the assessment of capillary density, vessel calibre and blood flow patterns. Monitoring these retinal vascular parameters through OCTA may offer insights into systemic vascular changes, serving as a valuable non-invasive tool to investigate and understand central capillary tone dynamics. Retinal scans were obtained using Optovue Avanti XR3 Widefield OCT with AngioVue, offering ~20 µm transverse resolution. Each participant's eyes underwent four scans (two right, then two left): duplicate 3x3mm fields of view (2x sampling density). Parameters measured included foveal avascular zone and vessel densities in various retinal areas. Scans took 5-7 seconds each, minimising interscan time per visit. Given that OCT analysis is sensitive to captured image quality, we only used image quality 7 or above. The quality scores seemed to be already evenly distributed between the treatment groups.

BLOODS

The selection of our biomarkers was based on their relevance to endothelial function and migraine, considering their roles in vasodilation and vasoconstriction. We utilised validated enzyme-linked immunosorbent assay (ELISA) technology to measure plasma concentrations of nitrites and nitrates (NOx), asymmetrical dimethylarginine (ADMA), symmetrical dimethylarginine (SDMA) and endothelin-1 (ET-1). NOx (Sapphire Bioscience) served as proxies for NO-induced vasodilation due to their superior bloodstream stability. ADMA (Jomar Life Research) and SDMA (Jomar Life Research) were employed to assess interictal migraine propensity by reflecting the inhibition of vasodilatory NO production. ET-1 (R&D Systems) enabled the evaluation of vascular tone changes in migraine subjects through its antagonism with NO.

ADVERSE EFFECTS

Participants were instructed to promptly report any capsule-related effects during the trial. We documented these in Microsoft Excel and maintained an adverse-event log (Appendix 15) for negative outcomes.

STATISTICAL ANALYSIS

Data analysis was performed using IBM SPSS® Statistics (version 24.0) by a biostatistician (SD). Intention-to-treat analysis included completed migraine diaries for at least two months. Non-parametric tests were used due to the non-normal distribution of scores. Wilcoxon's test assessed within-group changes, and the Mann-Whitney U test compared between groups. Chi-Square tested demographic data. Significance was set at $p<0.05$, with no multiple-comparison correction due to its pilot nature. Treatment groups were organised as a 2x2 factorial for sex-by-treatment effect. A general linear model analysed continuous variables with baseline as covariate, exploring sex-treatment interaction. A logistic regression model assessed categorical outcomes. Treatment effects were expressed as mean differences or odds ratios with 95% confidence intervals. Residuals were examined to validate assumptions.

ROLE OF THE FUNDING SOURCE

A philanthropic donation by the McCusker Charitable Foundation is gratefully acknowledged.

RESULTS

There were 127 participants recruited between 27 May 2021 and 2 February 2023, with 71 participants included in the analysis. The trial profile is shown in Fig. 1, and the analysed participants' relevant baseline characteristics are given in Table 2. Baseline statistical analyses showed comparable demographics and anthropometrics between the treatment groups, including F-test variabilities.

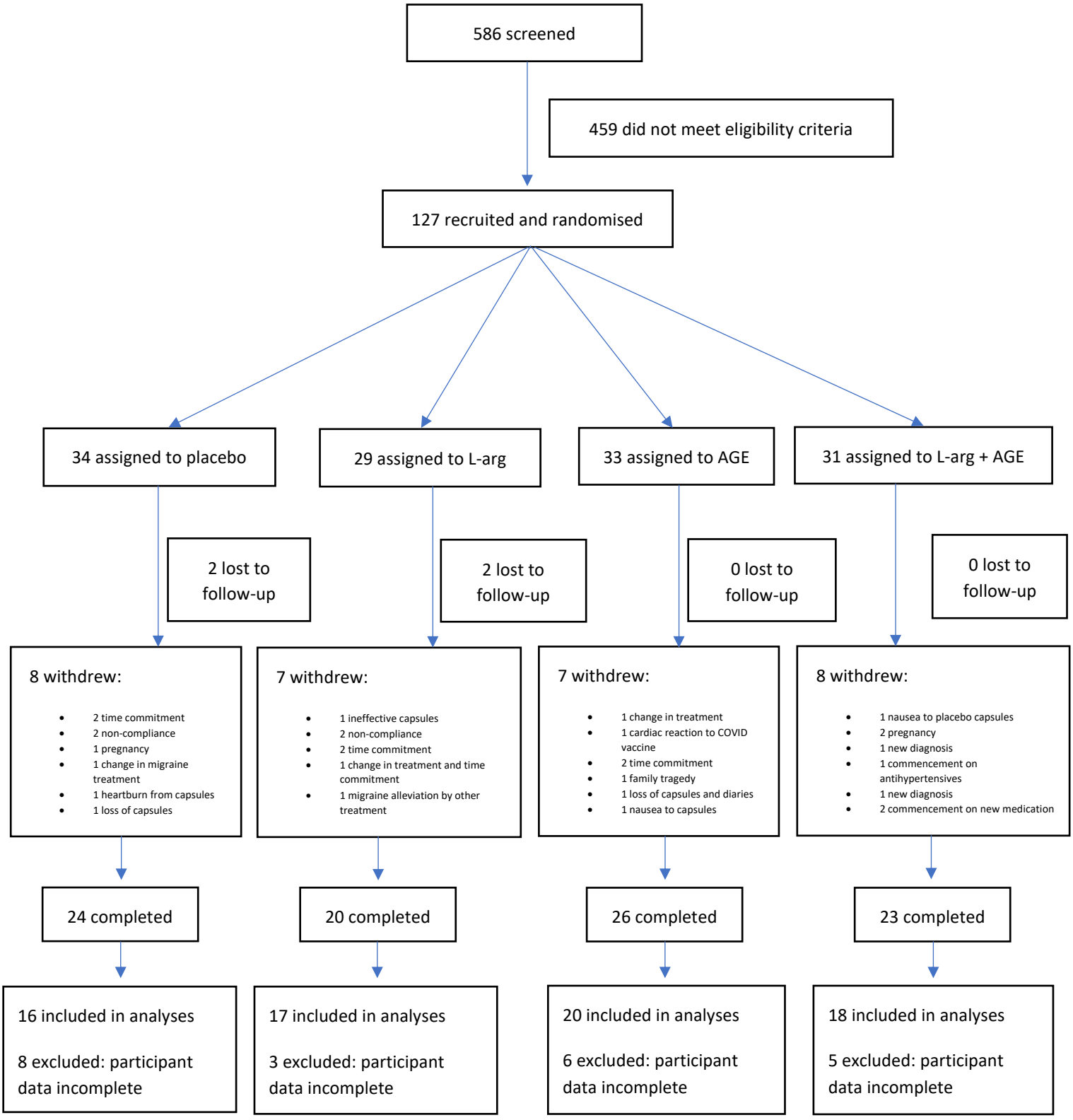


Fig. 1. *Trial profile. AGE: aged garlic extract; COVID: coronavirus disease; L-arg: L-arginine.*

Table 2. *Baseline demographics and anthropological baseline characteristics of the trial-complete participants, N = 71.*

	Placebo (n=16)		**L-arginine** (n=17)		**AGE** (n=20)		**L-arginine + AGE** (n=18)	
Sex								
Male	6 (37.5%)		4 (23.5%)		2 (10.0%)		5 (27.8%)	
Female	10 (62.5%)		13 (76.5%)		18 (90.0%)		13 (72.2%)	
Age (years)	55.2 (34-71)		51.7 (20-77)		44.0 (18-68)		49.0 (20-68)	
Ethnicity								
Caucasian	14 (87.5%)		15 (88.2%)		20 (100.0%)		16 (88.9%)	
Non-Caucasian	2 (12.5%)		2 (11.8%)		0 (0.0%)		2 (11.1%)	
Migraine family history								
Present	9 (56.3%)		11 (64.7%)		18 (90.0%)		9 (50.0%)	
Not present	7 (43.8%)		6 (35.3%)		2 (10.0%)		9 (50.0%)	
Smoking in last 3 months								
Present	0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)	
Not present	16 (100.0%)		17 (100.0%)		20 (100.0%)		18 (100.0%)	
Menstruation (out of females only)								
Present	5 (50.0%)		7 (53.8%)		11 (61.1%)		8 (61.5%)	
Not present	5 (50.0%)		5 (38.5%)		7 (38.9%)		5 (38.5%)	
Regular hormone-replacement therapy (out of females only)								
Present	2 (20.0%)		1 (7.7%)		2 (11.1%)		2 (15.4%)	
Not present	8 (80.0%)		12 (92.3%)		16 (88.9%)		11 (84.6%)	
Body-mass index (kg/m²)	25.02 (21.7-25.5)		22.93 (22.9-23.0)		24.58 (20.6-26.9)		26.14 (21.0-25.0)	
Blood pressure (mmHg)	**SYS**	**DIA**	**SYS**	**DIA**	**SYS**	**DIA**	**SYS**	**DIA**
Baseline	125.3 (103.5-151)	78.9 (64-102.5)	127.8 (99.5-157.5)	78.8 (64-96.5)	127.3 (103-154.5)	81.2 (65.5-98.5)	120.3 (96-139.5)	79.1 (65-94.5)

Delta change (end of study – baseline)	-8.2	-4.1	-4.5	-0.5	-1.9	-1.5	-1.3	-2.0
Minimum change	-12.5	-2.5	-2.0	2.5	0.5	2.0	-3.5	-2.5
Maximum change	-14.0	-13.0	-2.5	-4.0	28.0	4.0	9.5	-0.5

Categorical variables are presented as n (%); continuous variables are presented as mean (range). All values are to 1 d.p. where applicable. SYS: systolic; DIA: diastolic.

Three validated migraine instruments were employed to comprehensively assess potential verum effects on migraine burden over the 12-week study period. Primary outcome results revealed improvements in long-term questionnaire scores as measured by the Migraine-Specific Quality-of-Life Questionnaire (MSQ). Unlike generic quality-of-life questionnaires, the MSQ is tailored specifically to evaluate how migraines impact various aspects of an individual's life including physical functioning, role functioning (such as work or household responsibilities) and emotional well-being. The MSQ effectively captures these migraine-specific burdens, providing insight into the extent to which migraines interfere with activities, relationships and overall quality of life. When comparing the MSQ scores between the baseline and final visit, reporting the previous 4-week lived experience, Fig. 2a displays the median with interquartile range at the measured baseline and at the conclusion of the study for all treatment groups across the three domains of MSQ – “preventive”, “limiting” and “emotional”. Group comparison suggests that L-arginine and combination treatments significantly improved the preventive domain scores, L-arginine alone significantly improved the limiting domain score, and no verum treatments significantly changed the emotional domain scores. However, normalising for interindividual variability, Fig. 2b-e illustrate score differences for participants between the pre-treatment and post-treatment visits in each domain. Placebo demonstrated no significant effects in the preventive, limiting or emotional domains (Fig. 2e). L-arginine intervention significantly enhanced scores in the preventive, limiting and emotional domains (Fig. 2b). Meanwhile, AGE improved only emotional domain scores (Fig. 2c). The combined treatment also significantly improved preventive and emotional domain scores (Fig. 2d).

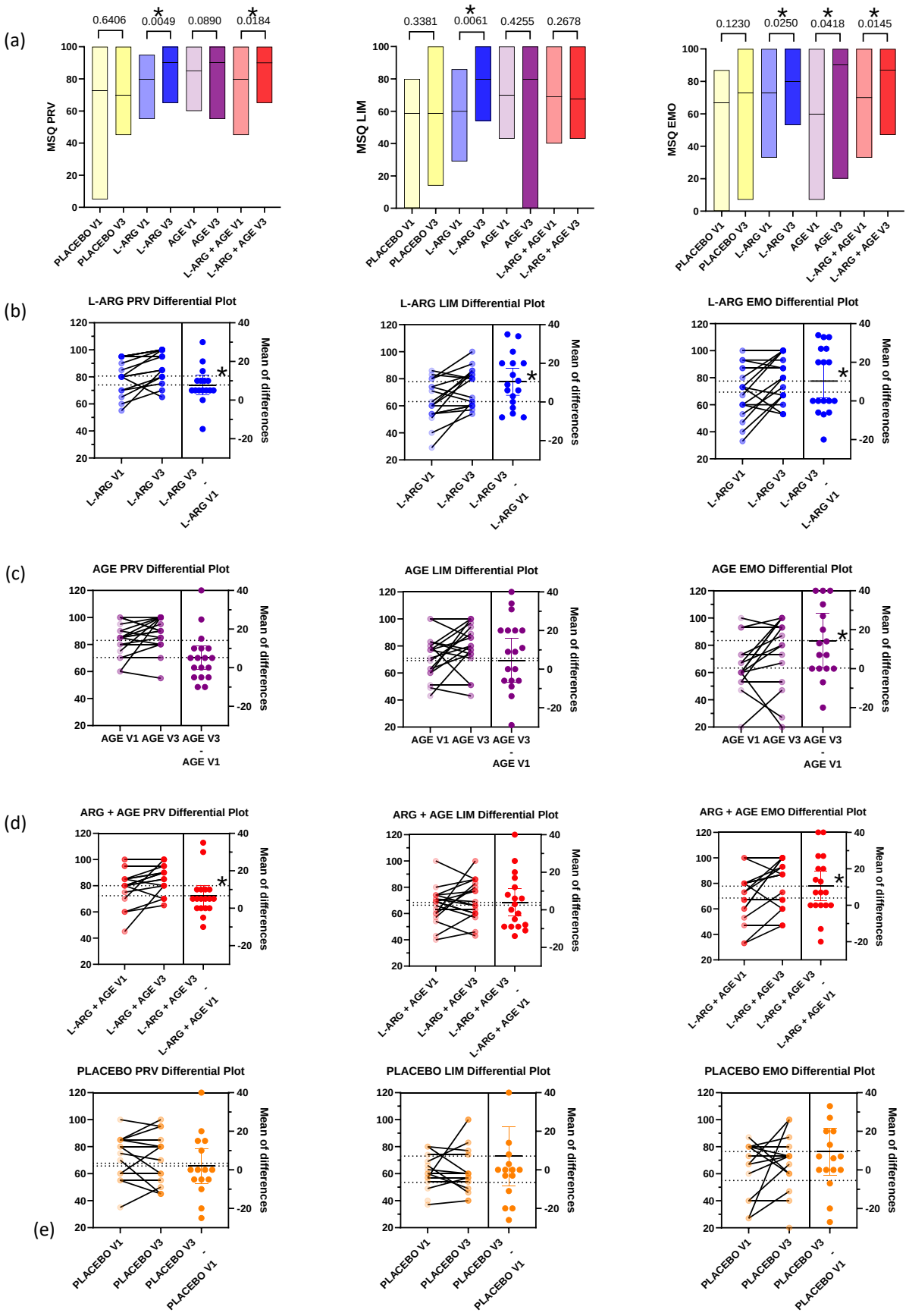


Fig. 2. *(a) Box plots of the preventive (PRV), limiting (LIM) and emotional (EMO) domain scores of the MSQ. (b) Differential plots of the (b) L-arginine group's, (c) AGE group's, (d) L-arginine + AGE group's, and (e) placebo group's scores in the MSQ preventive (PRV), limiting (LIM) and emotional (EMO) domains. AGE: aged garlic extract; L-arg: L-arginine. V1: Visit 1; V3: Visit 3. *p<0.05.*

The SF-MPQ was the second validated instrument selected to assess the putative effect of intervention on migraine burden. The most compelling attribute of the SF-MPQ is its multidimensional approach to pain assessment. The SF-MPQ not only evaluates the intensity of pain experienced by migraineurs, but also captures various qualitative aspects of pain. This assessment provides a nuanced understanding of the migraine experience beyond just pain intensity. Fig. 3a depicts the treatment effect for the affective + sensory score shown as the mean slope per group overlaying weekly individual reporting. The slope for L-arginine was significantly steeper than for placebo, with comparable effects suggested in combination. Allowing for interindividual variability, the delta score change (treatment effect size) appeared doubled in the L-arginine group compared to placebo, but this was not statistically significant (Fig. 3b). Treatment with AGE alone had no remarkable effect, and no synergic action with L-arginine was detected.

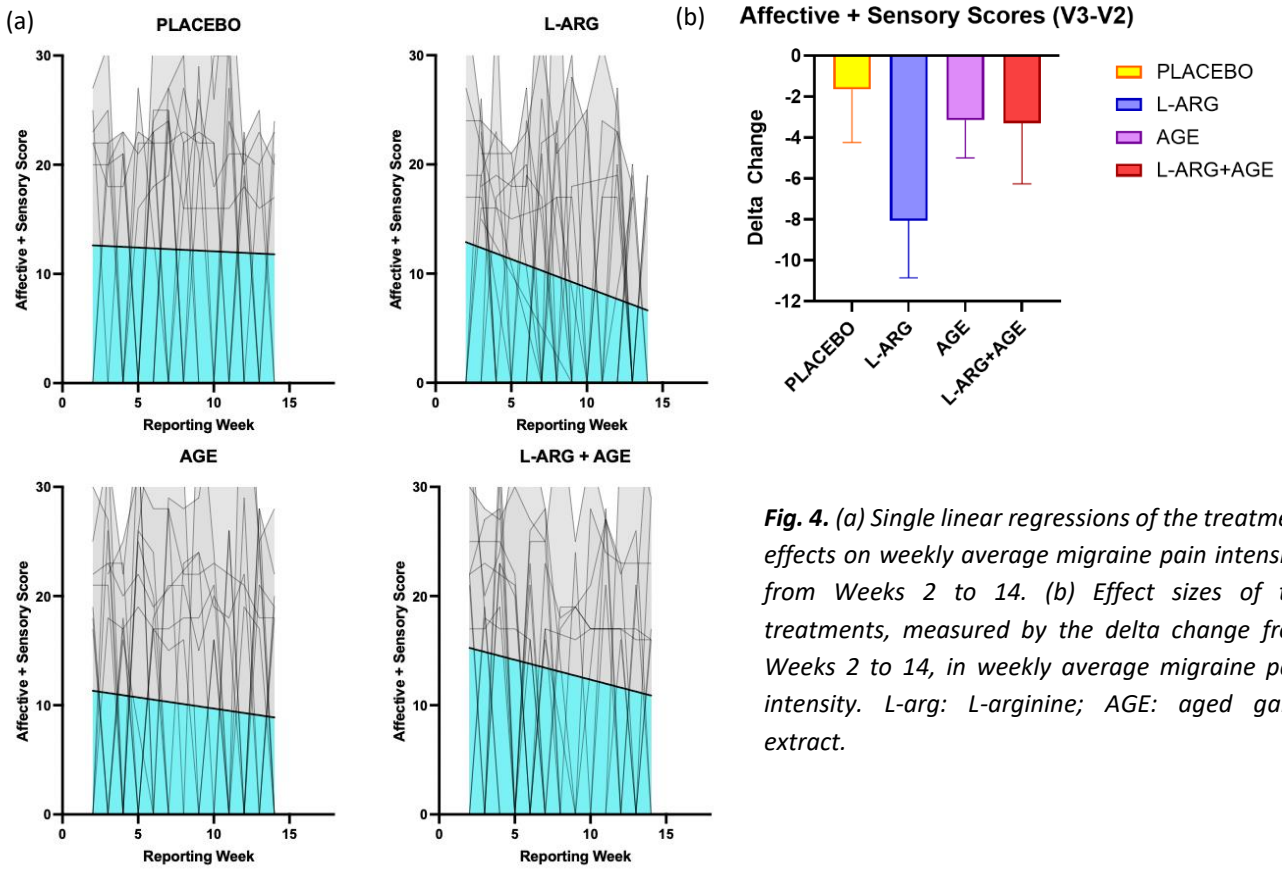


Fig. 4. *(a) Single linear regressions of the treatment effects on weekly average migraine pain intensity, from Weeks 2 to 14. (b) Effect sizes of the treatments, measured by the delta change from Weeks 2 to 14, in weekly average migraine pain intensity. L-arg: L-arginine; AGE: aged garlic extract.*

The visual analogue score captured via SF-MPQ suggested a potential positive effect with combination therapy (Fig. 4a-b). However, adjusting the treatment effect size for interindividual variability was not sufficiently sensitive with the number of subjects studied.

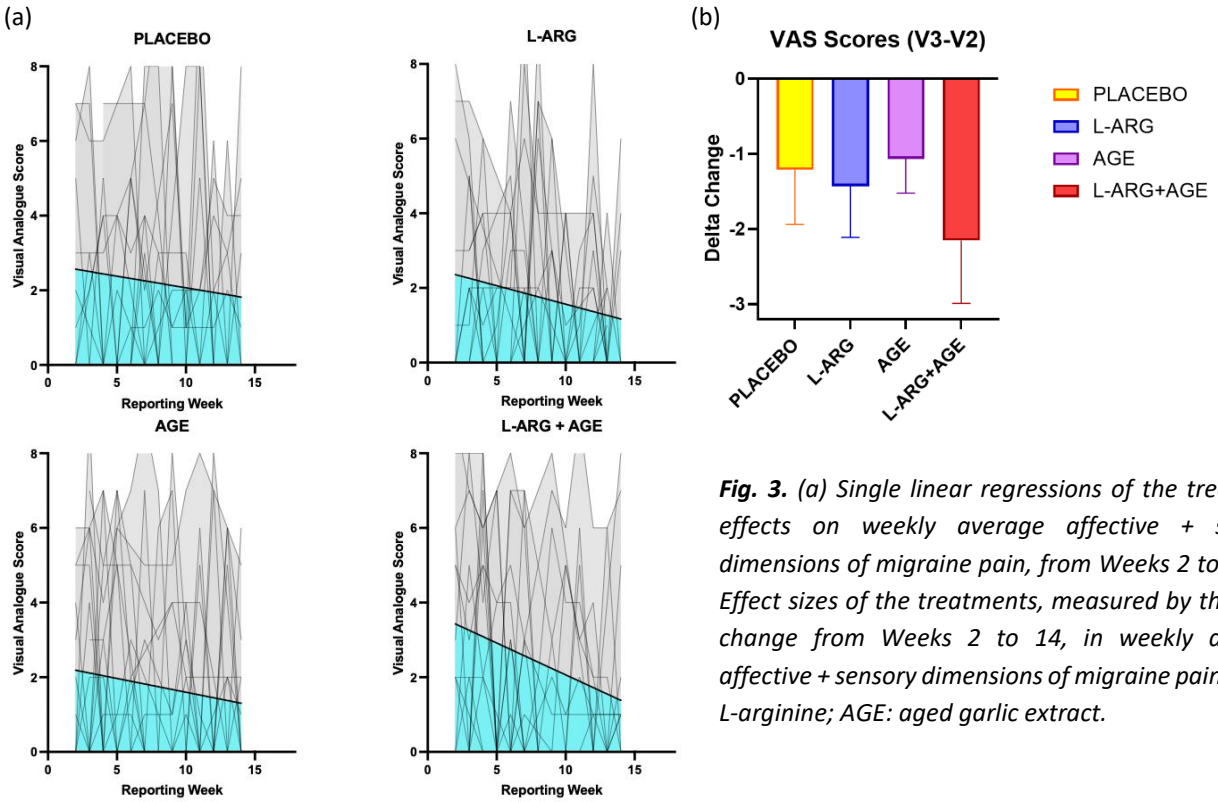


Fig. 3. *(a) Single linear regressions of the treatment effects on weekly average affective + sensory dimensions of migraine pain, from Weeks 2 to 14. (b) Effect sizes of the treatments, measured by the delta change from Weeks 2 to 14, in weekly average affective + sensory dimensions of migraine pain. L-arg: L-arginine; AGE: aged garlic extract.*

The third validated instrument adopted to assess migraine burden were daily diaries following IHS guidelines. The strength of these is their ability to provide detailed and real-time information about migraine symptoms, triggers and associated factors over an extended period. Daily diaries offer valuable insights into key migraine phenotype

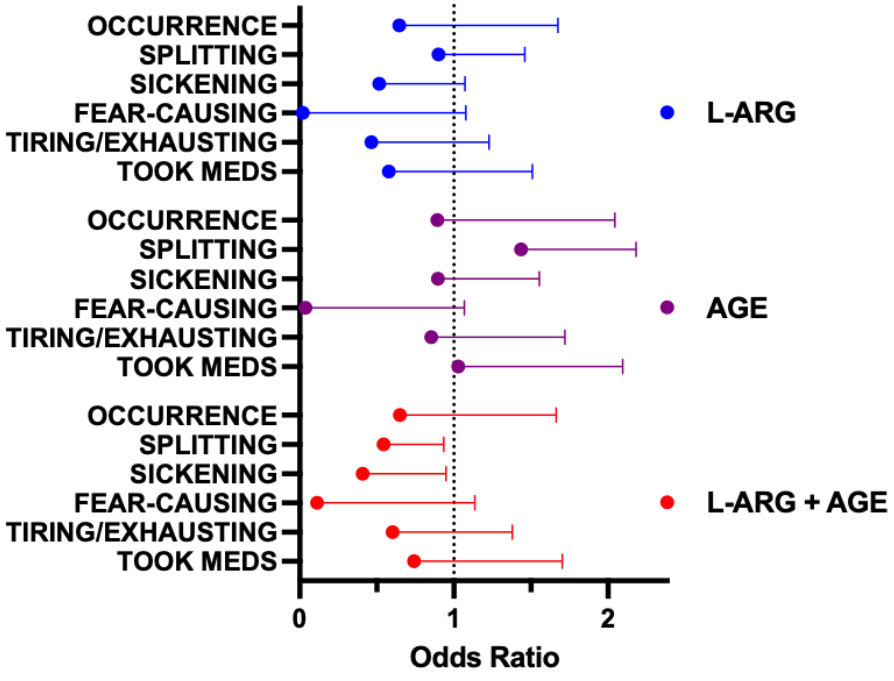


Fig. 5. *Odds-ratio graph of positive effects on migraine subphenotypes of the daily diary. AGE: aged garlic extract; L-arg: L-arginine; meds: abortive medication. Odds ratios for each verum treatment are the probabilities, compared to placebo (OR = 1), of the likelihood of that subphenotype.*

experiences that significantly impact the quality of life. Fig. 5 shows that strong positive effects were observed for the pain subphenotypes of occurrence, splitting, sickening, fear-causing and tiring/exhausting, as well as for the use of abortive medication. Specifically, the L-arginine and combination treatment groups demonstrated the most improvement, with less effect realised for the AGE treatment group. Notably, the photosensitivity test and questionnaires (L-VISS and VLSQ-8) provided inconclusive results in this pilot trial.

OCTA enables quantitative analysis of various parameters related to retinal microvasculature, including vessel density, perfusion density and vascular morphology. These quantitative metrics can serve as objective markers of treatment response and help elucidate the precise effects of capillary vasodilators on microvascular function within the retina and potentially the brain. This study is the first to adopt OCTA (176) in a migraine randomised clinical trial (RCT) as an extension of the proposed mechanism of action of our verum treatments being capillary vasodilation. Treatment effects on retinal vascular density (the surrogate marker for capillary dilation in the absence of angiogenesis in superficial and deep retinal capillaries), taken at resolution 3x3 on OCTA, are indicated in Fig. 6. Vessel density (VD) is taken as the length/area of flowing vessels as a percentage of total area scanned, or the percentage area of vessels with active (OCTA-detectable) blood flow (141, 145). Fig. 6 demonstrates that in the left eye, vessel density, a surrogate marker of capillary diameter, was markedly augmented in both the superficial and deep retinal microvasculature by L-arginine treatment. Remarkably, there was a substantially different treatment effect realised for the right-eye assessment of vessel density, with a significant effect of density reported more so with combination therapy for both the superficial and deeper microvasculature regions. Even so, L-arginine's effect in the left eye was greater in magnitude than that of combination treatment in the right eye. Placebo and AGE had similarly negative effects on vessel density in both eyes, placebo more so in the left eye.

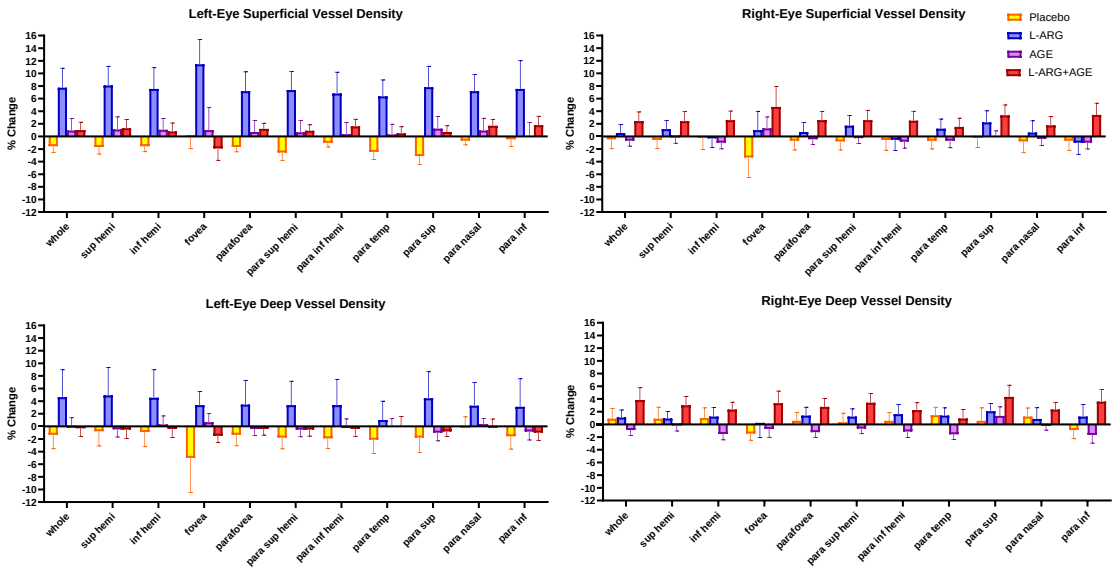


Fig. 6. *Bar charts showing within-group percentage change, due to treatment, of retinal vascular densities, taken via 3x3 OCTA. AGE: aged garlic extract; hemi: half; inf: inferior; L-arg: L-arginine; para: surrounding; sup: superior; temp: temporal. *p<0.05.*

The selected blood biomarkers of ADMA, SDMA, ET-1, nitrate and nitrite offer valuable insights into NO-induced vasodilation by reflecting key aspects of NO metabolism, endothelial function and vascular tone regulation. Monitoring these biomarkers can provide insight of capillary function and the potential effectiveness of interventions targeting NO signalling pathways in promoting vasodilation and mitigating vasoconstriction-related disorders, including migraine. For analysis and to allow for interindividual variability, we considered the ratio difference and delta change between start (Visit 2) and end (Visit 3) of trial. The table below shows the delta change and within-group paired difference analyses (Table 3).

Table 3. *Difference in plasma vascular tone marker concentrations from baseline (Visit 2) to end of study (Visit 3). ADMA: asymmetrical dimethylarginine; AGE: aged garlic extract; L-arg: L-arginine; NOx: nitrites and nitrates; SDMA: symmetrical dimethylarginine. Significant values are written in bold. *p<0.05.*

Metabolite	**Treatment group**	**Mean at baseline**	**Mean delta change**	**Interindividual p-value**
ADMA	Placebo	372.97 ng/ml	-100.17 ± 13.42	0.097
	L-arginine	324.34 ng/ml	-114.15 ± 30.34	**0.002**
	AGE	232.26 ng/ml	-43.96 ± 13.88	**0.006**
	L-arginine + AGE	239.47 ng/ml	-29.90 ± 4.934	0.31
SDMA	Placebo	286.08 ng/ml	-36.58 ± 0.07025	0.59

	L-arginine	292.87 ng/ml	-62.39 ± 0.06341	0.47
	AGE	332.96 ng/ml	-15.71 ± 0.07192	0.57
	L-arginine + AGE	267.24 ng/ml	66.29 ± 0.07791	0.99
ET-1	Placebo	1.42 pg/ml	0.39 ± 0.1422	0.07
	L-arginine	1.29 pg/ml	0.23 ± 0.08266	**0.04**
	AGE	1.54 pg/ml	0.04 ± 0.07026	0.73
	L-arginine + AGE	1.34 pg/ml	0.31 ± 0.1365	0.08
Nitrite	Placebo	1.87 µM	-0.71 ± 0.2071	0.28
	L-arginine	2.53 µM	-1.47 ± 0.2190	**0.009**
	AGE	1.58 µM	-0.14 ± 0.2538	0.85
	L-arginine + AGE	2.52 µM	-0.87 ± 0.3196	0.33
Nitrate	Placebo	25.86 µM	-0.27 ± 0.1652	0.94
	L-arginine	18.00 µM	3.63 ± 0.1303	0.174
	AGE	16.56 µM	3.91 ± 0.1390	0.09
	L-arginine + AGE	16.97 µM	3.35 ± 0.2116	0.19

Table 3 shows that L-arginine had a highly significant reduction in blood nitrite (p=0.009), concomitant with a significant reduction in ADMA and minimal increase in ET-1. Intervention with AGE also significantly decreased ADMA, but without remarkable effect on NOx or ET-1. Interestingly, ingestion of the formulated combination nutraceuticals abolished the strong effects of single treatment with L-arginine on nitrite and ADMA, and AGE on ADMA, respectively.

Regarding adverse events and discontinuation rates, 12 participants experienced adverse effects: placebo (6), L-arginine (2), AGE (3) and combination (1). Among these, 3 withdrew (2 from placebo due to nausea and heartburn, 1 from L-arginine due to non-specific gastrointestinal symptoms). The remaining 9 continued after resolving mild, mostly gastrointestinal issues. Attrition due to other reasons was 23.8% (30 out of 126).

DISCUSSION

We undertook a study to explore a novel migraine-prevention mechanism. To this end, we used the vasodilatory and anti-inflammatory agents, L-arginine and AGE, to mitigate constrictive and inflammatory pain pathways leading to migraine episodes. Briefly, L-arginine provides fuel for NO synthesis in blood vessels systemically, and this NO causes vessel dilation, precluding capillary

constriction which we hypothesise effectuates malperfusion underlying headache pain; and AGE contains various anti-inflammatory compounds alongside a heightened concentration of the active vasodilatory ingredient S-allyl-cysteine. Our primary outcomes align with our vasoconstrictive hypothesis. In general, vasodilatory treatments, especially L-arginine followed by combination, may improve migraine parameters, with AGE showing some effectiveness.

Our primary outcomes consisted of validated subjective questionnaires from the migraine literature. The MSQ demonstrated the expected effect, with no significant improvement observed in the placebo group between the initial and final visits. In contrast, L-arginine exhibited improvements across all three domains of the questionnaire, while AGE showed enhancement only in the emotional domain. Interestingly, combination treatment led to improvements in both preventive and emotional domains, although no notable change was observed in the limiting domain. We question whether this discrepancy might stem from a genuine effect, or rather, from the limited statistical power of this interim-analysis pilot trial. Should this disparity indeed represent a genuine effect, it is plausible that L-arginine's putative superior efficacy could be impeded by the presence of the combination treatment, potentially due to interference in absorption within the digestive tract. Furthermore, it is conceivable that the preventive effects of L-arginine might pre-empt the downstream anti-inflammatory effects typically targeted by AGE.

We used the validated SF-MPQ to monitor migraine pain manifestations on a weekly basis among our migraineurs, and the results from the weekly diaries pertaining to the sensory and affective domains were consistent with both our findings from the MSQ and the daily-diary records. Through the weekly diaries, the visual analogue scale assessing pain severity demonstrated the expected trend, with the combination treatment exhibiting the most substantial preventive effect, followed by L-arginine. This observation aligns with the composition of the combination treatment which includes both vasodilatory L-arginine and anti-inflammatory AGE. However, in the sensory and affective domains of the questionnaire, L-arginine and the combination treatment showed comparable effectiveness. Once again, this similarity in efficacy could either indicate a genuine effect, or reflect the limitations imposed by the sample size.

The daily diaries characterise common experiences of chronic migraineurs. The interim findings demonstrate that strong positive effects were observed for the pain subphenotypes of occurrence, splitting, sickening, fear-causing and tiring/exhausting, as well as for the use of abortive medication. The daily diaries suggest an inferior effect of AGE compared to L-arginine, with the caveat of potential interactive effects of formulated concomitant administration.

This study represents the first endeavour to incorporate longitudinal OCTA measures within the framework of a migraine RCT. The deeper retinal capillary network, particularly within the inner plexiform and inner nuclear layers, assumes a pivotal role in meeting metabolic demands. Simultaneously, the superficial capillaries form part of the intricate microcirculatory network essential

for sustaining vision. The proximity of these capillaries to the nerve fibre layer and ganglion cell layer underscores their significance in transmitting visual information from photoreceptor cells to the brain. Maintaining an adequate blood supply from the superficial capillaries is critical for meeting the energy requirements of ganglion cells and supporting their neural functions.

OCTA assessments of both the left and right eyes revealed that in the left eye, L-arginine markedly increased superficial and deep vascular density, serving as a surrogate marker of capillary dilation. Intriguingly, in the right eye, combination therapy involving L-arginine + AGE consistently demonstrated superiority in enhancing both superficial and deeper capillary networks.

The paradoxical differences observed between the left and right eyes may be associated with lateral or contralateral aspects of migraine pathology, particularly concerning the focal centre of central migraine pain. Migraine often presents with unilateral headache pain, and this unilateral manifestation may be linked to lateralised alterations in vascular dynamics or to neural processing. Further investigations are warranted to elucidate the specific mechanisms driving these differential responses and their association with lateral aspects of migraine symptomatology. We did not compare raw differences at baseline; rather, we were interested in the interindividual changes between start and end of treatment period. It would have been interesting to see if baseline values were related to migraine severities/frequencies. We would expect higher severity/frequency to correlate negatively with retinal vessel densities.

Blood biomarkers relevant to endothelial function and migraine, reflecting roles in vasodilation and vasoconstriction, were employed to evaluate the putative effects of L-arginine and AGE. Asymmetric dimethylarginine (ADMA) is an inhibitor of endothelial nitric-oxide synthase (eNOS), and increased plasma levels may indicate lower rates of eNOS activity and reduced production of vasodilatory nitric oxide (NO). Previous studies have reported that chronic migraine patients have low plasma L-arginine levels and higher levels of ADMA, ornithine and monomethyl arginine (318). Additionally, a study showed an increase of tyramine, a TAAR1 agonist, in migraineurs, which may be significant because endothelial TAAR1 inhibits L-arginine metabolism into NO synthesis and release (319).

In this study, considering interindividual variation through analysis of end-of-study measures versus baseline for each patient, both L-arginine and AGE were found to significantly decrease plasma ADMA by 33% and 17% respectively, potentially contributing to central microvascular dilation via increased NO production. Symmetric dimethylarginine (SDMA) may also impact L-arginine availability by competing with transport to eNOS. However, in contrast to the ADMA findings, paired analysis found no suggestion of a significant treatment effect on SDMA homeostasis.

Endothelin-1 is a vasoconstrictor protein that directly inhibits eNOS synthesis and regulates intracellular signalling pathways. Allowing for interindividual variability, the study suggests a small 2.1% increase in ET-1 in L-arginine-treated patients, significant at $p=0.04$. We suggest this is not

physiologically relevant and may reflect a type-I error due to the small sample size. Rather, consistent with the hypothesis of increased NO anabolism, we found that L-arginine significantly decreased plasma abundance of nitrites, the intermediary of nitrate conversion to NO, by approximately 49%. Surprisingly, the effect was not achieved when both agents were ingested simultaneously, suggesting that some components of AGE interfere with the metabolism of L-arginine to NO, consistent with the potential for contraindicated effects. Further research is needed to elucidate the specific interactions between these two agents and their impact on endothelial function and microvascular dynamics.

To summarise across all measures, we believe that L-arginine followed by combination treatments may have been the most effective in alleviating migraine symptoms over the 12 weeks of treatment, with AGE alone showing a more limited effect. The Migraine-Specific Quality-of-Life Questionnaire (MSQ), Short-Form McGill Pain Questionnaire (SF-MPQ), daily diaries and OCTA results all indicate that L-arginine is effective, followed by combination treatment. We initially expected the combination treatment to have the strongest effect, followed by L-arginine alone and then AGE. However, there may be absorption or interference effects between L-arginine and AGE that we are unaware of, which could have hindered the combined efficacy. Alternatively, the study may have simply been underpowered. Indeed, the research team received 12 unsolicited testimonials from participants essentially describing "life-changing" effects. Of these, ten of the testimonials were from patients on combination therapy and one each for patients on single treatment. No such testimonials were received from participants on placebo treatment.

Key strengths of our study and interim analysis include the assessment of interictal outcomes alongside ictal burden, thereby recognising the significant impact of migraine beyond the acute attack phase. Our longitudinal use of OCTA enabled direct visualisation of changes in capillary tone, albeit with unexpected differential findings for each eye. By extending the eligible participant age range beyond the standard 18-65 years old, we ensured inclusivity for older patients meeting the eligibility criteria. Our treatments were effectively aimed at enhancing function, decreasing attack frequency and severity, and mitigating disability, all aligning with migraine-preventive goals.

To further strengthen the study, incorporating objective measures of migraine severity and disability – such as neuroimaging techniques – could provide further insight into treatment efficacy. Moreover, exploring potential mechanisms underlying the observed treatment effects – such as changes in neurovascular coupling or inflammatory markers – could deepen our understanding of migraine pathophysiology and treatment response.

Study limitations include a limited subject pool, resulting in underpowered results. This was due in part to the large dropout of potential candidates for study participation. Our eligibility criteria were stringent, and most candidates were rejected due to vascular comorbidities which could pose as confounders to the study given our vascular measurements, change in anti-migraine or vascular medication during the trial, Botox treatment or use of migraine polypharmacy which had the potential

of masking our treatment effects. Another contributor to the small sample size analysed would have been the number of dropouts during the trial after enrolment. We noticed that these were mostly due to timetabling issues or non-compliance on the part of the participants. In hindsight, intention-to-treat analysis could have been performed to undermine the effects of sample size compromise. Other limitations include unexplored influences of diet and exercise on vascular tone; non-isolated effects of L-arginine within AGE; indistinct separation of anti-inflammatory, antioxidant and vasodilatory impacts of AGE; and omission of exploration of neural contributions in the neurovascular pathophysiology of migraine. Our age range was 18-80 years, excluding children and adolescents for legal reasons. We focused solely on certain migraine types, excluding tension headaches and various other minority types. Participants with specific comorbidities, vascular disorders, and medications or Botox treatment were excluded. Dosage studies for L-arginine and AGE were not conducted; future trials may explore this. Other systemic vasodilators were not studied, and the potentially altered bioavailability of oral treatment in combination may need exploration. Conventional and potential migraine biomarkers, as well as other biomarkers of endothelial dysfunction, were not investigated. Exploring inhibitory and excitatory components of the L-arginine-NO pathway, as well as investigating additional symptoms like phonophobia and nausea, would provide valuable insights. We did not utilise other available migraine pain subjective instruments or outcomes. In selecting questionnaire instruments, we excluded MIDAS due to its 3-month timeframe overlapping our trial, and HIT-6 due to its double-barrelled questions. There are also new instruments in development that could be investigated to address current limitations or add migraine-management features. Comparing L-arginine and AGE's efficacy to conventional migraine medications (calcitonin gene-related peptide (CGRP) antagonists, triptans/ditans etc.) was beyond this pilot study scope. Combining L-arginine and AGE (or other vasodilators) with established non-pharmacological migraine therapies or studying drug interactions with conventional medications would be intriguing directions. Extending the 14-week trial and including a post-capsule-cessation observation period would also provide valuable insights. Comparing therapeutic results with migraine relapse time after stopping CGRP antagonists or other mainstream medications, as well as studying chronic vasculature changes, would also be insightful. We also did not measure treatment effect on duration of migraine, as the participants could take an abortive medication at any time and sleep patterns interfere with participants' ability to distinguish between migraine episodes. Indeed, we recently published a clinical case report where combination treatment progressively improved the patient's migraine duration (161). Defining a "migraine day" more concisely and using appropriate treatment-assessing instruments could also enhance future trials. Including participants with medication-overuse headaches and exploring nutraceutical effects on various headache/migraine types could also expand our understanding.

Our sample exhibited a female bias which is commonly observed among migraineurs. The literature indicates that females tend to experience more severe and prolonged migraine episodes, along with greater disability and a higher risk of becoming chronic. These differences between genders are attributed to hormonal, genetic, neuronal and brain structural factors. Mechanisms include prolactin

inducing migraine through neuronal excitability and enhancing calcitonin gene-related peptide (CGRP) levels, estrogen withdrawal impacting vascular tone and sensitisation, and oxytocin withdrawal affecting trigeminal neuronal activity, both in menstruating and post-menopausal women.

Further investigations could explore additional OCTA measures such as intercapillary space and retinal arteriolar thicknesses for vascular wall integrity. Additionally, assessing interictal choroidal thickness and retinal thickness measurements could offer insights into the structural integrity of different ocular layers, including the nerve fibre layer, ganglion cell layer, inner and outer plexiform layers, and the photoreceptor layer. Changes in retinal thickness can indicate structural alterations or damage to these layers. OCTA provides high-resolution imaging of retinal thickness, enhancing the ability to detect early signs of vascular dysfunction including capillary dropout. Therefore, in future studies, adding retinal thickness to the list of measures could provide valuable information regarding vascular and structural changes associated with migraine.

An adaptive trial design will now be considered based on the generally positive findings of this pilot study, because it allows for flexibility in modifying the trial protocol based on accumulating data as the trial progresses. Moreover, an adaptive trial design allows for the exploration of additional research questions or hypotheses that may arise during the trial. An adaptive trial design will offer the flexibility to optimise trial efficiency, maximise the likelihood of detecting treatment effects and generate more robust and informative results, advancing our understanding of the efficacy and safety of the interventions under investigation.

An ideal prophylactic treatment for migraines should be orally administered, have lasting effects and minimal side effects, effectively preventing migraine attacks. The lack of such effective and well-tolerated preventive treatments, combined with patient preference for oral options over injections, motivated this investigation. Existing migraine clinical trials lack standardised endpoints and outcomes, hindering comparisons and clinical relevance. Recent evidence suggests that some herbal supplements and nutraceuticals can reduce migraine frequency, with favourable safety profiles. Participants were attracted to our natural-treatment approach due to its safety and tolerability. Evidence for this can be found in studies such as those of McNeal and Macan et al. (99, 129).

There is a pressing need for improved migraine prophylactic agents due to treatment failures, adverse effects and poor adherence which lead to healthcare inefficiencies and elevated costs. Existing treatments often fall short, resulting in increased healthcare expenses and productivity losses, particularly with rising migraine frequency (320-325). Healthcare professionals' outlook on treating refractory patients is pessimistic, contributing to the disease's underappreciation (326, 327). Treatment-resistant patients bear higher migraine and treatment costs, whilst effective treatment and adherence can swiftly reduce healthcare resource use and expenses (328, 329).

EVIDENCE BEFORE THIS STUDY

We used Curtin University Library access to search online articles with the keywords, “migraine”, “vessel” and “vascular”, to first explore the current vascular theory of migraine pathogenesis. We then used the same to access online articles with the keywords, “L-arginine”, “aged garlic extract”, “S-allyl-cysteine”, “vascular” and “vessel”, to explore the nutraceuticals’ cardiovascular uses in patients, prior to this study. These searches took place in April-June 2020. We thus compiled our hypothetical literature review to propose these nutraceuticals as a potential vascular preventive treatment for migraineurs.

ADDED VALUE OF THIS STUDY

Our randomised placebo-controlled trial results show that L-arginine, followed by AGE, may be effective in reducing the frequency and severity of future migraine episodes, and may represent a safer, cheaper and more accessible alternative to the current migraine-preventive drugs available.

IMPLICATIONS OF ALL THE AVAILABLE EVIDENCE

In phase-II clinical trialling, L-arginine and aged garlic extract have shown efficacy, safety and tolerability. Its oral administration is convenient, and it is easily obtainable cheaply over the counter. Their dosages can be adjusted to suit the individual patient’s physiological response, also taking into account their inherent aversities to each (e.g. garlic allergy requiring L-arginine-only treatment). Further studies are still required, including in child/adolescent/pregnant subjects and dosage studies. In terms of comparison studies with other preventive migraine drugs, the minimum significant differences are similar to other treatments using the same questionnaires.

CONTRIBUTORS

DC executed the clinical trial and drafted the manuscript. JM developed the project, supervised the development of the manuscript and led the data analysis. CE performed the blood assays. PB extracted the OCTA data; CM collated the OCTA data; MN processed the OCTA data; RT analysed the OCTA data. SD performed the statistical analysis for the daily diaries. MV, VL, PD and EV supervised. All authors had final access to all the output data in the study, and had final responsibility for the decision to submit for publication.

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DECLARATION OF INTERESTS

We declare no competing interests.

DATA-SHARING

Individual participant data will not be made available publicly. For those participants seeking their particular results after their part in the trial is completed, JM and SD will unblind themselves and relay the requested information to each participant, while keeping DC blinded as the continuing practitioner of the clinical trial. De-identified mass participant data will remain with the biostatistician (SD) for 7 years after study completion, according to Curtin University human-ethics guidelines. The study protocol, including statistical analysis plan and participant forms, have been published as a separate paper (330).

CHAPTER 6: GENERAL DISCUSSION, STRENGTHS AND LIMITATIONS, AND FUTURE DIRECTIONS

GENERAL DISCUSSION

MIGRAINE EPIDEMIOLOGY

Migraine is the most common and 3rd most costly neurological disorder to treat (1, 2), affecting more than 1 billion people worldwide (116, 120, 331, 332), with a female:male ratio of 2-3:1 (333). Migraine has a worse day-to-day impact than tension-type or other headaches, with less work efficiency and more monthly days off work (334). 1.18 million working-age Australians are afflicted, with a 10-year expectancy of 2.49 million quality-adjusted life-years, 533 000 productivity-adjusted life-years, $1.54 billion in direct healthcare costs, and $94.45 billion in GDP lost in total (335). In people younger than 50 years old, it is a leading cause of years with disability, especially in women (336, 337), negatively affecting work productivity, career potential, lifestyle and social opportunities, relationship health; and there is low resource allocation to mitigate (116). Episodic migraines have more net direct costs than chronic migraine due to higher prevalence of the former (116) and it is episodic we focus on in this study. In addition, there is an increased risk of disorders with current migraine regimens (e.g. MOH or opioid addiction) (116), so better treatments need to be developed. There is also a strong correlation between migraine and the presence of comorbidities. Psychiatric illnesses are common, with 75% migraineurs co-affected in one study (especially with anxiety) (338). Worryingly, migraines can increase suicidal ideation and behaviour (339-341). Also associated with migraines are sleep disorders (342), cyclic vomiting syndrome and motion sickness (343). Further, migraine biology could compound the disability in patients with comorbidities already involving nausea and vomiting (343). In addition, overuse of medications may exacerbate and confuse the contribution of migraine symptoms.

MEDICATION-OVERUSE HEADACHES

Medication-overuse headache (MOH) results from frequent use of migraine medications and poses a risk factor for migraine chronicity (344, 345). Surprisingly, it may counterintuitively indicate inadequate migraine management (346). MOH entails regular use of acute medications thereby increasing the frequency and intensity of headaches, perpetuating a cycle of acute medication intake and increased headache frequency (347). Therefore, preventive medication assumes additional importance. A study on L-arginine metabolism revealed that MOH might stem from a defect in NO synthesis, characterised by low plasma arginine, high plasma ADMA but normal SDMA in chronic migraineurs (348). Here, we explore the current migraine medications contributing to this phenomenon.

CURRENT MIGRAINE DRUGS AND ASSOCIATED SIDE EFFECTS

The current overuse of medications, particularly of the non-steroidal anti-inflammatory drugs (NSAIDs) and triptans (11, 12), can paradoxically induce headaches and impede ongoing migraine treatment (349). Abortive/acute medications for adults have only around 50% response rate (350), with most being ineffective for aura (351). Remarkably, 90% patients treated with beta-blockers, antidepressants, antiepileptics and Botox have discontinued after only a year, citing low efficacy and/or adverse effects (352). Only 13% migraineurs are using preventive therapies (353), mostly due to insufficient efficacy, adverse effects and consequent poor adherence (354, 355). Numerous potential adverse effects of the commonly prescribed synthetic drugs to treat tension headaches and migraines, with cardiovascular contraindications and invasive modes of administration being notable shortcomings (120, 356-363). Below, we list common migraine drugs.

MIGRAINE-PREVENTIVES

Beta-blockers and calcium-channel blockers are associated with gastrointestinal adverse effects (364). Flunarizine is a calcium-channel-blocker which can cause weigh gain, fatigue, nausea, constipation and parkinsonism (120).

Anticonvulsants have been used, but valproate can cause teratogenicity and is therefore contraindicated in pregnant migraineurs (120). Another anticonvulsant, topiramate, can induce weight loss, fatigue, nausea, depression, cognitive deficits and paraesthesia (120). Both topiramate and divalproex sodium are associated with gastrointestinal issues (364), while topiramate and sodium valproate are associated have teratogenic and neurodevelopmental risk potential *in utero* (365).

Among the tricyclic antidepressants, amitriptyline causes weight gain, dizziness and gastrointestinal disorders (120, 364).

Peripheral nerve blocks are parenterally invasive, and may also induce dizziness, light-headedness and headache exacerbation; when combined with corticosteroids, they may also lead to alopecia and lipoatrophy (366). Botulinum toxin type A (Botox) has only been established for chronic and not episodic migraine, and may cause neck pain, muscle weakness and injection-site pain – with the injection mode of administration being invasive (367).

Central nerve blocks include the sphenopalatine-ganglion (SPG) which can cause sensory disturbances, pain (including of the tooth), swelling, trismus/lockjaw, headache, dry eye and haematoma (368).

CALCITONIN GENE-RELATED PEPTIDE

Calcitonin gene-related peptide (CGRP) has been implicated in migraine pathogenesis through neurogenic inflammation and meningeal irritation (369, 370). Two classes of antagonists have been designed to counteract its effects: monoclonal antibodies and small-molecule antagonists (gepants) (352, 371). There are 4 monoclonal antibodies available against the CGRP molecule (fremanezumab, galcanezumab, eptinezumab) or its receptor (erenumab). Erenumab, fremanezumab and

galcanezumab are subcutaneously injected, and eptinezumab is intravenously injected. This is invasive, especially when having to be monthly or quarterly. Furthermore, they are expensive and regulated restrictively, reserved for only second-line therapy (120, 372).

CGRP has been implicated in migraine pathogenesis through vasodilatory mechanisms (373), along with pituitary adenylate-cyclase-activating peptide (PACAP) (374, 375). PACAP is also a migraine-triggering vasodilator (281, 375), but therapeutics targeting this pathway have not yet been successful (376, 377). It is unknown whether this is because, unlike CGRP which acts more on the cerebral arteries, PACAP acts more on the meningeal arteries (375). The recently discovered antagonists against calcitonin gene-related peptide (CGRP) and its receptor (CGRPr) have already accumulated some adverse events recorded across different organ systems. These include hypersensitivity reactions, and they are contraindicated in coronary heart disease, cerebrovascular disease and inflammatory bowel disease (120, 378-386). They are also not safe in pregnancy (387), and even non-placebo-related beneficial effects may be minor (388).

In particular, erenumab has high discontinuation rates, induces constipation and wears off in 1 week (389), fremanezumab more commonly has dose-dependent local injection-site reactions (390), and ubrogepant and rimegepant have only modest effectiveness (120). Ubrogepant and rimegepant (along with lasmiditan) also cannot be combined with certain common drugs (391). Injection-site reactions are common throughout the literature (122, 371, 392), and other adverse effects reported include nasopharyngitis, sinusitis, back/neck/extremity/joint pain, constipation, gastroenteritis, immunogenicity, nasopharyngitis, nausea, fatigue, urinary-tract and upper-respiratory-tract infections, hypersensitivity, dry mouth, hepatotoxicity, dizziness, anxiety, hypertension, anaphylaxis and insomnia (120, 352, 382, 391, 393, 394). Besides, gepants only have 20% effectiveness, are not recommended in pregnancy/lactation, and may induce fatigue, nausea and constipation at higher doses (395). Gastrointestinal symptoms can be attributed both to migraine as well as to CGRP treatment, particularly gepants (364, 394).

An additional concern is that such antagonists would inhibit important physiological functions attributed to CGRP. For example, CGRP is needed physiologically to vasodilate and so cardiovascular concerns have also been expressed (121). Perhaps the physiological CGRP release associated with migraine attacks is a compensatory mechanism to vasodilate to replenish adequate cerebral perfusion pressure (281). The fact that it is required to maintain healthy lungs (396) may explain the respiratory-tract-infection adverse effects. It is also required for efficient gut motility and defence (364). As CGRP helps regulate bone mass, and knockout mice were found to have reduced bone formation and mass, there are osseous concerns especially for women (predominant migraine demographic, also hormonally prone to osteoporosis) taking CRRP antagonists (397). In fact, long-term CGRP therapy can also have the opposite effect of over-increasing systemic CGRP levels, as a study showed 40% increase from baseline after 6 months of CGRP therapy (398). Downstream effects of increased CGPR include

flush, hypotension, and catecholamine release leading to tachycardia (399). Loss of treatment effect can occur with these drugs, possibly from endogenous neutralising antibodies (391).

MIGRAINE-ABORTIVES

Vasoconstrictors such as triptans cannot be used for patients with disorders involving restricted blood supply to tissues, such as cardiovascular disorders, or in patients where vasoconstriction is likely to exacerbate hypertension (104, 120). Triptans have adverse events such as transient paraesthesia, flushing and palpitations, chest/neck tightness, peripheral and coronary vasospasm, myocardial ischaemia, arrhythmias, nausea and vomiting (120, 400). Since triptans are vasoconstrictors, they are contraindicated for patients with cardiovascular cerebrovascular or hypertensive issues (120, 401). Triptans vasoconstrict mainly via vascular smooth muscle cell receptors (402), raising questions about whether this action on the larger cerebral vessels lead to downstream dilation of the microvessels, leading to replenished blood flow to alleviate migraines. Vasoconstriction is not even required for migraine abortion (394, 403). In addition, as allodynia severity increases, stress and resultant disability worsen (404). This is when migraine treatment is most needed, but patients becomes more resistant to triptan therapy (405). One study found that 75% of chronic migraineurs did not use triptans, also due to unknown reasons besides the above (406).

Non-steroidal anti-inflammatory drugs (NSAIDs, e.g. ibuprofen, naproxen) may cause peptic ulcers (thereby gastrointestinal bleeding, so aspirin is contraindicated), obstruction, perforation, rash, nausea, heartburn, vomiting, hepatotoxicity and cardiovascular issues (364, 407-409).

Opioid and barbiturate analgesics have limited efficacy and can also predispose to addiction and medication-overuse headache (410). Opioids can also cause bowel dysfunction and many associated gastrointestinal sequelae (364), as well as habituation, addiction, tolerance, withdrawal syndromes, sedation, dizziness and constipation (394).

Ergot alkaloids have low tolerability (commonly causing nausea), poor oral bioavailability, unpredictable efficacy, high overuse risk and fatal vasoconstriction risk (ergotism) (120, 411, 412).

Melatonin may cause liver injuries, reproductive system dysfunction and hyperimmune stimulation (413).

Ditans can cause sedation, vertigo, paraesthesia and anxiety (400). In fact, lasmiditan poses the risk of paraesthesia, drowsiness, dizziness and vertigo, driving impairment and lack of insight into the level of impairment (120, 394, 414). These are also expensive, have restricted availability, and are the most laden with adverse-effect risk (120, 415).

MIGRAINE-PREVENTIVE TREATMENT

While acute treatment should offer pain relief within 2 hours (120), preventive treatment is needed to reduce migraine frequency, severity or duration of migraine, decrease migraine-related medical costs and is required for people with migraines occurring 2 or more days a months, improving quality of life (412, 416, 417). Preventive treatment may even reverse cognitive decline which has been associated with migraines (418-424). It can increase acute-therapy response, but is not curative; therefore, abortive treatment would still be needed at times (417).

Choosing a preventive treatment relies on consideration of cost and efficacy, any comorbidities present, tolerability, availability and patient's own preference (120). Migraine duration, frequency and severity are predictors of disability and quality-of-life detriments in migraineurs (425), but selecting a treatment based foremost on attack severity is recommended (410). With preventive medication, patients consider effectiveness most important, then quickness of relief onset and lack of adverse effects (426). The success of the preventive efficacy of this treatment may be measured by migraine frequency stability at 6-12 months post-treatment, when treatment could be stopped to check for a sustained effect (116), as well as how much it precludes having to use acute/abortive medication (134, 427). This is particularly crucial as using it no more than 2 days a week or 10 days a month is recommended to prevent development of medication-overuse headaches (428).

Current migraine-preventives have poor effectiveness, tolerability and adherence (394). In a 2020 Italian study, only 10.7% patients received prophylactic treatment, with only 26.2% adhering to treatment (429). Due to low effectiveness and/or availability, people could resort to non-migraine medications. Unregulated non-migraine-medication use can also lead to MOH, thereby to migraine chronicity (344). To find more effective and safer migraine-preventives, we will need to look at the basics of migraine pathogenesis.

MIGRAINE PATHOGENESIS

THE CURRENT DOGMA

The pathogenesis leading to migraine is complex, multifactorial and varies widely from person to person, with equal genetic and environmental causative elements (374, 430-432), suggesting a multidisciplinary tailored treatment and management plan for each patient (precision medicine) (120). Its exact origins are still unclear, and it has a variety of different triggers for different individuals, primarily stress (433-435). Migraines commonly occur through a stress-induction pathway, and are commonly considered clinically within the spectrum of tension headaches (19).

THE PUTATIVE ROLE OF VASODILATION

Vasodilation has been traditionally associated with migraines, and various vasodilatory molecules such as CGRP, PACAP-38, NO and glycerol trinitrate have been used to induce migraines (e.g.) (376, 436). Accumulating evidence that suggests that tension headaches and migraines occur as a result of

vasodilation of cerebral arteries, but with no net change in cerebral blood flow (22, 437). Nociception occurs around these blood vessels (374), specifically at the endothelial, vascular smooth muscle and mast (inflammatory) cells and nerve fibres (374).

However, recent papers reported clues that, although vascular factors seem to be a causative component of migraines (every migraine-inductive substance used is a vasodilator (374)), vasodilation may not be the causative factor (394, 436), and treatment do not necessarily involve vasoconstriction (293). For example, pain occurred after the vasodilation had subsided (438). Previous studies suggest that the vasodilation is not the source of head pain, but a response to continually activated trigeminal fibres (439).

The trigeminovascular system is implicated in maintaining cerebral perfusion pressure (directly influencing cerebral blood flow/supply), whereby a hypoxic threat could result in a migraine attack (281). This may explain patient behaviour limiting further physical exertion during a migraine, if this helps replenish adequate cerebral pressure for tissue perfusion (281). Reflex arteriolar vasodilation (through CGRP and PACAP release) would then maintain perfusion pressure (281), but this would set off the periarteriolar trigeminal pain receptors in migraineurs. This is why the vasodilation traditionally associated with migraines may be due to neurogenic activation and inflammation (440). Correspondingly, functional-ultrasound-measured blood flow has shown a direct correlation with neuronal firing (neurovascular coupling) (441). The throbbing pulsating aspect of the migraine may be due to mechanical vascular changes (436). Additionally, major migraine treatments and inducers act via their effects on the cerebral vessels (436). Dilated brain blood vessels would then trigger pain onset via stretching of cerebral perivascular nerves, whereby the efferent sympathetic nerve fibres release nociceptive ATP, and mast cells degranulate further vasodilatory pro-inflammatory and nociceptive molecules (374). Compensatory constructive sympathetic activity downstream at the microvasculature counteracts the upstream vasodilation, again releasing nociceptive ATP (374). Energy supply, therefore, becomes a serious contender as a contributor to migraine pathogenesis.

THE ENERGY-DEFICIT MODEL

The brain's energy reserve and workload seem to be at the crux of migraine pathogenesis, triggering the trigeminovascular system (442-444). In fact, hypoxia alone has been able to trigger migraine attacks and aura (445). A study even developed a mathematical model for systemic vasoconstriction and neurovascular coupling disruptions affecting blood flow dynamics (446). Plasma glucose levels are elevated during migraines (447), which may be a compensatory response to low nutrient supply from downstream small-vessel constriction as well as impaired glucose metabolism (448).

Paolucci, Altamura and Vernieri (2021) propose that endothelial dysfunction (mostly caused by oxidative stress) of the intracranial vessels may be involved in migraine pathophysiology via increased vascular tone, thrombosis, inflammation and permeability (154). The endothelium excretes NO, ET-1,

homocysteine and other factors with lead to contraction of the smooth muscle cells. When this is imbalanced, endothelial dysfunction can precipitate vasoconstriction and inflammation (154, 449). Migraine inflammation may be caused by endothelial NO release (154), which we wonder could be a compensatory response to dilate for more blood supply to the brain (AGE's anti-inflammatory properties may help here). NO increases during a migraine attack (450). NO is the main blood-vessel tone regulator, causing endothelium-dependent vasodilation (154). The large vessels' NO moves downstream to smaller, resistance vessels – causing the latter to dilate (451), increasing oxygen supply to the end parenchyma.

Oxidative stress decreases NO bioavailability, which allows ET-1 and angiotensin-2 vasoconstrictor activity to overcome the balance (154). ET-1 could act as migraine trigger (452). Increased oxidative stress and decreased NO bioavailability have been reported surrounding migraines (453). NO could also be a biomarker for migraine status (154). NOS inhibition or decreased NO bioavailability can be countered by L-arginine (154). NOS (NO synthase) oxidates intracellular L-arginine (154). It also facilitates release of calcitonin gene-related peptide (CGRP) which can itself sustain endothelial NO release (154). Cellular oxidative imbalance, especially involving the NO system, has been considered a possible pathway (454). From oxidative stress, vasoconstrictive ET-1 may not be sufficiently offset by vasodilatory NO, which could lead to net vasoconstriction, which could lead to the cortical spreading depression (CSD) (154) associated with compromised blood flow and is well-known to herald the aura (394, 431, 455). The CSD can itself induce NO (456, 457), which could be a compensatory response, since there is a homeostatic requirement for stable perfusion pressure to be maintained within the cerebrum (154). It possibly also triggers trigeminal pain receptors (394, 431), which may be a result of the compensatory larger-vessel dilation. Indeed, the CSD-associated potassium efflux vasodilates arteries, and the extracellular increased potassium could sensitise the surrounding trigeminal pain receptors (394, 431).

Consistent with the indicated hypothesis, living at high altitudes where there is less oxygen saturation despite normal-pressure conditions is associated with increased risk for migraine (458-460). Longer duration in these conditions accordingly leads to increasing CGRP levels (459). Below 60 mmHg oxygen partial pressure, compensatory mechanisms kick in, including increased vasodilation and blood flow, vasodilatory NO release, more oxygen extraction from the bloodstream and upregulation of transcription factors for angiogenesis and ATP synthesis for energy (459). This may be associated with tissue hypoxia could cause cortical spreading depression, the starting element in aura (459, 461) and ischaemia-associated white-matter-associated hyperintensities are similarly linked to migraineurs with aura as opposed to those without (394).

Increased water intake has been linked to decreased migraine frequency and duration (462), supporting the hypothesis of compromised blood flow contributing to migraine pathogenesis. Other clues may be that migraine throbbing worsens with exertion/movement,

hunger/fasting/exhaustion/hypoglycaemia can trigger migraine (463) and that migraine may be caused by an energy deficit (464). Oxidative stress, glucose-metabolic disturbances and mitochondrial dysfunction are also linked to migraine (448, 465, 466).

OUR ALTERNATIVE HYPOTHESIS

The above observations seem at odds with the current dogma of migraine pathogenesis. Therefore, we propose the alternative hypothesis that stress-induced heightened sympathetic tone results in a chronically constrictive microvascular phenotype, both centrally and within the periphery. Suboptimal central parenchymal blood flow, or hypoxia, could activate nociceptors and cause headache pain onset. The hypothesis would then be that preventing or attenuating chronic microvascular constriction and promoting a more dilatory phenotype, would reduce frequency or severity of vascular-mediated tension headaches. Accordingly, we need to consider benign vasodilating alternatives to prevent and treat patients with vascular-induced headaches. Nitric oxide (NO) is the primary endogenous vasodilator synthesised by vascular endothelial cells (33), which stimulates arterial smooth muscle cells and capillary-adjacent or arteriolar pericytes to relax by regulating cellular ionised-calcium (Ca^{2+}) homeostasis.

In migraines, distal cerebral vessels may be prone to constriction, according to our hypothesis. Even outside migraines, migraineurs have reported vasoconstrictive systemic symptoms (241). Blood behaves as a non-Newtonian fluid in stenotic vessels due to the red cells and cellular-interaction-derived structures (467). We wonder if this increase in viscosity may exacerbate migraines. Oxidative stress could lead to a hypercoagulable state (154) and white-matter hyperintensities (468). Since blood protein content differs between people, different people may be more prone to this effect than others. For example, free-floating (low-density) cholesterol or its derivatives are known to increase non-Newtonian factors in blood (469) and are also associated with increased frequency of migraines (470). Cholesterol affects vessel junctions especially (471) and the brain has more of them; therefore, this effect may be enhanced when it comes to the cerebrovasculature. Therefore, using L-arginine and AGE to dilate those blood vessels may further increase blood flow by also decreasing viscosity of the blood. In relation to this, a gene involved in cerebrovascular malformations is also associated with migraine (472): the angiotensin-converting enzyme (ACE) gene's DD allele (473) increases ACE enzyme serum levels, which increases vasoconstriction. This further supports our hypothesis of a vasoconstriction-based migraine aetiology. This vasoconstriction mechanism may also explain the female bias in migraine epidemiology, as explained below.

GENDER BIAS

Being female predisposes one to having migraines, with females being affected up to three times as much as men (474, 475). Migraine frequency, duration and consequent disability are higher in women then in men (476). There are even differences in oestrogen-receptor expression between male and female rats (477, 478). Oestrogens induce vasodilation via different pathways (154), both neuronal

and vascular (477, 479). One example is such as the NO-mediated pathway through oestradiol receptors in the endothelium (154). Progesterone may have a net vasoconstrictive effect, partly from reduced NO release (480). There are also higher interictal prolactin levels in migraineurs than controls, more so in chronic than episodic migraineurs (481). Prolactin can promote pain in females, especially in times of stress and could be implicated in migraine induction through neuronal excitability in females, also enhancing CGRP release (481).

MENSTRUAL MIGRAINES

Migraines may well be hormonally linked, as its prevalence peaks at 35-39 years old (reproductive years) (20), and is most prevalent in women under 50 years old (116, 425). 18-25% female migraineurs associate their migraines with their menstrual cycle, possibly due to oestrogen withdrawals and fluctuation during that time (479, 482). Hormonal migraineurs also have worse cerebrovascular function (483), and with dementia in women (484).

Oestrogen increases oxytocin which decreases CGRP release – so oestrogen withdrawal means that that protective effect against migraines is lost (485). Oestrogen may be directly involved in risk for migraine via a microvascular axis as it promotes vasodilation by antagonising calcium-channel activity in vascular smooth muscle cells and increasing NO synthesis in endothelial cells (479). However, it is not as simple as administering oestrogen to prevent migraines, as inflammatory migraine and ischaemic stroke risk can worsen dose-dependently from oestrogen (486). This area is controversial, and such considerations are still under debate. It should be noted that migraineurs already have a higher stroke risk (487). Oestrogen receptors α and β are found on cerebral arteries (477), and since high oestrogen levels increase vasoconstrictive vasopressin expression (485), we wonder if the oestrogen withdrawal opens up the central cerebral arteries such that the distal capillaries constrict to maintain cerebral pressure. Oestrogen treatments are available to counteract oestrogen drops and can be effective in some women, although more quality research is needed (488). Oestrogen drops are more common in menopausal women, and it is during this midlife phase that migraine frequency and burden often increase, with frequency linked to more oestrogen withdrawals (489).

There are two subtypes of menstrual migraine – pure menstrual and menstrually related (346). After stress which can lead to vicious cycles of migraine occurrence and eventually chronicisation, menses is the most common trigger, due to late luteal oestrogen withdrawal (489). Menstrual migraines are usually not related to auras, and seem to be an abnormal central-nervous-system response to cyclical changes in ovarian-hormone ratios. Oestrogen withdrawal leads attacks, as oestrogen is vasodilatory; but oestrogen also activates the trigeminal pain pathway. Migraines seem dependent on – not absolute but – relative amounts of oestrogen and progesterone together. Prostaglandin appears exacerbating, testosterone protective. Oestrogen appears to enhance neurotransmission within the TNC in rat models of trigeminal activation, while progesterone has been shown to inhibit neurotransmission (482, 490, 491).

PREGNANCY

There is often a decrease in migraines during pregnancy (492). Indeed, one of our participants recalled that the only time she recalled not having a migraine was during pregnancy. The risk of miscarriage or congenital malformations means that even simple analgesics like ibuprofen, diclofenac and naproxen are not recommended and current preventive medications should be avoided altogether (493).

Migraines may also predispose to hypertension, hypercoagulability and thromboembolism in pregnancy (494), therefore blood-thinners such as AGE may be especially considered in this population. Hypertension and hypercoagulability themselves predispose to migraines (495), thereby a pregnancy-benign treatment is required, such as our nutraceuticals as opposed to current pharmacological treatments which are contraindicated in pregnancy. For these reasons, it was of utmost importance to find the safest treatment for migraine prevention in our study. In the literature, we found evidence to support use of the following nutraceuticals. It is important to note, however, that this study cannot and *should* not be extrapolated to pregnant patients without actually investigating the nutraceuticals on them as subjects.

L-ARGININE AND AGE

L-arginine and AGE have been used to alleviate cardiovascular disorders in the clinical literature, using these vasodilatory mechanisms. L-arginine is a precursor for the vasodilator NO (496, 497), and studies have shown an association of vascular tone with plasma concentrations of L-arginine (40, 124). Aged garlic extract (Kyolic, *Allium sativum*) is a capillary inflammation inhibitor (41) with the relevant active component, S-allyl-cysteine (42), which enhances NO synthesis (44), promoting vasodilation (45). Indirect positive effects on vessel diameter have also been proposed because of its potent antioxidant, antithrombotic and antiatherosclerotic effects (46, 498).

Both L-arginine and AGE have been used clinically to alleviate vasoconstrictive disorders, but surprisingly, neither have been investigated for vascular constriction-caused headaches. In both animals and humans, L-arginine has been shown to induce vasodilatory (and anti-inflammatory) effects, by a variety of different measures (40, 48-54, 56-63, 65-69, 71-75, 78). Similarly, in both animals and humans, AGE also has been shown to induce vasodilatory effects, by a variety of different measures (47, 80-84, 87-92, 94). In a landmark study, Gruenwald et al. found that arterial elasticity markedly increased with AGE treatment and blood pressure (especially diastolic) was decreased, possibly due to S-1-propenylcysteine (499, 500).

Aged garlic extract is a blood-thinner, therefore may also mitigate thromboembolisms and not just the inflammation associated with migraine (344, 501). Recent studies found that AGE's vascular effect may not be limited to the S-allyl-cysteine dilatory effect (502) and general anti-inflammatory effect, but that increased arterial elasticity and decreased vascular resistance could also cause a decrease in blood pressure (499), which in itself may counteract migraine pathogenesis. AGE also confers fewer

gastrointestinal adverse effects than does raw garlic with the latter's harsh organosulphuric compounds (503). Indeed, we only had one AGE-treated participant with an adverse effect which was mild heartburn (499).

A significant advantage these two nutraceuticals have, over currently used conventional drug treatments, is that both have only mild, short-lived, easily managed gastrointestinal side effects. Based on reviewed studies (111), we used a combination of 1.5 g L-arginine + 1 g AGE, per day, to prevent migraines in 18-80-year-old Australian migraineurs. We assessed their efficacy using a number of different subjective and objective migraine measures, as listed below.

OUR STUDY

We decided to conduct a study investigating a newly hypothesised treatment mechanism to prevent migraines, as expounded in detail in our preceding literature review (111) and protocol paper (330). This decision was driven by the significant preventive-treatment gap for migraines, stemming from concerns about safety, tolerability and efficacy, as well as patient preference of oral over injectable treatment options (504). Furthermore, there is no standardised set of endpoints and outcomes in current migraine clinical trials, which would otherwise facilitate intertrial comparisons, as well as provide clinical meaningfulness to inform patient care (505, 506).

A recent review suggested that herbal supplements and nutraceuticals can reduce migraine frequency, on top of their benign tolerability/safety profile (507). Many participants in our study were drawn to the natural aspect of our treatment, especially with the favourable safety profile (508, 509).

SUBJECTIVE MIGRAINE MEASURES

LONG-TERM INSTRUMENTS

The long-term questionnaires consisted of the Migraine-Specific Quality-of-Life Questionnaire (MSQ) (137), Leiden Visual Sensitivity Scale (L-VISS) (139) and the Visual Light-Sensitivity Questionnaire 8 (VLSQ-8) (140). The MSQ has 3 domains measured over the past 4 weeks. The role function-restrictive (MSQ-RFR) or limiting (LIM) domain measures the limiting effect of migraine on daily social and work-related activities (7 items), the role function-preventive (MSQ-RFP) or preventing (PRV) domain assesses whether migraine prevents the individual from performing these activities (4 items), and the emotional functioning (MSQ-EF) or emotive (EMO) domain measures emotions associated with migraine (3 items). Each item measures on a Likert scale of 1-6 ("None of the Time" to "All of the Time"), the scores are inverted, the domain sums are calculated and these scores are transformed to percentages. If half or more questions are answered, the missing-value item(s) can be estimated using the average of the other items in same domain. If fewer than half the questions are answered, those items must be considered missing. The MSQ has the following limitations: it does not specifically

measured ability to move the heads or body, do activities requiring physical effort or self-care tasks; it was not developed assessing conceptual fit; and day-to-day variation was not accounted for (510).

The L-VISS measures the impact of light- and pattern-sensitivity on daily functioning ("visual allodynia"). Nine identical questions/items are measured for 2 domains – within (ictal) and for between (interictal) migraines. Each is on a Likert scale of 0-4 ("Not at All" to "Very Severely"), and the scores are summed for each domain. The VLSQ-8 measures the presence and severity of photosensitivity symptoms, and has only 1 domain of 8 items, each on a Likert scale of 1-5 (Questions 1-3 and 5-8: "Never" to "Always"; Question 4: "None" to "Severe").

When selecting our questionnaire instruments, we excluded the frequency-oriented Migraine Disability Assessment test (MIDAS) (511), as it measures over the last 3 months which was basically the course of our trial. We also excluded the severity-oriented Headache Impact Test (HIT-6) (512), because it contains double-barrelled questions which may provide ambiguous answers (for example, migraine impacts could differ between work and home). The MIDAS questionnaire also may not accurately reflect disability, also being difficult to complete according to a study on Spanish migraineurs (513). The HIT-6 and MIDAS have also been shown to be particularly sensitive to clinical changes after prophylactic drug cessation, and ours is a long-term therapeutic. However, MSQ and HIT-6 scores were found to be highly correlated (514). Notably, Item 12 ("frustration") has been shown to perform poorly in validation studies of the MSQ (136, 137), so caution must be practised in including it.

SHORT-TERM INSTRUMENTS

The weekly summaries consisted of the Short-Form McGill Pain Questionnaire (SF-MPQ) which measured the intensity of different pain types (138). There was a sensory domain of 7 items, an affective domain of 4 items, with each item scored on a Likert scale of 0-3 ("None" to "Severe"). These scores were totalled for each domain and there was an additional visual analogue scale of 0-10 for average migraine pain over the past week. Additionally, there was a Likert scale of 0-5 ("No Pain" to "Excruciating") for concurrent headache pain while filling in the questionnaire (as that would affect answers).

The daily diaries consisted of outcomes according to the International Headache Society guidelines (17, 134): date reported, whether or not they had migraine that day, how many migraines if they did, how severe the pain was on average (visual analogue scale of "No Pain" to "Worst Possible Pain", measured as 0-100 cm), whether they took anything (medication or otherwise) to relieve the pain, list of medications (including over-the-counter) taken, checklist of prodromal symptoms (presence of changes in vision, changes in smell, changes in sound, change in taste, excessive sleepiness, trouble talking, confusion, nausea, vomiting, anxiety/irritability, low mood and neck ache), whether they had prodromal numbness/tingling in the face/arms, checklist of prodromal/dromal sensations (presence

of throbbing, shooting, stabbing, sharp, cramping, gnawing, hot/burning, aching, heavy, tender, splitting, tiring/exhausting, sickening, fear-causing and punishing/cruel).

PHOTOSENSITIVITY

Photophobia has been shown to be the most common most bothersome symptom in migraineurs (515) and is defined as light-induced headache exacerbation, abnormal light-sensitivity and light-induced eye discomfort (516). Migraineur photosensitivity may be mediated by the melanopsin-containing intrinsically photosensitive retinal ganglion-cell light-irradiance pathway (517, 518), probably sustained by trigeminovascular activity (516).

In various studies, 69% patients report light as a migraine trigger in different studies, especially in migraineurs with aura and seasonal migraineurs (516). Triggering aspects of the light include flickering, glare, brightness, sunlight, contrasting light and shade, and lower and higher wavelengths (516, 519). Even with unilateral pain, photophobia usually occurs bilaterally (516, 519), although may or may not be more pronounced on the headache side (519, 520). Photophobia prevalence decreases with age, aura prevalence increases with age (521). Photophobia is much more prevalent in migraineurs with aura than without (522). Increased light intensity is correlated with increased pain burden (516). Patients are more affected during attacks than between (519), and we always made sure our participants did not have a migraine during our light challenge.

Current management involves avoiding light exposure (516). However, narrow wavebands of the light spectrum may decrease pain intensity, however (516). Excluding the harmful wavelengths may be a beneficial treatment to be investigated in the future (516). The beneficial versus the harmful light wavelengths varies greatly between different individuals (516), necessitating a tailored clinical treatment approach. Interestingly, green light may even alleviate headaches (523), which could be worth investigating in future trials.

Alternative instruments we could have used include the Utah Photophobia-Symptom Impact Scale (version 2) which measures the impact of the photophobia on daily living (524), or the 4-point-Likert longer Visual Discomfort Scale for interictal photophobia with or without headache, or the simple yes/no Migraine Photophobia Scale for ictal or headache-related light-sensitivity (525, 526). Similarly simple are the ID-Migraine and Migraine Photophobia Score (MPS) for photosensitivity (ictal) and less quantitative (only yes/no) diagnostic measures of migraine-related photosensitivity (527).

We found, during the acute light challenges, that discomfort gradually decreased through graduated increase in bulb brightness, similar to a previous study (519). Participants were less likely to detect further brightness changes as the LED panel got brighter, which was expected given the constant brightness increase in each step was a smaller percentage of the absolute brightness at each increased-brightness step. Precision ophthalmic tints reduce cortical hyperactivation, so we asked them to remove tinted glasses or contact lenses prior to testing (528). Migraine-drug use did not affect

photosensitivity threshold in a previous study (519), which may be an indication that current migraine drugs are ineffective at reducing this major migraine symptom, although CGRP is linked to light aversion (529).

To reduce participant burden and likelihood of migraine induction, we only performed the light challenge once on each before and after visit, especially as a study showed that repeated stimulation increased light-sensitivity (519). Striped patterns and flicker are worse for migraines (530-534), but we did not want to give them a proper headache, of course.

Photophobic migraineurs are more photophobic during than outside an attack (increased by migraine pain), but are still more light-sensitive than controls interictally (519). All are ictally sensitive, but only most are interically sensitive (519). There is little difference between migraineurs with and without aura, with light-sensitivity (519). Photophobia is usually not liked to nausea, attack severity or pain character/laterality, however (519). Interestingly, light exacerbates migraine in even blind people, indicating the non-image-forming retinal pathway is dysregulated, affecting dura-sensitive thalamocortical neurons (535).

OBJECTIVE MIGRAINE MEASURES

PHYSIOLOGICAL BIOMARKERS

There is a paucity of reliable and precise biomarkers for migraine progression or its treatment response (154, 536, 537). Flow-mediated dilation is not a reliable marker of peripheral vascular dysfunction (154), but arterial stiffness may be used as a marker of migraine status via arterial tonometry (154). Subclinical cochlear impairment measurement has also been proposed to measure migraine progression, but more data is needed (537).

IMAGING BIOMARKERS

There are not many robust imaging biomarkers for migraine. White-matter hyperintensities have shown conflicting results (538), therefore they may not be a reliable biomarker. MRI studies have shown a thicker visual cortex linked with aura (539) and white-matter lesions (more so in migraineurs with aura) (540, 541), the latter of which are permanent and cannot be used to track treatment response. An fMRI study showed glial activation in migraineurs with aura (542). During migraine aura, fMRI has shown acute hyperperfusion, followed by chronic hypoperfusion in aura-associated brain regions (543). Although there are ictal imaging markers available (376), imaging during the headache phase would probably be too burdensome for patients.

Previous studies examined migraine patients using OCTA (Table 3). Although white-matter hyperintensities are permanent, we anticipated treatment-related improvements in retinal vascular tone. Our prior literature search yielded results comparing migraineurs to healthy controls (Table 3).

Table 3. *Key ocular areas examined by OCTA are shown alongside the expected results (compared to controls) in migraineurs with and without aura. The lower retinal vein density was seen in cluster-headache patients (544), but we expect the same in our migraineurs if the same vasoconstrictive mechanism applies.*

Vessel density areas	**Migraine without aura**	**Migraine with aura**
Macula	• Lower superficial and deeper retinal foveal, parafoveal, perifoveal vessel densities (142, 145, 545) • Larger foveal avascular zone (145)	• Lower superficial and deeper retinal foveal, parafoveal, perifoveal vessel densities (141, 142, 545) • Larger foveal avascular zone (141, 210, 269)
Optic nerve	• Lower whole optic disc, peripapillary, superior hemisphere, superior layer and temporal layer vessel densities (142, 545)	• Lower superior peripapillary vessel density (141) • Lower whole optic disc, peripapillary, superior hemisphere, superior layer and temporal layer vessel densities (142, 545) • Larger optic disc rim area (210)
Choroidal thickness	• Increased during migraine attack (143, 146) • Decreased outside migraine attack (143, 147, 148)	• Increased during migraine attack (143, 146) • Decreased outside migraine attack (143, 147, 148)
Retinal thickness	• Lower ganglion cell complex thickness (149) • Lower foveal thickness (150) • Lower retinal nerve fibre layer thickness (146, 153, 214, 222) • Lower retinal vein density (544)	• Lower ganglion cell complex thickness (149) • Lower foveal thickness (150) • Lower retinal nerve fibre layer thickness (146, 153, 214, 222) • Lower retinal vein density (544) • Thinner inferior hemisphere and nasal sectors of retinal vein (142)

Regarding secondary measures, we focus on OCTA findings. Our study uniquely employed OCTA longitudinally, unlike previous cross-sectional studies (241). Assessing the choroid's relevance is uncertain, given recent findings showing no difference in thickness or vascularity index between chronic migraineurs and controls (287). Various studies have reported conflicting results on choroidal thickness (143, 146, 277). Vessel density can be measured as blood vessel length divided by scan area (141), or the percentage of vascularised tissue within the area (145).

BLOOD BIOMARKERS

We employed the enzyme-linked immunosorbent assay (ELISA) to measure blood concentrations of nitrites and nitrates (NOx), ADMA, SDMA and endothelin-1 (ET-1). The rationale behind this selection of biomarkers is elaborated in detail in Paolucci, Altamura and Vernier's excellent review on endothelial dysfunction and migraine (154). Nitrates and nitrites are more stable derivatives of reactive NO in the bloodstream (155), so we chose to use them as the proxy measure of vasodilation. ADMA and SDMA additionally would be valuable measures of interictal propensity to migraine, as higher levels of both vasoconstrictive molecules would reflect inhibition of vasodilatory NO production from L-arginine (154). If certain participants exhibit increased levels of these markers, it could elucidate why these participants may not respond as well to our treatment. Finally, the antagonism between vasodilatory NO and vasoconstrictive ET-1 should allow us to assess the changing vascular tone in our migraine subjects.

The principles (including limitations) of the ELISA are outlined in Gan and Patel (2013) (546). We ensured control for sensitivity, specificity, inter- and intra-assay variability and matrix interference (376). Plasma was utilised as specified in the protocols for each biomarker, minimised time delays, protease inhibitors and freeze-thaw cycles, and ensured all sample values fell within the calibrated linear assay range (376).

In our blood analyses, we take note of previous studies have found that chronic migraine patients have low plasma L-arginine levels and higher ornithine, ADMA and monomethyl arginine (NMMA) levels (348). ADMA and NMMA are inhibitors of NO synthase, and increased plasma levels may indicate the L-arginine is not being metabolised by NO synthase into vasodilatory NO as much (348). The decreased levels of L-arginine and increased ornithine may indicate the arginine is being shunted instead to ornithine production via arginase (348). In this scenario, the origin of migraine pain (at least in the migraineurs in the D'Andrea study) may not even be trigeminal vascular dilation (348), which supports our proposed hypothesis of a vasoconstrictive origin instead. The study also showed an increase of tyramine, a TAAR1 agonist (TYR), in migraineurs – which may be significant because endothelial TAAR1 inhibits L-arginine metabolism into NO synthesis and release (319). Future trials could also include ornithine, tyramine and NMMA in their set of vasoactive migraine biomarkers.

Biomarkers to predict and monitor treatment response are needed, and blood biomarkers in particular would shed light on the molecular mechanisms behind this (376). A panel of biomarkers may be better

to better delineate intergroup results, allow greater reproducibility (376), and be more reliable due to multiple molecular pathways investigated. Migraine is associated with small-vessel diseases, where vascular alterations could lead to neuronal dysfunction, with circulating endothelial progenitor cells decreased and aberrant in migraineurs (547).

One study found lower plasma L-arginine levels and higher plasma ADMA levels in chronic migraineurs than in controls, which they attribute to a possible NO synthesis defect (318). Raised ornithine and monomethylarginine (NMMA) plasma levels (318). The authors suggest that ADMA and NMMA, which convert L-arginine to vasodilatory NO and are NOS inhibitors, may explain the low L-arginine levels, suggesting inhibited vasodilatory NO synthesis in chronic migraineurs (318). Further investigation is required to elucidate the role of arginase, which hydrolyses L-arginine to ornithine, potentially explaining the elevated ornithine levels in chronic migraineurs (318).

NOS inhibition may not directly occur in chronic migraineurs, explaining the absence of an accompanying increase in SDMA in plasma (318). SDMA only competes against L-arginine transport, and does not directly inhibit NOS (548). Cubital (peripheral) and cranial (jugular) blood PACAP levels fall between attacks (549, 550), with longer the disease duration, the lower the interictal PACAP cubital plasma level (549). Both peripheral (cubital) and jugular (cranial) levels rise during attacks (549), suggesting PACAP should not be collected ictally to avoid increasing participant burden. Peripheral PACAP levels between attacks could serve as a disease duration biomarker, with increased levels indicating positive treatment response. However, chronic migraineurs exhibit higher interictal PACAP than episodic migraineurs, suggesting PACAP may better be used as a biomarker of migraine chronicity instead (551). Serum PACAP is already a proposed biomarker for migraine (551).

Vasoactive intestinal peptide (VIP) is also proposed as a biomarker for migraine (551) with higher levels observed in migraineurs than controls (552), indicating parasympathetic activation. Although ET-1 could serve as a migraine biomarker (154). studies have found that endothelin levels are not different between migraineurs and controls (140), making its utility debatable – an idea worth exploring in our study.

There are also many blood markers of inflammation that can be used for migraine patients (431), but these are not specific to migraine and may depend too closely on time of attacks. Other metabolites and serotonin may be more sensitive and specific to migraines, but the evidence base around its long-term use in migraineurs (such as for treatment progress) is not yet established. However, as there is systemic inflammation in migraine, including interictally (553), there have been found increased serum levels of IL-6, IL-1ß, CRP and TNF-a in migraineurs (554), possibly explaining AGE's therapeutic effect as an anti-inflammatory. Endogenous glucocorticoids in both serum and cerebrospinal fluid could serve a biomarkers for migraine chronicisation (555). Substance P, indicative of neurogenic inflammation and pain signalling, is unreliable (537). Colocalised with substance P is CGRP (370). Predictably, CGRP is elevated only ictally in migraineurs compared to controls (370, 556), but only in

cerebral and not peripheral blood (557). Jugular CGRP is elevated during rather than after attacks (370), and it is undesirable to collect CGRP during attacks for obvious patient-burden reasons. CGRP jugular blood collection itself is invasive and valid for only a half-life of 7-9 mins. (558). Serum CGRP is also a proposed biomarker for migraine (551), but peripheral blood CRGP shows conflicting results, and only a small fraction of CGRP appears there (370, 376, 459).

Plasma biomarkers of oxidative stress are not yet established to track treatment response (537). Headache episodes lead to long-term cellular damage, possibly continuing interictally, as shown by serological S100B and neuron-specific enolase (NSE) biomarkers (559). In a different study, among the episodic-migraine biomarkers were NO, S100B and NSE which did not depend on whether the participant was currently suffering a migraine (560). S100B and neuron-specific enolase are markers of glial and neuronal damage respectively (431), but these changes may be too permanent to be used to track treatment response. However, asymmetric dimethylarginine (ADMA) is a NOS inhibitor, indicates oxidative stress, and could also be used as a migraine (and possibly also white-matter-lesion) biomarker (154). Symmetric dimethylarginine can reduce NO production from L-arginine, also being a potential biomarker (154). ADMA decreases vasodilatory NO production and release (318). The presence of white-matter lesions (indicating ischaemia) have been found to correlate with higher ADMA levels in migraine patients (561). This is because retinal vascular density may also be related to brain white-matter hyperintensities (142) which have been visualised in migraineurs using magnetic resonance imaging (MRI) (267). White-matter hyperintensities are considered a marker of focal hypoperfusion, and are associated with aura (255). They are believed to arise from microvascular damage, are made of myelin and gliosis (255), and are correspondingly associated with ischaemia (142). While using MRI to detect these fell beyond the scope of our project, we may find OCTA-detected vessel areas of reduced density and thickness corresponding to MRI-detected areas of white-matter hyperintensities. Symmetric dimethyl-arginine (SDMA) also decreases intracellular L-arginine availability, but does not directly inhibit NOS as does ADMA, instead competing against L-arginine transport to NOS (562). It has been found that endothelin levels are not different between migraineurs and controls (563), but endothelin-1 may play a role in migraine (564).

Other potential biomarkers are scattered across the literature. For example, plasma glutamate is elevated interictally in episodic and chronic migraineurs (565). Increased serum prolactin may indicate increased migraine frequency (566). Migraineurs with aura often have higher homocysteine levels, where homocysteine is a vasoconstrictor released by the endothelium (154). Angiogenin, vascular endothelial growth factor (VEGF), circulating endothelial progenitor cells (EPCs), stromal-cell-derived factor 1α (SDF-1α) and endothelial microparticles (EMPs) could also be investigated as future biomarkers of migraine progression, as they indicate endothelial dysfunction (154). EMP release is triggered by hypoxia (567), so it would be interesting to investigate that to support our hypoxia-related migraine hypothesis.

NON-PHARMACOLOGICAL TREATMENTS

These have been used alone or on top of pharmacological treatment in migraineurs. Neuromodulation and biobehavioural therapies have the strongest evidence base so far (120). In terms of neuromodulation therapies, studies have investigated anodal transcranial direct current stimulation, physiotherapy, remote neuromodulation and music therapy (568-570). But some of these are expensive and involve inpatient hospital stays, with equipment expertise needed. Transcranial direct current stimulation can be used to prevent episodic migraine, but the effect is slow and short-term (571). Neuromodulation studies are of poor quality, invasive procedures and hospital stays are required, and adverse effects such as treatment intolerance, non-compliance, tingling and itching are common (572). Given the safety and cost-effectiveness of behavioural interventions, they would be good adjuncts to pharmacological treatments (573), especially since older patients are more susceptible to adverse effects of pharmacotherapies (120).

Among the biobehavioural therapies, cognitive behavioural therapy, biofeedback, relaxation training, yoga, mindfulness and headache education have been proposed (574-577). Episodic migraineurs seem to benefit more than chronic migraineurs with mindfulness-based cognitive therapy (578). Yoga has been observed to decrease migraine frequency (579), as it may enhance cerebral oxygenation (580) and angiogenesis (581), prevent oxidative stress (582), and potentially reduce general peripheral vascular resistance (575). However, yoga also reduces stress, depression and anxiety independently, and these factors independently influence migraines (575), so these need to be controlled in future studies. Other exercise interventions have also been investigated, their efficacy also enhanced when in conjunction with medication (583). Relaxation therapy with massage is more effective than mindfulness for migraine severity (584). Dietary interventions have also been proposed, but with lack of control and poor adherence, varied evidence of efficacy (583). Acupuncture studies so far have low reporting quality and increased bias risk (585).

Social support, sport and surgery are other non-pharmacological therapies worth a mention. Since migraine is also associated with depression and anxiety, social support may be critical (586, 587). Sport may play a protective role at least in females (588), which is interesting because endurance training causes vascular adaptations to increase perfusion and vascular flow (589). Women with aura have shown increased vessel pulsatility, abnormal cerebrovascular reactivity and decreased cerebral blood flow velocity in central cerebral large vessels (590). So this may be an additive/adjunct to our treatment, especially because exercise-trained animals show increase vascular response to endothelial NO-mediated vasodilators (589). Surgery is resorted to with relentless migraines, but this is expensive and invasive, also requiring prolonged inpatient stays. An example is nerve decompression surgery (591, 592).

STRENGTHS AND LIMITATIONS OF THE STUDY

STRENGTHS

The strengths of our study are as follows. We used a prospective randomised double-blind controlled trial design to minimise bias in interpreting the results in terms of comparing intervention efficacy, and to maximise possibility of causal inference from treatment to effect. The randomisation minimised selection and allocation bias; the double-blindness via allocation concealment minimised performance and assessment bias; and the prospective design minimised recall error and selection bias. The randomisation specifically minimised confounding factors by ensuring maximal uniformity in baseline measures between the treatment groups.

We measured interictal outcomes in our migraine trial, whereas most migraine trials assess only ictal burden (593). This is especially important, as there is a significant interictal burden from migraine (536, 594-596), on top of its being a major cause of emergency-department admissions (597). We used OCTA, for the first time in the literature to our knowledge, to directly visualise the change in capillary tone as a reflection of the progression of our treatment. Our age range went beyond the usual 65-year-old maximum age limit in other trials, because we realised there was no reason not to include older patients if they fulfilled our extensive inclusion and exclusion criteria. We used an oral mode of administration, which is not only less invasive than parenteral, but also has less of a placebo response rate (598). Since migraine preventive therapy aims to increase function and decrease attack frequency, severity and disability (599), we feel our treatment were successful in these outcomes. L-arginine treatment appeared to be most effective, followed by combination, followed by AGE.

We did not have major participant compliance issues with our migraine diaries. However, 23 participants were excluded due to incomplete data. The incomplete data generally included missed days and omission of weekly summaries. Common reasons for non-compliance are belief that it is unnecessary, forgetfulness, limited knowledge on recording, no time to record, unable to remember and too lazy/bored to use it (600). Effective reminders are reviewing the diary, keeping it beside the medication, and recording it elsewhere first when the diary is not with them (600). Therefore, it seems to have helped that we discussed filling out the diary with the participants on the first visit.

LIMITATIONS

There are multiple factors we did not test, due to the limited scope of our study.

1. We did not evaluate different doses of our treatments. Tailoring dosage to patient needs and preferences would also increase patient adherence to treatment (601). Mixed-dose combination pills and unit-of-use packaging may be optimal to increase patient adherence in general (602).

2. We did not follow participants up after their 14 weeks of treatment, whereas active follow-up is recommended within weeks of treatment change (412).
3. Children and adolescents were not tested. It may be noted that preventive medication has not been established in children, especially with the high placebo rates in children and adolescents (603). Our benign nutraceutical treatment offers an appealing investigation candidate for later paediatric migraine trials.
4. We did not explore other bothersome symptoms such as vertigo, osmophobia, phonophobia movement-sensitivity and allodynia which can sometimes be more debilitating than the headache pain itself (343, 604).
5. We did not delve into animal studies. While human and animal models may be inherently different, animal models could offer insight into a wider range of molecular investigations at a pre-clinical stage (605, 606), as well as sex-related differences in migraine (607). For example, attacks could be more ethically induced by infusing endogenous signalling molecules found to induce migraine in human patients; multiple behavioural experiments could be performed on an animal, with neural activity in the trigeminal system recorded (529).
6. This is not intended to be an acute/abortive migraine treatment and the oral route also tends to be slower ictally than interictally (due to gastric stasis during a migraine) (608).
7. There is potential for a higher placebo response rate in parallel studies compared to crossover studies (609), which may compromise clinical trial power (610). 30% placebo participants have adverse effects in migraine drug trials (609), and many current migraine drugs are not safe for the foetus during pregnancy (387, 611).
8. This was an interim analysis with limited subjects, possibly leading to underpowered results.
9. We did not explore contributory roles of diet and exercise on vascular tone.
10. L-arginine is already present in AGE, and we did not test the isolated effects of the different compounds present in AGE.
11. The nature of the migraine pathology means that we could not clearly differentiate anti-inflammatory, antioxidant and vasodilatory effects of our active treatments.
12. We focused on solely the vascular side of the neurovascular theory of migraines, as it was beyond the scope of this study to also investigate the neuronal factors involved.
13. The design would have greatly benefited from the involvement of patient partners or community-organisation partners. A discussion or consultation with institutions such Migraine & Headache Australia would have elevated the design, facilitated recruitment and made the knowledge-translation plan more robust.
14. Recruitment did not meet the power calculation, which is a significant concern for the validity and reliability of the study's findings, limiting its impact and usefulness in the scientific community. Partnering with clinical colleagues or community associations may have prevented this.

15. We did not use other available migraine pain instruments or outcomes, while there are also new ones potentially being developed – so other instruments (612), data-collecting techniques and outcomes could also be investigated, especially to address any shortcomings of the current techniques or to add migraine-management facilities (600, 613-641). Some of these are listed below:
    a. The Migraine Physical-Function Impact Diary (MPFID) may be used instead of the MSQ to assess quality of life in migraineurs, using everyday-activity and physical-impairment domains, and an overall global impact item (642). The MSQ we used may focus participants on only the salient points of their migraine experiences, rather than the required average migraine occurrence and characteristics, since it is over 4 weeks of recall (643). Also, since it was developed initially for episodic migraines, it may underestimate chronic migraineur burden (644). The MFID may compensate for these shortcomings. Therefore, we could use the MFID as a daily diary of migraine-induced disability in future such trials, avoiding the long recall bias.
    b. The Activity-Impairment-in-Migraine Diary (AIM-D) assesses physical impairment and performance of daily activities (617), capturing difficulty walking and thinking clearly, focusing on severity rather than frequency (643).
    c. The Physician's Global Impression of Change (PGIC) and the Subject's Global Impression of Change (SGIC) are non-migraine-specific 7-point Likert-scale questionnaires which could also be used at the end of a trial, as previously done in migraine prophylactic trials (645).
    d. The Patient-Reported Outcomes Measurement Information System (PROMIS) Pain-Interference Scale Short-Form 6b measures pain interference in daily activities over the past week, via a 6-item 5-point scale (646), and has been used in a migraine trial before (647).
    e. The mMIDAS is the MIDAS altered to have a 4-week recall period (improved accuracy, reduced recall bias, but not yet validated). It could be used for future studies like ours, to measure missed or productivity-reduced work and leisure days due to migraine (599). Monthly assessments are still subjected to recall bias, however.
    f. The Headache Disability Inventory/Index (HDI) is a 3-point 25-item instrument with functional and emotional domains with an unspecified recall period (648).

New headache instruments continue to be developed and published in the literature. These include the Medical Outcomes Study (MOS)'s Short-Form Health Survey SF-20 and SF-36 (649, 650), the Headache Scale (651), the Pain Behaviour Questionnaire (PBQ) for chronic migraineurs (652), the Headache Disability Inventory (HDI) (648, 653), the Minor-Symptoms Evaluation Profile (MSEP), the Subjective-Symptom Assessment Profile (SSAP), the Psychological General Well-Being (PGWB) Index for interictal migraine impact (654), the Fear-of-Attacks-in-Migraine Inventory measuring attack-

related fear in migraineurs (626), the Visual-Aura Rating Scale relevant for migraineurs with visual aura (616), and the Loneliness-of-Migraine Scale which assesses migraine-caused loneliness in individuals (632).

FUTURE DIRECTIONS

In terms of future directions, several additions and amendments could be made to upcoming trials:

1. **Combination treatments:** combining pharmacologic and non-pharmacologic treatments is more effective than either alone, especially with comorbid mental disorders which are more common than uncommon in migraineurs, and could prevent migraine chronicisation (655). Future trials could use a non-pharmacologic adjunct to this treatment.
2. **Hypoxic model:** a normobaric hypoxic model of migraine induction could be used for future migraine treatment trials, as it is non-pharmacological, has fewer adverse effects, and higher attack and aura induction rates (281, 459).
3. **Vitamin D and magnesium supplementation:** Vitamin D and magnesium supplementation could be explored. In endothelial cells, Vitamin D has been shown to increase NO production, combined with L-arginine and Q10 (656). Vitamin-D deficiency has also been linked with migraine (657), and supplementation has led to its improvement in various studies (658). Low Mg has been shown in tension-type headaches which have been relieved by subsequent Mg treatment (659). This has been linked to low Vitamin D, as Vitamin D is required for intestinal Mg absorption (659). Low Mg has been associated with migraine frequency indicating future studies exploring Mg to prevent and treat migraines (660-663). Low Mg may promote cortical spreading depression, platelet hyperaggregation and altered neurotransmitter release (664), but also reduces NO production and increases circulating CGRP (663). Magnesium may also prevent migraine by limiting vasoconstriction and promoting NO production (665).
4. **Gender differences:** women and men can respond differently to the same medication (666), so expanding our study to include higher male numbers may be sensible.
5. **Cerebrovascular function:** we could include advanced neuroimaging, potentially using MRI.
6. **Citrulline investigation:** an interesting recent paper claimed that citrulline-containing watermelon ingestion triggered migraine attacks due to increased blood nitrites, activating the L-arginine-NO pathway (667). Citrulline is a precursor of L-arginine (668), thereby more indirectly activating the NO pathway as in our hypothesis. It may be acting on the proximal larger vessels, in that case. However, Silva-Néto et al. only looked at nitrates, not L-citrulline or L-arginine; the study also had no placebo group and a small sample size. Another paper actually hypothesised a beneficial effect of citrulline on migraines, due to increased cerebral blood flow in rats (669). Regardless, citrulline may also work to trigger migraine in other ways

uninvestigated as yet. In addition, watermelon does not seem to work in tension headache which is on same pathophysiological spectrum (670). Watermelon may have other migraine-triggering components – pure citrulline was not used in this study, nor was it quantified. However, El-Hattab et al. speculate that citrulline may more effectively trigger NO release than L-arginine (through L-arginine itself) (668), so it may be worth investigating citrulline as our next vasodilatory migraine-preventive agent.

7. **Other vasoactive agents:** alternative vasoactive agents could also be tested for migraine prophylaxis in the wake of the success of this study, such as CoEnzyme Q10 (671, 672). Riboflavin has also been proposed as a migraine prophylactic, due to its stress-alleviating effects on the mitochondrial respiratory chain (673, 674).
8. **Definition of a migraine day:** there is some ambiguity regarding what constitutes a migraine day. Whilst this was not such a problem in our study, as each participant kept their definition of a migraine constant throughout the trial looking at individual changes longitudinally, a recent paper provides a more concise definition (675), which could be used for future migraine trials, especially those comparing between participants.
9. **Assessment instruments:** there are also treatment-assessing instruments that could be used to more accurately determine efficacy and safety of this treatment (676, 677). Although acute-treatment-relevant items such as response time would not be applicable here, investigating other components of L-arginine-NO pathway, both inhibitory and excitatory of the vasodilation pathway could be beneficial. We could investigate not just photophobia, but phonophobia and other migraine bothersome symptoms that we have not already, given there is such a huge variety of migraine symptoms between different people. Other studies also mentioned nausea as among the most bothersome symptoms (678, 679). In future, we could include participants with medication-overuse headaches, given that the view that medication overuse must be tempered for preventive therapy to be successful is outdated (680, 681). We have considered migraines as the extreme end of the same pathophysiological spectrum as tension headaches. However, we could still investigate these nutraceuticals for other types of headaches/migraines (vestibular, occipital, cluster, cervical etc.) to see if they have any effect despite their different pathophysiologies.
10. **Additional OCTA measures:** we could investigate other OCTA measures. For example, intercapillary space or retinal arteriolar thicknesses could indicate vascular wall integrity (682, 683) or interictal choroidal thickness (143, 277). Our OCTA machine was not equipped to take these measurements. One paper managed to directly measure vessel diameter with the Spectralis OCT (147), which would have been most useful to our study.
11. **Neural activity:** it would be interesting to couple migraine-related nerve activity with central blood vessels, to see if they correspond with the changes in vascular tone in those vessels as a downstream effect of capillary dilation. Studies have developed implantable neural probes in rodents, with minimal vascular damage or inflammatory/immune response (684, 685). If

this were developed for safe accurate use in humans, we could more closely see the therapeutic intervention mechanism at play, inclusive of the neural side. Similarly, we could use neurovascular coupling (686) to track preventive therapeutic treatment progression by seeing how much more similarly to healthy brains, migraineur brains respond to stress, over time (687). In contrast, BOLD functional MRI is where neural activity is detected indirectly via blood flow at a certain brain region at a more macroscopic level (688), therefore the above methods may be more suitable. However, fMRI can still provide biomarkers to track preventive therapy progress overall. Arterial spin labelling could be used to check perfusion areas within the brain, to see if there is indeed increased perfusion with our treatment (689, 690). However, its resolution is insufficient to work beyond the macrovascular to the capillary level. However, dynamic contrast-enhanced MRI may assess tissue perfusion at the capillary level (691). The drawback here is that the contrast agent must be injected, which is invasive.

The ideal prophylactic treatment drug should be orally active, have a long-lasting effect, be benign in terms of side effects and prevent future migraine attacks rather than only lessening their severity (692). Many of our dropouts were due to medication non-adherence. The most common reasons for non-adherence to migraine medications seem to be concern about long-term and adverse effects and simply missing the medication (693). This implies that the reduced occurrence of adverse effects and continued toleration over 3 months with our treatments are advantages, but they still need to be investigated longer-term. Each treatment plan would be tailored to each patient's individual physiological needs, with early treatment encouraged (694, 695). Not to be forgotten are important aspects such as safety in driving and other common occupational/lifestyle habits (696). For instance, L-arginine would be contraindicated in cancer patients (697). AGE is also fibrinolytic, so the patient dose must be monitored for potential bleeding events (98, 99). Patients themselves prioritise the following as treatment factors influencing their drug preferences: fewer milder adverse effects, longer and quicker treatment effect, lower costs, and reduction of symptom severity and frequency (698-704).

At present, there is a dire need for more and better prophylactic agents, due to inadequate or failed treatment, unacceptable adverse effects or mode of administration, and (often resultant) poor patient adherence to treatment (116, 698, 705-712). These issues lead to resource misallocation/wastage and increased healthcare costs, as well as productivity and employer costs, especially with increasing migraine frequency (320-325, 713-717). Even doctors' attitudes towards treating currently treatment-refractory patients seem pessimistic, and not much attention is given to the disease (326, 327). Treatment-refractory patients often even have a greater migraine and treatment cost burden (713, 718). When this is successfully treated and treatment adhered to, there is already reduced healthcare resource utilisation and costs already in 6 months (328, 329, 719).

ABSTRACT

Investigating the preventative effects of L-arginine and aged garlic extract on migraines

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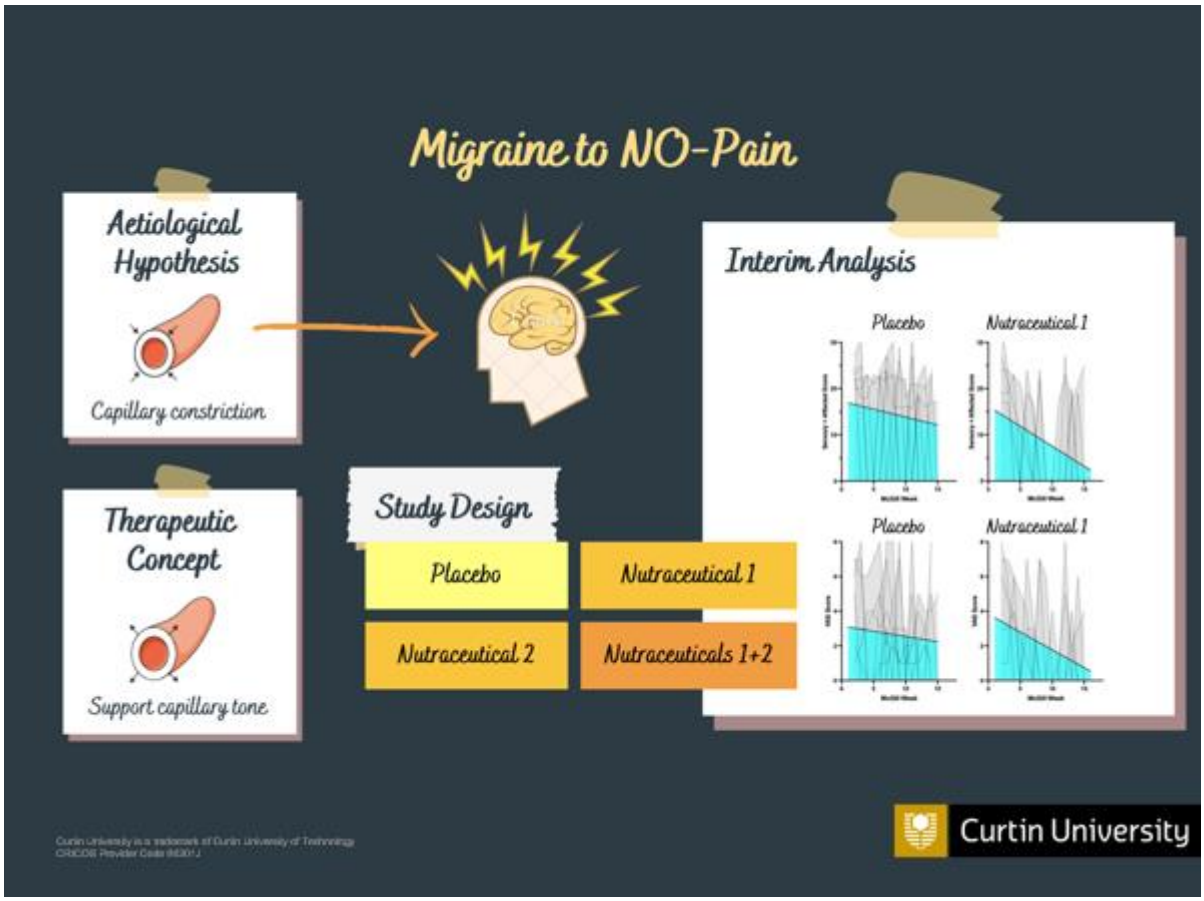
We used an oral nutraceutical approach to increase brain capillary tone, thereby reducing migraine frequency and severity in migraineurs. This was a double-blind randomised controlled clinical trial where we randomised ~90 migraineurs into 4 treatment groups: placebo, L-arginine, AGE, or combination L-arginine and AGE. Following a 2-week placebo run-in, treatment interventions were for 12 weeks. We used validated headache diaries and instruments, retinal optical coherence tomography angiography as a surrogate marker of central capillary tone, an acute light challenge to assess threshold sensitivity to headache induction, and blood collections to determine concentrations of compounds affecting vascular tone. We collected these data at baseline and post-intervention.

The 4-week-recall Migraine-Specific Quality-of-Life Questionnaire shows the L-arginine group having a decline in migraine frequency, and improvement in lifestyle measures commonly affected by migraine. The weekly-pain-recall Short-Form McGill Pain Questionnaire (SF-MPQ) shows a continuous improvement in patients randomised to L-arginine. Total sensory and affective domains, and visual analogue score were positively affected. Similar data were found with Daily Diary analysis. There is a suggested positive effect of AGE alone, as well as strong synergistic effects with combination L-arginine and AGE.

Migraine is the predominant cause of disability in young people worldwide, but there are challenges with under-treatment, patient adherence and access to treatments. This trial addresses a new pathophysiological hypothesis and prophylactic treatment opportunity, supported by pilot data with potency effects possibly greater than contemporary migraine trials. The proposed interventions may provide a more effective, cheap, easily attainable and safe method of attenuating migraine pain.

Word Count: 250

PRESENTATION SLIDE



PRESENTATION TRANSCRIPT

“Welcome to my skull. A truck is rolling over the bones in my neck and head. The vertebrae break, the brains pop and ooze. A thousand flashlights shine in my eyes. The world tilts. I throw up. I black out. This happens all the time. It’s nothing but an ordinary day.” This is a testimonial from a real migraineur. Migraine is a common distressing neurological condition that features throbbing headaches, nausea and distorted vision. It is the sixth highest cause of disability worldwide.

The prevailing dogma is that migraines are caused by excessive widening of large arteries supplying the brain. However, we published a controversial hypothesis that instead, migraine is caused by focal and exaggerated constriction of brain capillaries – the microscopic blood vessels that supply the nutrients and oxygen to the brain tissue. If our hypothesis is correct, then there may be an opportunity to reduce migraine frequency and severity through formulated nutraceutical interventions to support capillary dilation.

My study is a double-blind placebo-controlled diet in chronic frequent episodic migraineurs. Participants are randomised to placebo, Nutraceutical 1, Nutraceutical 2, or a combination of both. Intervention is for 14 weeks where participants complete comprehensive migraine diaries using validated questionnaires. They complete a light challenge at start and end of study, and also undergo optical coherence tomography angiography which is a potential marker of brain capillary integrity.

We recently completed interim analysis on 90 people, and here is a snapshot: this graph shows the effect of Nutraceutical 1 on a combination score of migraine frequency/severity and on quality of life. The slope of the solid line depicts the trend, with downward showing improvement. We can see that migraine severity was reduced over the intervention period, significantly greater than a placebo effect. Similar data was realised for visual sensitivity scores. In fact, the level of improvement was even greater than that reported for contemporary drug interventions for migraine!

We are now receiving unsolicited testimonials which we hope are indicative of the trial findings, including the following I'd like to read out to you:

"I am a 64-year-old female. I have suffered from debilitating chronic migraines most of my life. These have impacted on my daily life and thus quality of life, for many years and in many ways. With sharp, crippling, throbbing pain, aching muscles, very poor sleep, days of inactivity and a dull head, I struggled to function in everyday activities. The only relief was bed rest, ice packs, drugs and time.

I participated in the 3-month clinical trial run at Curtin University. The results have been outstanding! As a result of this trial, I have had one headache in 10 weeks compared to almost daily occurrences. This has led to gaining a regular sleep pattern, awakening pain-free and feeling "alive". I honestly feel I am gaining my life back!

The daily diary I have kept for months before and during the trial are evidence of this. It is my belief this intervention is responsible for this turnaround, and it has the potential to provide long-term relief for the millions of chronic migraine sufferers".

Thank you.

ABSTRACT

Themes: Neurological and Psychiatric Disorders, Neurovasculature and Metabolism

Method

We used an oral nutraceutical approach to increase brain capillary tone, thereby reducing migraine frequency and severity in migraineurs. This was a double-blind randomised controlled clinical trial where we randomised ~90 migraineurs into 4 treatment groups: placebo, L-arginine, AGE, or combination L-arginine and AGE. Following a 2-week placebo run-in, treatment interventions were for 12 weeks. We used validated headache diaries and instruments, retinal optical coherence tomography angiography as a surrogate marker of central capillary tone, an acute light challenge to assess threshold sensitivity to headache induction, and blood collections to determine concentrations of compounds affecting vascular tone. We collected these data at baseline and post-intervention.

Key findings

The 4-week-recall Migraine-Specific Quality-of-Life Questionnaire shows the L-arginine group having a decline in migraine frequency, and improvement in lifestyle measures commonly affected by migraine. The weekly-pain-recall Short-Form McGill Pain Questionnaire (SF-MPQ) shows a continuous improvement in patients randomised to L-arginine. Total sensory and affective domains, and visual analogue score were positively affected. Similar data were found with Daily Diary analysis. There is a suggested positive effect of AGE alone, as well as strong synergistic effects with combination L-arginine and AGE.

Conclusion

Migraine is the predominant cause of disability in young people worldwide, but there are challenges with under-treatment, patient adherence and access to treatments. This trial addresses a new pathophysiological hypothesis and prophylactic treatment opportunity, supported by pilot data with potency effects possibly greater than contemporary migraine trials. The proposed interventions may provide a more effective, cheap, easily attainable and safe method of attenuating migraine pain.

Word Count: 250

Presenting author: Devahuti Chaliha

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Using L-Arginine and Aged Garlic Extract to Prevent Migraine

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Background

- Migraine is the predominant cause of disability in young people worldwide, and is more common in women than men
- There are challenges with under-treatment, patient adherence, adverse reactions to conventional treatments, and treatment access

Hypothesis

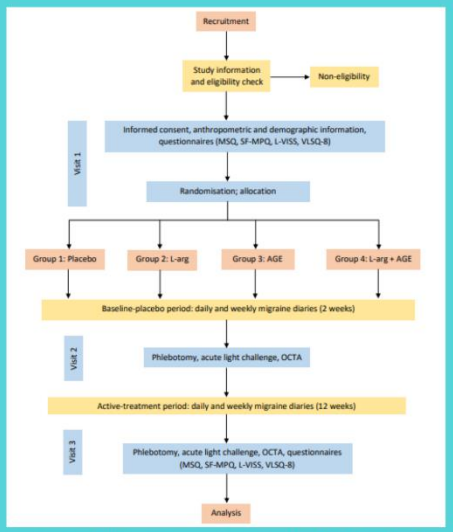
- Reduced microvascular blood flow may activate nociceptors and trigger headache pain onset
- The maintenance of central capillary blood flow may be a therapeutic opportunity to attenuate migraine frequency and severity

Objectives

- We are investigating two dietary nutraceuticals which have previously been shown to dilate systemic capillaries: L-arginine and aged garlic extract (AGE) (ACTRN12621001476820)
- L-arginine and AGE enhance nitric oxide. AGE is reported to reduce pro-inflammatory cytokines, which may be relevant here

Design

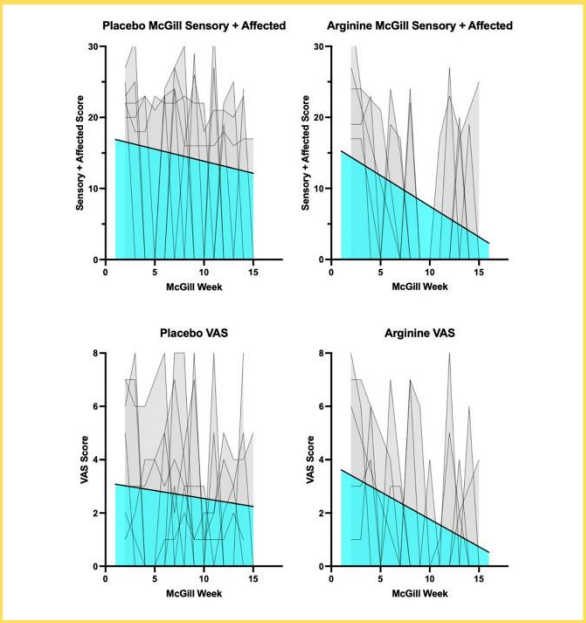
- Ongoing randomised double-blind placebo-controlled phase-2 single-site 14-week trial
- Targeting n=240 x 18-80-year-old female and male chronic frequent episodic migraineurs



Questionnaires: MSQ = migraine-specific quality-of-life; SF-MPQ = short-form McGill pain; L-VISS = Leiden visual sensitivity; VLSQ-8 = visual light-sensitivity

Results

- Interim analysis of n=90 on L-arginine treatment



- Top row: L-arginine shows significant improvement in sensory pain and mood characteristics, compared to placebo (regression slope)
- Bottom row: L-arginine shows significant improvement in pain intensity (VAS), compared to placebo

Study Objective Measures

Primary

- Change in migraine frequency and severity via daily and weekly migraine diaries
- Migraine-associated disability via long-term migraine surveys

Secondary

- Retinal capillary diameter via optical coherence tomography angiography (OCTA)
- Photosensitivity threshold via acute light challenge
- Blood biomarkers of vascular tone via enzyme-linked immunosorbent assay (ELISA)

Interim Study Findings

- L-arginine treatment decreases headache severity in chronic frequent episodic migraineurs
- L-arginine improves visual analogue score - a surrogate marker for threshold sensitivity for migraine induction
- Preliminary analysis suggests that AGE may have synergistic beneficial effects in combination with L-arginine (data not shown)
- A fully powered study may provide evidence that targeting central capillary dilatation could be a therapeutic target to reduce chronic frequent episodic migraine burden

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APPENDICES

APPENDIX 1. ANIMAL STUDIES OF L-ARGININE ON VASCULAR TONE

Study	Animal	Dose	Mode of Administration	Treatment Frequency	Treatment Duration	Medical Condition	Effect on Serum Measures (unless otherwise stated)
Javanmard 2009 **(72)**	Rabbit	3%	P.o.	Dietary	1 month	Hypercholesterolaemia	Increased eNOS expression intensity in aorta
Howell 2009 **(62)**	Rat	95 mM	P.o.	Dietary	2 weeks	Hypoxia	Maintained pulmonary arterial pressure, inhibited increased precapillary resistance, maintained decreased vascular resistance
Kharazmi 2015 **(50)**	Rat	0.01 M	Postmortem vascular infusion	Single	8 weeks	Diabetes	Increased GABA-induced vasodilation in both diabetic and non-diabetic animals
Mendrinos 2010 **(51)**	Minipig	30 µl 1 mM	Intravitreal	Single	30 mins.	Retinal vein occlusion	Persistently increased arteriolar diameter
Morita 2014 **(71)**	Rat, rabbit	2.85 m M/kg	P.o.	Single	4 hours	None	Increased blood NOx (nitrite + nitrate) and cGMP
Ou 2010 **(73)**	Rat	500 mg/kg	I.p.	Once daily	3, 5 weeks	Pulmonary hypertension	Decreased pulmonary artery wall thickness. Increased NO production, eNOS expression and phosphorylation

Saleh 2011 **(720)**	Rabbit	2.25%	P.o.	Dietary	28 days	Hypercholesterolaemia	Prevented aortic intimal thickening
Schreiber 2017 **(61)**	Rat	300 mg/kg	P.o.	Once daily	14 days	Pulmonary hypertension	Decreased pulmonary artery pressure, increased vascular elasticity, palliated small vessel disease
Feng 2013 **(60)**	Rat	3%	P.o.	Dietary	4 weeks	Insulin-resistance hypertension	Reversed vessel thickness, increased NO and cGMP back to non-hypertensive controls, decreased systolic blood pressure

Study first authors and years are listed with animals used, doses and modes of administration, treatment frequencies and durations, induced medical conditions of the subjects, and drug effects on serum measures. P.o.: per os*; i.p.: intraperitoneal; eNOS: endothelial nitric-oxide synthase; GABA: gamma aminobutyric acid; cGMP: cyclic guanosine monophosphate; NO: nitric oxide.*

APPENDIX 2. HUMAN STUDIES OF L-ARGININE ON VASCULAR TONE

Study	**Registration No.**	**Dose (g) P.o.**	**Treatment Frequency**	**Treatment Duration**	**Medical Condition**	**Effect on Serum Measures (unless otherwise stated)**	**Adverse or Side Effects**
Ast 2010 **(68)**	NA	2, 4	Thrice daily	4 weeks	Primary hypertension	Decreased blood pressure with 12 g daily (4 g x3)	None reported
Camarena Pulido 2016 **(66)**	NCT 02363348	3	Once daily	20 weeks	Risk of pre-eclampsia	Decreased cases of pre-eclampsia	Mild dyspepsia
Chen 2010 **(78)**	NA	5.2	Once daily	3 weeks	Old age	Increased anaerobic threshold	None
Deveaux 2016 **(52)**	NCT02354794	1.5	Thrice daily	4 weeks	Overweight, cardiometabolic risk	In those with lower baseline L-arginine, attenuated decrease in flow-mediated dilation	None
Fahs 2009 **(54)**	NA	7	4 times, before exercise, 72 hours apart	16 days	None	After exercise: Decreased brachial stiffness. Increased central aortic stiffness, reactive hyperaemia, forearm blood flow	None reported

Jahangir 2009 **(721)**	NA	9	Once daily	4 days	Coronary artery disease	Increased homocysteine:methionine ratio	None reported
Lucotti 2009 **(57)**	NCT00408577	6.4	Once daily	6 months	Cardiovascular disease, after aortocoronary bypass	Decreased ADMA. Increased cGMP, L-arginine:ADMA ratio, reactive hyperaemia	None
Morris 2013 **(75)**	NCT01796678	0.1/kg	Thrice daily	5 days	Sickle cell disease	Decreased vaso-occlusive pain score	None
Neri 2010 **(69)**	NCT00974714	4	Once daily	12 weeks	Pregnant, chronic mild hypertension	No change in blood pressure, but fewer received antihypertensives	None reported
Orozco-Gutiérrez 2010 **(49)**	NA	4	Twice daily	2 months	Heart failure	Decreased pulmonary artery, diastolic and systolic artery pressures. Increased duration on treadmill	Gastrointestinal distress
Pahlavani 2017 **(48)**	IRCT2013121515807N1	2	Once daily	45 days	None	Increased maximal oxygen uptake	Skin dermatitis, stomach problems
Siasos 2009 **(53)**	NA	7	Thrice daily	3 days	Smoking	Increased flow-mediated dilation. Decreased carotid-femoral pulse-wave velocity	None reported
Vadillo-Ortega 2011 **(67)**	NCT00469846	3.3	Twice daily	19 weeks	Risk of pre-eclampsia	With antioxidant vitamins: Decreased pre-eclampsia incidence	Nausea, dyspepsia, dizziness, palpitations, headache

Study first authors and years are listed with clinical trial registration numbers, oral doses in grams, treatment frequencies and durations, existing medical conditions of the subjects, drug effects on serum measures, and adverse/side effects. "None reported": study did not mention adverse/side effects; "none": there were no adverse/side effects from the treatment; p.o.: per os*; NA: not available.*

APPENDIX 3. ANIMAL STUDIES OF AGE ON VASCULAR TONE

Study	Animal	Dose	Mode of Administration	Treatment Frequency	Treatment Duration	Medical Condition	Effect on Serum Measures (unless otherwise stated)
Aguilera 2010 **(83)**	Rat	1.2 ml/kg	I.p.	30 mins. before, at onset of, or 1 hr. after reperfusion	2 hrs. infarct, 4 hrs. reperfusion	Middle cerebral artery occlusion causing focal ischemia	Onset of reperfusion: Decreased 2-hr. infarct area
Cemil 2016 **(722)**	Rat	250 mg/kg	P.o.	Once daily	15 days	CNS injury	CNS tissue: Decreased ischaemia
Colin-Gonzalez 2011 **(84)**	Rat	1.2 ml/kg	I.p.	Single (reperfusion onset)	1 hour infarct, 24 hours reperfusion	Middle cerebral artery occlusion causing focal ischemia	After 1 hr. ischaemia, 24 hrs. reperfusion: Decreased infarct area
Perez-Torres 2016 **(82)**	Rat	125 mg/kg	I.a.	Twice daily	1 month	Metabolic syndrome	Decreased systolic blood pressure and coronary vascular resistance. Increased vascular function, heart NO, citrulline, NOx

Study first authors and years are listed with animals used, doses and modes of administration, treatment frequencies and durations, induced medical conditions of the subjects, and drug effects on serum measures. I.p.: intraperitoneal; p.o.: per os*; i.a.: intra-abdominal; CNS: central nervous system.*

APPENDIX 4. HUMAN STUDIES OF AGE ON VASCULAR TONE

Study	Registration No.	Dose (g) P.o.	Treatment Frequency	Treatment Duration	Medical Condition	Effect on Serum Measures (unless otherwise stated)	Adverse or Side Effects
Ahmadi 2013 **(87)**	NA	0.25	Once daily	1 year	Medium cardiovascular risk	Decreased homocysteine. Increased temperature rebound	None reported
Budoff 2009 **(80)**	NCT01534910	0.25	Once daily	1 year	Medium cardiovascular risk	With supplements including 0.1 g L-arginine: Decreased homocysteine. Increased temperature rebound	None reported
Larijani 2013 **(88)**	NCT00860847	0.3	Every 3 months	1 year	None	With 30 mg CoQ10: Improved pulse-wave velocity and digital thermal monitoring	None reported
Ried 2010 **(89)**	ACTRN126090 00151235	0.96	Once daily	12 weeks	Systolic hypertension	Containing 2.4 mg S-allyl-cysteine: In hypertensive patients, systolic blood pressure decreased. In normotensive patients, no change	Belching, reflux, taste sensations
Ried 2013 **(91)**	ACTRN126110 00581965	0.24, 0.48, 0.96	Once daily	12 weeks	Systolic hypertension	Containing 0.6, 1.2, 2.4 mg S-allyl-cysteine: Decreased systolic blood	Constipation, bloating, flatulence, reflux, garlic taste,

						pressure in patients taking 0.48 g AGE (1.2 g S-allyl-cysteine)	difficulty swallowing the capsules, dry mouth, cough
Ried 2016 **(47)**	ACTRN12613000747729	1.2	Once daily	12 weeks	Hypertension	Containing 1.2 mg S-allyl-cysteine: Improved central blood pressure, central pulse pressure, mean arterial pressure, augmentation pressure, pulse-wave velocity, arterial stiffness	Reflux, burping, bloating. No increased bleeding risk in patients on blood-thinning drugs. Improved digestion
Reid 2018 **(90)**	ACTRN12616000185460	1.2	Once daily	12 weeks	Hypertension	Containing 1.2 mg S-allyl-cysteine: Decreased mean, central and pulse blood pressures, arterial stiffness	Garlic taste, burping
Seo 2012 **(723)**	NA	0.08	Once daily	12 weeks	Postmenopausal	Decreased homocysteine	None reported

Study first authors and years are listed with clinical trial registration numbers, oral doses in grams, treatment frequencies and durations, existing medical conditions of the subjects, drug effects on serum measures, and adverse/side effects. "None reported": study did not mention adverse/side effects; "none": there were no adverse/side effects from the treatment; NA: not available.

APPENDIX 5. CURRENTLY COMMONLY USED APPROACHES TO TREAT MIGRAINES

Migraine Treatment	**Examples**	**Considerations**
Acute medication	Analgesics (11, 12, 105, 724, 725) Antiemetics (101, 105, 726, 727) Barbiturates (12, 724, 725, 728, 729) Bolutinum toxin and muscle relaxants (102, 724, 727, 729, 730) CGRP receptor antagonists (gepants) (731) Ergot derivatives (11, 104, 105, 725-727, 729) Local anaesthetics (105)	Can induce migraine chronicity/recurrence, contraindicated in pregnancy, adverse effects, tolerance and dependence, serotonin interaction effects, require multiple doses, expensive, contraindicated in valvular disorders, short-term efficacy, early migraine recurrence, contraindicated in bleeding disorders, contraindicated in asthma, contraindicated in urticaria, contraindicated in renal impairment, contraindicated in peptic ulcers or recent gastrointestinal bleeding, abuse, rebound headache, additional dietary and management requirements, contraindications with MAOIs or SSRIs, contraindicated in cardiovascular disorders, incomplete and inconsistent pain relief, inefficacy during aura/premonitory phase (11, 12, 104, 105, 725, 726, 728-731, 735-738)

	NSAIDs (12, 102, 105, 724, 725, 727, 730, 732) Opioids/opiates (11, 12, 101, 724-726) Selective serotonin reuptake inhibitors (SSRIs) (104, 732, 733) Triptans (11, 12, 104, 105, 725-727, 729, 731, 734)	
Prophylactic medication	Alpha-2 agonists (727, 728) Angiotensin antihypertensives (727, 729, 739) Anticonvulsants or antiepileptics (102, 104, 727, 729, 732, 739)	Adverse effects, ineffective at low doses, contraindicated in pregnancy, expensive, contraindicated in diabetes, long time to take effect, contraindicated in myocardial conduction blockages, drug interactions, contraindicated in bleeding disorders, contraindicated in asthma, contraindicated in urticaria, contraindicated in renal impairment contraindicated in peptic ulcers or recent gastrointestinal bleeding, additional dietary and management requirements, contraindications with MAOIs, contraindicated in glaucoma, anticholinergic effects,

	Beta blockers (104, 727, 729, 732, 739) Bolutinum toxin and muscle relaxants (102, 724, 727, 729, 730) Calcium-channel blockers (104, 729, 732, 740) Monoamine-oxidase inhibitors (MAOIs) (104) NMDA receptor antagonists (730, 741) NSAIDs (12, 102, 105, 724, 725, 727, 730, 732) Parenteral nonsteroidal agents and corticosteroids (105, 726, 727, 742) Selective norepinephrine reuptake inhibitors (SNRIs) (729)	contraindicated in epilepsy, contraindicated in acute heart-attack recovery (11, 12, 104, 105, 726-730, 741-747)

	Selective serotonin reuptake inhibitors (SSRIs) (104, 732, 733) Serotonin antagonists (727, 728, 732) Tricyclic antidepressants (102, 104, 724, 727, 729, 730, 732, 739, 743)	
Acupuncture **(748-756)**	N/A	Insufficient or conflicting evidence, ineffective, small effect size (748-750, 752, 753, 755)
Meditation **(757-761)**	Distraction technique (760) Externally Focused Secular Meditation (757) Internally Focused Secular Meditation (757)	No effect on pain sensitivity, ineffective, insufficient evidence, distraction insufficient, practice takes time (757-761)

	Mindfulness (759, 761) Progressive Muscle Relaxation (757) Spiritual Meditation (757)	

Each treatment approach is listed alongside examples and their considerations. NSAID: non-steroidal anti-inflammatory drug; CGRP: calcitonin gene-related peptide.

APPENDIX 6. LARGE TRIAL INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria	Exclusion criteria
1. Migraine (with or without aura) diagnosed at least 1 year ago 2. Migraine onset occurred before 50 years of age 3. Adult males and females, aged 18-80 years (inclusive) at screening 4. 2-6 migraine episodes and fewer than 6 “other” headache types per month, averaged over the previous 3 months 5. Able to differentiate between migraine and “other” headache types 6. Able to commit to taking oral capsules once a day for 14 weeks 7. Able to fill out paper daily migraine diary over 14 weeks	1. Taking medications/drugs that affect vascular tone or blood pressure 2. Continuous residual headaches 3. Headache disorder or facial pain disorder other than migraine 4. Change in migraine treatment in previous 3 months 5. Taking >2 migraine-prevention drugs 6. L-arginine tablets or aged garlic extract in previous 3 months 7. Possibility of pregnancy or breastfeeding during study 8. Adverse reactions to food containing L-arginine or garlic 9. Clinical reports of kidney/liver dysfunction 10. Known risks of bleeding or coagulopathy, or currently on blood-thinning medications 11. Cancer, haemochromatosis or diabetes 12. Severe psychiatric disorder or other neurological disorders 13. Acute/chronic pain disorders, infections, cardiovascular or cerebrovascular disease 14. Poorly controlled depression or anxiety 15. Antipsychotic or antidepressant medications/drugs over past 3 months 16. Low blood pressure or complications arising from it 17. Diagnoses other than migraine as primary cause of headaches

	18. Changes in digestive system over past few months 19. Headaches cause fainting or other medical emergencies 20. Substance abuse/dependence/addiction in previous 3 months 21. Procedure or injection to treat migraine over past 3 months 22. Implanted or external nerve stimulator to treat migraines 23. Pain medications on most days a week for headaches 24. Opioid medications each day for chronic pain 25. History of overusing medications 26. History of eye pathology, surface disorder, surgery (except cataract extraction) or injury 27. Difficulty seeing clearly (with glasses or contact lenses, if required) 28. History of diabetes, hypertension, collagen vascular disease, vasculitis or renal disease/failure 29. Smoke >1 pack of cigarettes a day 30. Disorders of the optic nerve or retina 31. Reasons that retinae may not be clearly viewed 32. Poor image perception due to cataract or unstable fixation 33. Anaemia or iron deficiency 34. Participation in concurrent research trial

APPENDIX 7. LARGE TRIAL SCHEDULE OF ASSESSMENTS

	STUDY PERIOD					
Visit	**Screening /enrolment**	**Visit 1 (informed consent)**		**Visit 2**		**Visit 3 (EoS)**
TIMEPOINT	**-t_1 (1 day)**	**0 (1 day)**	**t_1 (2 weeks)**	**t_2 (1 day)**	**t_3 (12 weeks)**	**t_4 (1 day)**
ENROLMENT:						
Eligibility screen	X					
Informed consent		X				
Participant demographics		X				
Participant anthropometric measures		X				
Group allocation		X				
INTERVENTIONS:						

Placebo			X		X	
L-arg					X	
AGE					X	
L-arg + AGE					X	
ASSESSMENTS:						
Daily and weekly migraine diaries			X		X	
MSQ		X				X
SF-MPQ		X				X
L-VISS		X				X
VLSQ-8		X				X
OCTA				X		X
Acute light challenge				X		X
Blood markers				X		X

L-arg: L-arginine; AGE: aged garlic extract; t: timepoint; MSQ: Migraine-Specific Quality-of-Life Questionnaire; SF-MPQ: Short-Form McGill Pain Questionnaire; L-VISS: Leiden

Visual Sensitivity Scale; VLSQ-8: Visual Light-Sensitivity Questionnaire – 8; OCTA: optical coherence tomography angiography.

APPENDIX 8. PARTICIPANT INFORMATION SHEET

Migraine Treatment Research Project

You have been asked to participate in this study involving a new treatment for migraines. Please read this document carefully and ask any questions you wish. Knowing what is involved will help you decide if you want to take part in this research. Before deciding whether to take part, you might want to talk about it with a relative, friend or your local medical doctor. Participation in this research is voluntary. Do not sign this informed consent form unless you fully understand the nature of the study and any possible side effects. There will be ample opportunity for you to meet the investigators and have the study procedures and protocol fully explained to you.

Background information

Migraine is a very common and distressing neurological condition that causes symptoms such as recurrent throbbing headaches, nausea and disturbed vision. Migraines are caused by the interaction between the brain and blood vessels of the brain. Migraines are conventionally believed to occur because of the dilation (opening) of the large vessels in the brain, which leads to activation of pain receptors in the brain which causes the common symptoms of migraines. The medications currently offered do not seem to address the main cause of migraines, and have many adverse effects.

Recent research findings suggest that migraines may occur due to the constriction (tightening) of the smaller vessels of the brain, which leads to oxygen and nutrient insufficiency, causing vascular headaches; however, there are questions that still need to be answered. There are certain dietary compounds that can increase the dilation of smaller blood vessels, and our research project will investigate if these compounds can reduce the frequency and severity of migraine symptoms. These include L-arginine and aged garlic extract. L-arginine is an amino acid commonly found in food, that helps the body build protein. It is found in meat, dairy, eggs and seeds. Aged garlic extract is derived from garlic which is a common ingredient in food. Garlic is known to have beneficial antioxidant, antithrombotic and antiatherosclerotic properties. Neither have been commonly known to cause adverse effects when included in the human diet.

In this study, researchers are seeking participants to investigate the preventive/treatment effects of L-arginine and aged garlic extract in reducing migraines headaches both in the short- and the long-term. This study is a double-blind study. To ensure we test without bias whether each intervention is effective in reducing the severity of migraines, participants will be randomly assigned to one of four groups: Group 1 (placebo), Group 2 (L-arginine), Group 3 (aged garlic extract) or Group 4 (a combination of L-arginine and aged garlic extract). You will be randomly allocated (by computer) and have an equal chance to be in any of the groups. As this is a double-blind study, neither you nor the

research team will know which group you are allocated to, in order to reduce bias throughout the treatment.

Inclusion/exclusion criteria

To be eligible for this trial, you must fulfil the following inclusion criteria*:*

1. Migraine (with or without aura) diagnosed at least 1 year ago
2. Migraine onset occurred before 50 years of age
3. 2-6 migraine episodes and fewer than 6 "other" headache types per month, over the last 3 months
4. Able to distinguish between migraine and "other" headache types
5. Able to complete a daily diary about migraine experience
6. Able to commit to taking the 5 capsules a day for 14 weeks

If you meet any of the following criteria, you <u>will not be eligible</u> for the study:

1. Taking medications/drugs affecting vascular tone and/or blood pressure
2. Taking nitrate drugs or isoproterenol (often prescribed for angina or heart failure)
3. Taking more than two migraine-prevention drugs
4. Taking antidepressants or diuretics, herbs and/or supplements
5. Taking Sildafenil, Cialis, Spedra and any other PGE-5 inhibitor drugs
6. Taking drugs with potential blood-vessel effects (analgesics, decongestants and/or antihistamines)
7. Already taking L-arginine or garlic supplements for 3 months before the study
8. Having headaches causing fainting or another medical emergency
9. Having chronic daily headaches, medication-overuse headaches and/or other secondary headache disorders
10. Having diagnoses other than migraine as the primary cause of headache
11. A change in migraine treatment in the 3 months prior to or during the study
12. Clinical reports of renal or liver dysfunction
13. Clinical risks associated with bleeding or coagulopathy, or currently on blood-thinning medications such as warfarin/heparin therapy
14. Having any cardiovascular or neoplastic diseases
15. Having major chronic metabolic or neurologic disorders, or receiving current therapy for them
16. Being diagnosed with psychosis or bipolar affective disorder
17. Diagnosis of cancer
18. Substance abuse/dependence/addiction in the 3 months prior to or during the study
19. Having a history of diabetes, hypertension, collagen vascular disease, vasculitis and/or renal disease/failure

20. Having any low-blood-pressure-related issues or type-1 or -2 diabetes
21. Having the possibility of pregnancy or lactation
22. Being allergic to garlic or its constituents
23. Having gastric disturbances such as bloating, stomach pain, heartburn, diarrhoea, constipation, nausea and/or vomiting
24. Are a smoker of >1 pack a day
25. Having a history of eye pathology, surface disorder, surgery (except cataract extraction) and/or injury
26. Not being able to see clearly (with glasses/contacts, if need be)
27. Having disorders of the optic nerve (including glaucoma) or retina
28. The significant possibility of our not being able to see the inside of your eye clearly
29. Having poor image perception due to cataract or unstable fixation

What does the study involve?

This study is open to adults aged between 18 and 80, who currently experience migraines. This is a randomised controlled trial, which means that study participants will be randomly placed into groups and given different treatments in order for the results from each to be compared. You will be randomly allocated to one of 4 study groups. Your participation in this study involves a considerable time commitment with a total of **3 visits** over a period of 14 weeks. The 3 visits will take approximately 30 to 60 minutes each.

Before your appointment

Prior to your first visit, you will be sent a few questionnaires that need to be completed and brought with you to your first visit. These questionnaires will ask about your experience with migraines and the impact of migraines on your daily life. If you have not completed the questionnaires prior to your first visit, there will be time to complete the questionnaires during the visit.

Visit 1

You will be asked to attend an appointment at the Sarich Institute (8 Verdun St., Nedlands WA 6009). During this appointment, we will discuss the study requirements and go over the inclusion and exclusion criteria for the study. If you are eligible for the study, we will measure your body weight, height, waist and hip circumferences, blood pressure and heart rate. Next, we will provide you with a treatment pack of capsules to take every day for 2 weeks. During the next 2 weeks, we ask you to take **five (5)** capsules each day, and complete a hardcopy daily and weekly migraine diary we will provide you with. This session should take approximately 30 minutes.

Visit 2

2 weeks after Visit 1, you will be asked to attend the Sarich Institute again (8 Verdun St., Nedlands WA 6009). During this session, we will take a sample of your blood and measure your blood pressure, and you will be asked to complete a light-sensitivity task and undergo an eye (retinal) scan. For the blood collection, we will ask you to come in fasted (8-12 hours), only consuming water before we collect your blood. All blood samples will be collected by a certified phlebotomist. If we cannot collect a blood sample from you, you may be requested to visit PathWest for a blood test. In the rare event of a medical emergency, fully qualified physicians and nursing staff from the Sarich Institute will be available. The retinal imaging is completely non-invasive and pain-free (it does not even require eye drops), and it should only take 5 minutes to scan each eye. The reason for this retinal scan is that the small vessels of the eye (within the retina) are considered an extension of the tiny blood vessels of the brain, and therefore may serve as a surrogate marker of central nervous system (brain) small-vessel response to our treatment.

For the light-sensitivity task, you will be asked to sit in a dark room, and we will increase the luminosity of a light panel in front of you in a step-wise fashion until you feel the light is becoming uncomfortable, or continued exposure to the light would bring on a migraine. This may cause some discomfort; however, this will be aborted as soon as you indicate the light is uncomfortable.

Finally, you will receive your second treatment pack, comprising treatment doses to last the next 12 weeks. Again, we ask you to take the capsules and complete the migraine diary on a daily basis for the next 12 weeks. This session should last approximately 60 minutes.

Visit 3

After 12 weeks, you will be asked to visit the Sarich Institute (8 Verdun St., Nedlands WA 6009) one last time. Prior to the appointment, you will be sent questionnaires that need to be completed prior to your appointment. These are the same questionnaires that you completed prior to Visit 1. If you are unable to complete the questionnaires prior to the visit, there will be time during the visit to complete the questionnaires. Visit 3 will include the same assessments as Visit 2. This session should last approximately 60 minutes.

Possible adverse effects

The blood collection during each visit may cause discomfort, as we will use a needle to collect the blood. Although we will try to collect the blood from different sites, it is possible that you may find the number of blood samples collected an uncomfortable experience. Therefore, it is important to inform us if this is the case and to know that you are free to withdraw from the study at any stage. For us, your comfort during the procedure is of more concern than our ability to collect both blood samples. In some people, slight bruising and tenderness may appear afterwards at the site of venepuncture. These side effects are only minor and will return to normal in a matter of days. The volume of blood collected should not cause you any risk of becoming anaemic (approximately 6 tablespoons).

Moreover, the visits have been spaced 12 weeks between each visit, so that new red blood cells will be ready to enter the circulation. However, we advise you not to donate blood during the period of study. If you need to do any routine clinical test with blood collection, this is not a problem.

L-arginine and aged garlic extract have not been known to cause any serious adverse effects. You may experience some gastric disturbances, and in rare cases, nausea and vomiting. We will contact you after the first week of the first 2-week treatment period, and after the first week of the second 12-week treatment period, to check how you are going.

Ability to withdraw from the study

It must be stressed that your participation in this study is entirely voluntary; you are free to withdraw from the study at any stage. It is important that you do not feel any pressure to complete the study, particularly if it is not what you had originally anticipated. In the event that you withdraw or discontinue your participation in the project, we will ask your permission to use the data you have already provided. If you do not want your data to be retained, all data relating to your participation will be destroyed. In some instances, it may be required that we retain information about any adverse events you may have experienced. You will not be identifiable from this data. If you are currently enrolled as a student at Curtin University, there will be no impact on your studies if you were to withdraw from the study.

Benefits to the participant

This study may not be of direct benefit to you; however, by taking part in this study, you will gain useful information about your vascular tone as a migraineur. We encourage you to let your doctor know that you are taking part in our study. Any results that are out of clinical range will be sent to your general practitioner with your consent. Your participation in this study will provide us with important data, enabling us to investigate further the role of L-arginine and aged garlic extract in treating and/or preventing vascular headaches, especially migraines.

What happens when the research project ends?

Your data will be stored for a minimum of 15 years after the study, then destroyed. If published studies suggest benefits are realised via the indicated treatments being trialled, we will recommend participants discuss with their primary care provider, consideration for prevention and attenuation of migraine pain frequency and intensity. You will have the option of being contacted with the results of the study.

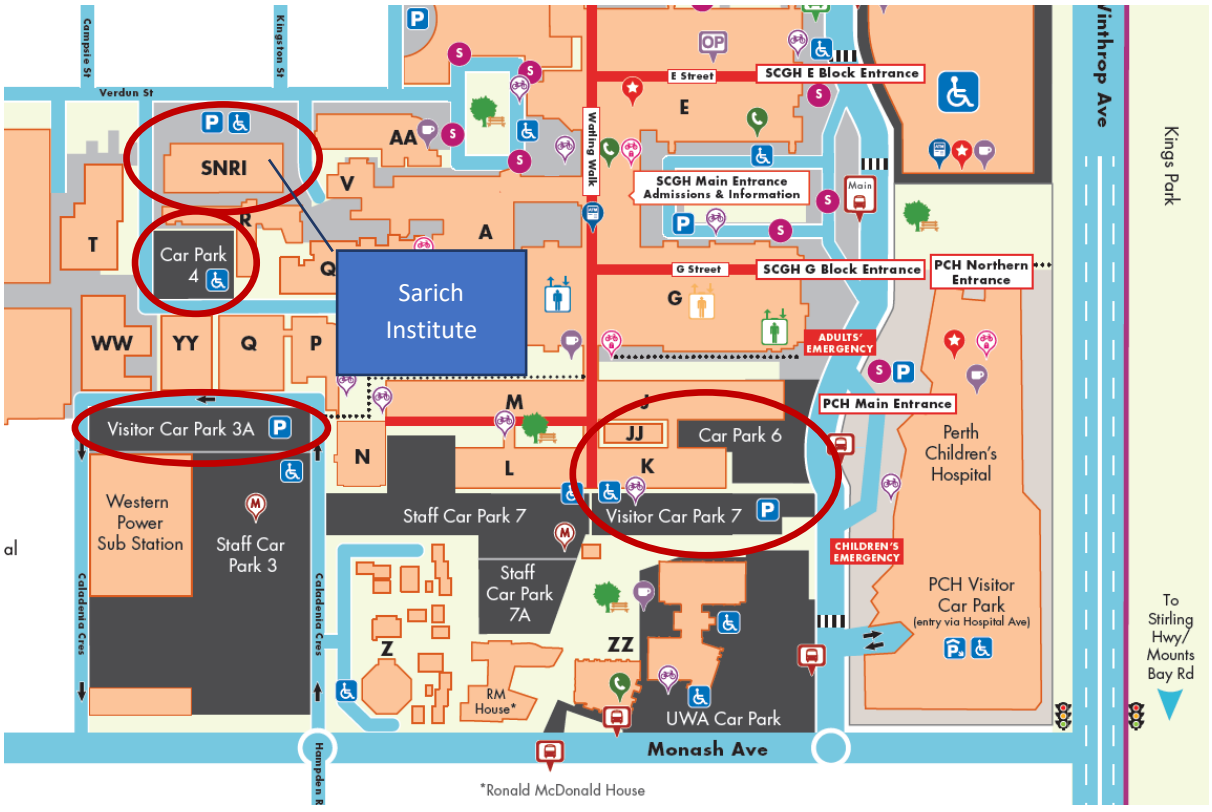
Confidentiality

Any information that you provide us will be stored securely, to protect your privacy. Some of your data may include personal information such as your name, date of birth and/or a reference number. This

information will be separate to your actual data (assessment scores) and your data will be de-identified. The security of standards of all personal information follows Curtin University data-management guidelines. All information will be held in secure, locked filing cabinets at Curtin University, or on a password-protected computer database held on a secure system, which allows access to authorised individuals only. It is possible that the results from this study will also be utilised in future studies on a similar topic; however, your identity will never be disclosed. All information will be strictly confidential, and any publications arising from this work will not include your name or any other identifying feature.

Site map and parking

Here is visitor-parking information around the Sarich Institute: https://qeiimc.health.wa.gov.au/travel-access/parking/visitor-parking/. Your visits will take up to an hour, and therefore will be $3.40 on the parking meter. Below is the site map, with the relevant areas circled in red. As you can see, there are 4 available carparks near the precinct.



Should you get lost on the way, please don't hesitate to call Debs on (04) 517 176 70.

Further information

If you have queries or require urgent contact, call Debs Chaliha on 0451717670.

If you have extensive queries or require further clarification, please contact the lead investigator:

Professor John Mamo

School of Public Health, Curtin University

Phone: (08) 9266 7232

Email: J.Mamo@Curtin.edu.au

Curtin University Human Research Ethics Committee (HREC) has approved this study (HRE2020-0466). The Committee is comprised of members of the public, academics, lawyers, medical doctors and pastoral carers. Its key role is to protect participants. If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University, GPO Box U1987, Perth, 6845, by telephoning 9266 2784, or by emailing hrec@curtin.edu.au.

HREC Project Number	HRE2020-0466
Project Title	Migraine Treatment Research Project (NOPAIN)
Principal Investigator	Prof. John Mamo (Director of Curtin Health Innovation Research Institute)
Student Researcher	Devahuti (Debs) Chaliha
Version Number	3
Version Date	24 Feb 2021

- I have read the information statement version listed above, and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I have had an opportunity to ask questions, and I am satisfied with the answers I have received.
- I understand that I will be randomly allocated to one of four possible treatment groups, and that neither I nor the trial coordinator will know which group I am in.

- I voluntarily consent to take part in this research project, and understand that I can withdraw at any time without prejudice.
- I understand that my data will be stored for 15 years following the completion of the study, after which time it will be destroyed.
- I understand that this project has been approved by Curtin University Human Research Ethics Committee, and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I have received a copy of this Information Statement and Consent Form.

Participant Name	
Participant Signature	
Date	

Declaration by researcher: I have supplied an Information Letter and Consent Form to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Researcher Name	
Researcher Signature	
Date	

Note: All parties signing the Consent Form must date their own signature.

APPENDIX 9. DAILY MIGRAINE DIARY

Patient ID:	**Date**:

Occurrence: Did you have a migraine today? Yes ☐ No ☐

- If **NO**, please leave the remaining of today's diary blank.
- If **YES**, please answer the questions below.

Frequency: How many migraine attacks did you have today, separated by 2 hours each?

Severity: On average, how severe was (were) the migraine(s)? The following line represents increasing pain intensity from "no pain" to "worst possible pain". Place a mark on the line that you feel best describes your level of pain.

No Pain **Worst Possible Pain** Score in mm (investigator's use only)

Did you take anything to help relieve the pain? Yes ☐ No ☐

If YES, please list all medications, including over-the-counter medications.

Medication name	*Number/amount of tablets taken*

BEFORE YOUR MIGRAINE BEGAN, did you experience any of the following? Select all that apply.

☐ Changes in vision	☐ Changes in smell	☐ Changes in sound	☐ Change in taste
☐ Excessive sleepiness	☐ Trouble talking	☐ Confusion	☐ Nausea (feeling sick)
☐ Vomiting	☐ Anxiety/irritability	☐ Low mood	☐ Neck ache
☐ Unsure	☐ None listed		

BEFORE YOUR MIGRAINE BEGAN, did you have numbness or tingling in your face or arms?

Yes ☐ No ☐ Unsure ☐

BEFORE AND/OR DURING YOUR MIGRAINE, did you experience any of the following sensations? Select all that apply.

☐ Throbbing	☐ Shooting	☐ Stabbing	☐ Sharp
☐ Cramping	☐ Gnawing	☐ Hot/burning	☐ Aching
☐ Heavy (like a weight)	☐ Tender	☐ Splitting	☐ Tiring/exhausting
☐ Sickening	☐ Fear-causing	☐ Punishing/cruel	

APPENDIX 10. WEEKLY MIGRAINE SUMMARY

Patient ID:	**Week:**
Did you have a migraine over the past 7 days? Yes ☐ No ☐	

Short-Form McGill Pain Questionnaire

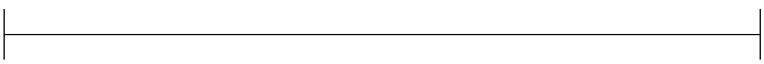
PLEASE DESCRIBE YOUR PAIN DURING THE LAST WEEK (Check off one box per line.)

	None	**Mild**	**Moderate**	**Severe**
1. Throbbing	0 ☐	1 ☐	2 ☐	3 ☐
2. Shooting	0 ☐	1 ☐	2 ☐	3 ☐
3. Stabbing	0 ☐	1 ☐	2 ☐	3 ☐
4. Sharp	0 ☐	1 ☐	2 ☐	3 ☐
5. Cramping	0 ☐	1 ☐	2 ☐	3 ☐
6. Gnawing	0 ☐	1 ☐	2 ☐	3 ☐
7. Hot-burning	0 ☐	1 ☐	2 ☐	3 ☐
8. Aching	0 ☐	1 ☐	2 ☐	3 ☐
9. Heavy (like a weight)	0 ☐	1 ☐	2 ☐	3 ☐
10. Tender	0 ☐	1 ☐	2 ☐	3 ☐
11. Splitting	0 ☐	1 ☐	2 ☐	3 ☐
12. Tiring-Exhausting	0 ☐	1 ☐	2 ☐	3 ☐
13. Sickening	0 ☐	1 ☐	2 ☐	3 ☐

14. Fear-causing	0 □	1 □	2 □	3 □
15. Punishing-Cruel	0 □	1 □	2 □	3 □

PLEASE RATE YOUR PAIN DURING THE LAST WEEK

The following line represents pain of increasing intensity from "no pain" to "worst possible pain". Place a vertical mark (|) across the line in the position that best describes your pain **during the last week.**



No Pain **Worst Possible Pain**

Score in mm
(investigator's
use only)

CURRENT PAIN INTENSITY

0 □ No pain

1 □ Mild

2 □ Discomforting

3 □ Distressing

4 □ Horrible

5 □ Excruciating

Questionnaire developed by: Ronald Melzack

APPENDIX 11. OCT AND OCTA STUDIES IN MIGRAINEURS

Study	Total No. Subjects	Sex of Subjects (% Female)	Adults or Children	Cross-Sectional/ or Longitudinal	Episodic or Chronic Migraineurs	Migraineurs With (MA) or Without Aura (MO)	Ictal or Interictal Measurements	Findings
Abdellatif 2018 **(211)**	130	Both (71% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	Migraineurs had thinner RNFL, superior and inferior GCL and all-except-central choroid than HC The longer the migraine history, the thinner the GCL, RNFL and especially choroid The more severe the migraine, the thinner the GCL and RNFL
Acer 2016 **(212)**	82	Both (91% female)	Adults	Cross-sectional	Episodic	MO	Interictal	Migraineurs had thinner temporal and nasal superior peripapillary RNFL than HC Migraineurs tended to have thinner GCL and maculae than HC
Altunisik 2021 **(213)**	155	Both (77% female)	Adults	Cross-sectional	Unspecified	Both	Both	RNFL tended to be thinner in migraineurs than HC

								Peripapillary RNLF in left-eye nasal, central, nasosuperior, nasoinferior quadrants, and right-eye temporoinferior quadrants, was thinner in migraineurs than HC GCL was thinner in migraineurs than HC Choroids were thicker in migraineurs than HC No difference in findings whether they had WMH or not The longer they had had migraines for, the thinner the choroid Choroids were thinner ictally than interictally No difference in findings between MO and MA IPL thicknesses did not differ between groups

Ao 2019 **(238)**	165	NA	NA	NA	NA	NA	NA	MA had thinner nasal peripapillary RNFL, inferior inner macula layer, and choroid, than HC No difference between MO and HC The longer the migraine history or the more frequent the attacks, the thinner the nasal peripapillary RNFL
Bing 2019 **(217)**	94	Both (83% female)	Adults	Cross-sectional	Unspecified	Unspecified	Interictal	Migraineurs had thinner average and superior RNFL than HC
Bulboacă 2020 **(236)**	77	Both (68% female)	Adults	Cross-sectional	Episodic	MO	Interictal	Migraineurs had more oxidative stress markers, and less antioxidative molecular markers, than HC. This was especially associated with the temporal quadrant The thinner the macular inner temporal RNFL, the less the catalase (antioxidant marker)
Burgos-Blasco 2023 **(239)**	37	Both (60% female)	Children	Cross-sectional	Episodic	Both	Interictal	MA had thinner temporal and inferior temporal RNFL than MO and

								HC (no difference between HC and MO) More the migraine frequency, thinner the nasal superior RNFL
Çam 2022 **(282)**	99	Both (66% female)	Adults	Cross-sectional	Unspecified	Unspecified	Interictal	Migraineurs had thicker iris sphincter and dilator muscle epithelia and stroma (and total, therefore), and choroids, than HC
Cankaya 2016 **(264)**	54	Both (56% female)	Adults	Cross-sectional	Episodic	Both	Interictal	MA had decreased foveal thickness compared to MO and HC
Chang 2017 **(141)**	49	Both (55% female) HC predominantly male, MA and MO predominantly female	Adults	Cross-sectional	Unspecified	Both	Interictal	Macula: MA had increased FAZ than controls, and decreased superficial foveal density than MO Optic nerve: MA had reduced superior peripapillary VD than MO and HC No differences between MO and HC
Colak 2016 **(257)**	90	Both (81% female)	Adults	Cross-sectional	Chronic	MA	Interictal	MA had thinner superior and inferior RNFL quadrants, and thinner

								subfoveal, temporal, and nasal choroids, than HC
Costello 2009 **(218)**	123	Unspecified	Adults	Cross-sectional	Unspecified	Both	Interictal	Temporal RNFL quadrant was thinner in migraineurs than HC In migraineurs, the thinner the RNFL, the more the migraine disability and frequency
Cunha 2008 **(219)**	1	Male	Adult	Longitudinal	Unspecified	MA	Interictal	Macula and peripapillary RNFL were thinner after 6 months post-episode, at papillomacular bundle ipsilateral to scotoma development, with mild localised RNFL loss temporal to optic disc
Dadaci 2014 **(146)**	29	Both (86% female)	Adults	Cross-sectional	Unspecified	Both	Both	Unilateral headaches: ictal choroidal thicknesses increased ipsilaterally from basal Bilateral headaches: foveal ictal choroidal thickness increased, more so in left eyes, compared to basal

Demircan 2015 **(150)**	95	Both (89% female)	Adults	Cross-sectional	Episodic	Both	Interictal	MA and MO had thinner nasal and nasal inferior RNFL than HC Macular: similar thicknesses between all groups MA and MO had thinner choroids than HC
Demirci 2016 **(222)**	150	Both (89% female)	Adults	Cross-sectional	Both	Both	Interictal	Superior, nasal and inferior RNFL thicknesses decreased in MO and MA, compared to HC No differences between MO and MA
Dereli Can 2021 **(240)**	101	Both (63% female)	Children	Cross-sectional	Unspecified	MO	Interictal	No difference between groups The less the capillary VD and RNFL thickness, the more the migraine disability
Dervisogullari 2015 **(283)**	59	Both (85% female)	Adults	Cross-sectional	Unspecified	Both	Ictal	Ictally, choroid was thinner in migraineurs than HC
Ekinci 2014 **(229)**	120	Both (67% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	MA had thinner RNFL, GCL and choroid than MO and HC

								MO and especially MA had thinner choroids than HC Migraineurs overall had thinner RNFL, GCC and choroid than HC
Ergiyit 2017 **(224)**	72	NA	NA	NA	NA	NA	NA	Migraineurs had thinner temporal upper, temporal lower, inferonasal and mean RNFL, and choroid than HC
El-Shazly 2017 **(252)**	90	Both (69% female)	Adults	Longitudinal	Episodic	MA	Both	Ictally, MA had thinner RNFL than HC, thickening interictally but remaining thinner than HC
Gipponi 2013 **(223)**	40	Female	Adults	Cross-sectional	Episodic	Both	Interictal	Migraineurs had decreased superior quadrant thicknesses compared to HC The thinner the RNFL, the longer the migraine history and attack and aura durations
González-Martín-Moro 2023 **(266)**	1	Male	Adult	Longitudinal	Unspecified	MA	Both	During left hemicranial pain and right-eye aura, there was right-eye macular hypoperfusion which

								resolved 2-7 days after aura and pain resolution
Guler 2020 **(202)**	50	Both (88% female)	Adults	Cross-sectional	Episodic	MO	Ictal	Migraineurs had less deep superior VD than HC
Gunes 2016 **(151)**	116	Both (84% female)	Adults	Cross-sectional	Episodic	Both	Interictal	Tendency for average and nasal thicknesses to be thinner in migraineurs than controls, more so on ipsilateral headache sides
Gunes 2018 **(234)**	81	Both (67% female)	Adults	Cross-sectional	Chronic	Both	Both	Choroidal thickness was increased, and GCL decreased, in chronic migraineurs than HC Choroid was thicker during than between migraines Cornea was thicker during migraines than between or HC
Hamamci 2021 **(209)**	90	Both (75% female)	Adults	Cross-sectional	Episodic	Both	Interictal	MA had lower superficial and deep foveal, whole optic disc, optic disc inside, peripapillary, superior hemisphere, inferior hemisphere, superior quadrant, and temporal

								quadrant VD, and larger FAZ, than HC The lower the VD, the higher the migraine frequency, disability and life impact
Hamurcu 2021 **(210)**	38	Both (89% female)	Adults	Cross-sectional	Unspecified	MA	Interictal	No thickness differences between groups MA had larger optic disc rim area than HC MA had larger FAZ than HC MA tended to have lower vessel densities than HC The longer MA had had migraines for, the lower the superior parafoveal VD tended to be
He 2022 **(260)**	86	Both (87% female)	Adults	Cross-sectional	Both	Both	Interictal	Migraineurs had reduced macular retinal vessel and perfusion densities than HC

								MA had reduced retinal vessel density at the optic nerve head than HC The lower the retinal vessel and perfusion densities of the macula and optic nerve head, the higher the migraine frequency and severity
Iyigundogdu 2018 **(762)**	NA	NA	NA	NA	NA	NA	NA	NA
Kanar 2021 **(230)**	127	Both (72% female)	Adults	Cross-sectional	Episodic	Both	Interictal	Foveal, nasal and temporal choroids were thinner in MA than MO or HC There was no difference in choroidal thickness between MO and HC Both MO and MA had thinner global, superior and inferior peripapillary RNFL than HC, with MO and MA having no difference Nasal quadrant peripapillary RNFL was thinner in MA than in MO or HC

								Superior and inferior macular GCL were thinner in MA and MO than in HC
Karaca 2016 **(148)**	64	Female (but 1 male HC, 1 male MO)	Adults	Cross-sectional	Both	Both	Interictal	MA and MO had thinner choroidal thicknesses than HC, with MA tending to be thinner than MO
Karahan 2021 **(259)**	116	NA	NA	Cross-sectional	NA	MA	Interictal	MA had decreased vessel densities at nasal and inferotemporal optic nerve head, inferonasal radial peripapillary capillaries, and deep macular plexus, than HC Most MA had deep FAZ enlargement
Karalezli 2015 **(143)**	46	Both (52% female)	Adults	Cross-sectional	Unspecified	Unspecified	Ictal	Migraineur choroids were ictally thicker than controls
Khosravi 2018 **(233)**	60	Both (65% female)	Adults (but youngest were less than 18	Cross-sectional	Unspecified	Both	Interictal	Migraineurs had thinner and less eye-eye symmetrical RNFL and choroid, smaller optic and neuroretinal rim disk area, and lower cup-disk ratio at optic nerve head, than HC

			years old)					
Kirbas 2013 **(247)**	80	Both (63% female)	Adults	Cross-sectional	Chronic	Unspecified	Interictal	Only superior RNFL thickness was lower in chronic migraineurs than HC No macular differences between groups
Kızıltunç 2020 **(265)**	1	Female	Adult	Longitudinal	Episodic	MA	Both	During aura preceding headache, diffuse retinal vessel narrowing, decreased radial peripapillary capillary density, decreased deep foveal VD. These changes occurred only ipsilaterally, and improved 3 hours after aura
Kızıltunç 2020 **(763)**	61	Female	Adults	Cross-sectional	Unspecified	Both	Interictal	MA had less choriocapillaris blood flow than MO and HC
Kurtul 2022 **(303)**	NA	NA	Children	NA	NA	NA	NA	NA

Labib 2020 **(243)**	60	Both (83% female)	Adults	Cross-sectional	Chronic	Both	Interictal	Chronic migraineurs had more thinning of average, superior, inferior, nasal, temporal RNFL than HC MA had thinner average RNFL and GCL than MO
Martinez 2008 **(153)**	123	Both (NA)	Adults	Cross-sectional	Unspecified	Both	Interictal	Migraineurs had thinner temporal RNFL than HC, and higher migraine frequency and disability were associated with thinner RNFL
Midelfart 2013 **(764)**	NA	NA	NA	NA	NA	NA	NA	NA
Nalcacioglu 2017 **(246)**	80	Both (75% female)	Children	Cross-sectional	Episodic	Both	Interictal	No difference between groups
Oba 2023 **(254)**	70	Females	Adults	Cross-sectional	Both	MO	Interictal	Chronic MO had thinner superior temporal RNFL and lower temporal and temporal inferior circumpapillary VD than HC (indicating optic nerve damage)

Özçift 2021 **(270)**	70	Both (76% female)	Adults	Cross-sectional	Unspecified	Unspecified	Interictal	Longer the migraine history, thinner the choroid
Panicker 2021 **(226)**	111	Both (81% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	Peripapillary, temporal and nasal RNFL quadrant thickness, and central macular thickness, were decreased in migraineurs than HC
Raga-Martinez 2022 **(235)**	180	Both (86% female)	Adults	Cross-sectional	Chronic	Both	Interictal	Chronic migraineurs had thinner superior quadrant peripapillary and macular RNFL, macula, GCL than HC
Reggio 2017 **(149)**	77	Both (82% female)	Adults	Cross-sectional	Both	Both	Interictal	Migraineurs had decreased RNFL, GCL and choroid thicknesses than HC Chronic migraineurs had more reduced RNFL and GCL than episodic migraineurs
Romozzi 2023 **(269)**	60	Both (77% female)	Adults	Cross-sectional	Both	Both	Interictal	MA had larger FAZ than HC MA had lower foveal choriocapillaris VD than MO
Salman 2015 **(249)**	120	Both (58% female)	Adults	Cross-sectional	Episodic	Both	Interictal	No thickness differences between migraineurs and HC

Sezer 2023 **(287)**	72	Both (83% female)	Adults	Cross-sectional	Chronic	Both	Interictal	No differences in choroidal thicknesses or vascularity indices between HC, MO and MA
Sim 2023 **(244)**	75	NA	Children	NA	NA	Both	NA	Optic disk RNFL was lower in MA than HC and MO
Simsek 2015 **(248)**	80	Both (73% female)	Adults	Cross-sectional	Both	Both	Interictal	No differences in RNFL thickness were found between MO, MA or HC – except nasal quadrant of right eye which was thicker in migraineurs than HC
Simsek 2017 **(152)**	80	Unspecified	Adults	Cross-sectional	Unspecified	Both	Interictal	Migraineurs with WMH had thinner RNFL than HC, but migraineurs without WMH did not
Sirakaya 2020 **(227)**	97	Both (85% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	MA and MO had thinner RNFL, and thinner and deeper lamina cribrosa, than HC The longer they had migraines for, the thinner the RNFL The thinner the lamina cribrosa, the higher the migraine disability

Slagle 2021 **(765)**	1	NA	NA	NA	NA	NA	NA	NA
Sorkhabi 2013 **(220)**	90	Both (71% female)	Adults	Cross-sectional	Chronic	Both	Interictal	Migraineurs had thinner RNFL in the nasal quadrant only, than HC, and there was no difference between those with and without aura
Tak 2018 **(216)**	120	Both (89% female)	Adults	Cross-sectional	Unspecified	Unspecified	Interictal	Migraineurs had thinner RNFL than HC No difference was found between migraineurs with and without WMH
Taşlı 2020 **(145)**	109	Both (78% female)	Adults	Cross-sectional	Episodic	MO	Interictal	MO had larger FAZ, and lower superficial and deep macular VDs, than HC No differences between MO with vs. without WMH
Temel 2021 **(228)**	56	Both (52% female)	Adults	Cross-sectional	Episodic	MO	Interictal	Migraineurs had thinner non-nasal RNFL, and increased choroidal vascularity, than HC
Torun 2023 **(251)**	138	Both (93% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	MO and especially MA had thinner choroids

								Those with visual aura had thinner RNFL than non-visual aura
Tunç 2017 **(215)**	120	Both (83% female)	Adults	Cross-sectional	Unspecified	Both	Unspecified	Migraineurs had thinner average, inferior and superior RNFL than HC MA had thinner average, inferior and superior RNFL quadrants than MO and HC; MO had thinner average and inferior RNFL than HC Migraineurs with >4 attacks a month had thinner macular parts and GCL than HC Migraineurs with WMH had thinner macular parts than those without
Uludag 2014 **(256)**	40	NA	Adults	NA	Chronic	NA	NA	RNFL and GCC were thinner in chronic migraineurs than HC
Ulusoy 2019 **(142)**	88	Both (72% female)	Adults	Cross-sectional	Episodic	Both	Interictal	Macula: superficial and deep retinal foveal VD reduced in MO and MA than HC Optic nerve: whole optic disc, peripapillary, superior hemisphere,

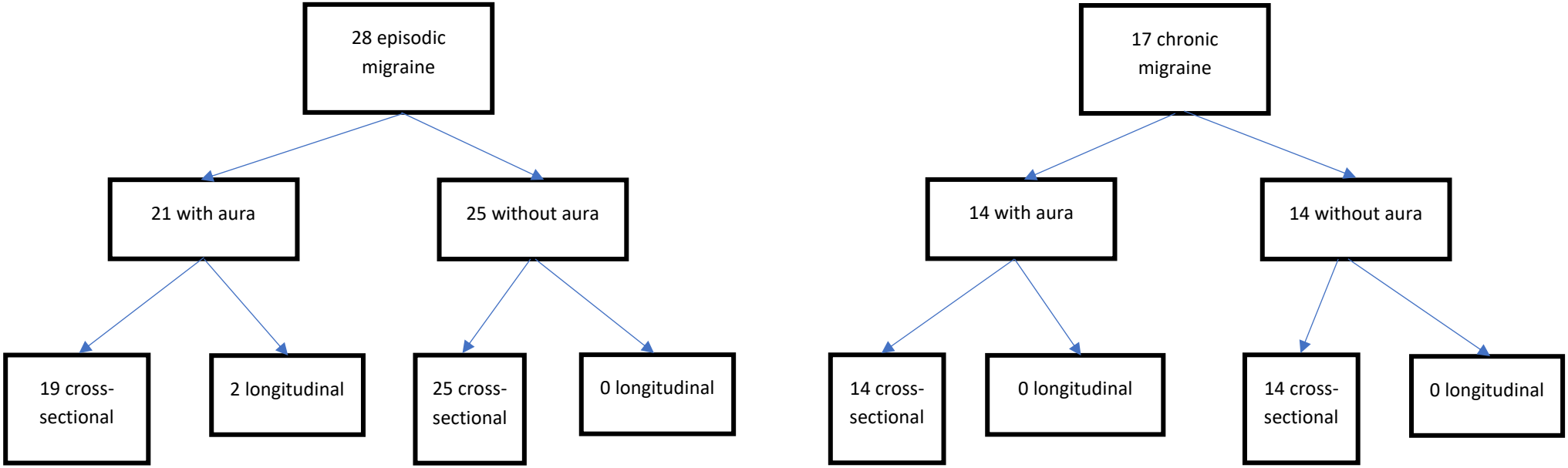
								superior layer and temporal layer VD reduced in MO and MA than HC In MA, those with WMH had reduced deeper foveal VD and superior hemisphere VD, average RNFL, superior hemisphere and superior layer than those without WMH
Ulusoy 2019 **(237)**	98	Both (66% female)	Adults	Cross-sectional	Episodic	Unspecified	Interictal	In temporal and medial quadrants, the thinner the RNFL, the higher the migraine severity and disability
Unlu 2017 **(147)**	104	Both (84% female)	Adults	Cross-sectional	Both	Both	Interictal	In chronic migraineurs but not episodic, retinal artery diameters increased and choroidal thicknesses decreased ipsilaterally to headache, compared to HC No changes in retinal vein diameters were found between groups
Verroiopoulos 2016 **(221)**	38	Both (92% female)	NA	NA	NA	Both	NA	MA had superior and inferior RNFL quadrants thinner than HC

								MO had superior RNFL quadrant thinner than HC
Yener 2019 **(766)**	70	Both (86% female)	Adults	Cross-sectional	Unspecified	Unspecified	Interictal	No differences between groups
Yener 2020 **(232)**	50	Both (76% female)	Children	Cross-sectional	Unspecified	MO	Interictal	MO had thicker nasal quadrant RNFL than HC MO had thinner temporal quadrant left-eye RNFL than HC MO had greater left-eye disc area than HC MO had larger cup volumes than HC
Yu 2022 **(245)**	100	Both (78% female)	Adults	Cross-sectional	Episodic	Both	Interictal	Vestibular migraineurs with WMH had thinner RNFL than HC Vestibular migraineurs without WMH had thicker RNFL than HC MO and MA had similar RNFL thicknesses

Yülek 2015 **(225)**	100	Both (71% female)	Adults	Cross-sectional	Unspecified	Both	Interictal	Migraineurs had decreased RNFL thicknesses compared to HC Longer the migraine history, thinner the RNFL
Yurtoğulları 2021 **(231)**	126	Both (79% female)	Adults	Cross-sectional	Both	Both	Interictal	MO and MA had thinner central and inner inferior macula; central macular RNFL; inner inferior and temporal, outer nasal and outer GCL, than HC
Zengin 2015 **(279)**	84	Both (86% female)	Adults (but lowest below 18 years old)	Cross-sectional	Unspecified	Both	Both	Migraineurs had thinner choroids than HC Ictally, migraineurs had thinner choroids than interictally

OCT and OCTA studies in migraineurs shown alongside study and migraineur types, measurement timings and vessel density results. Study authors listed in alphabetical order. FAZ: foveal avascular zone; RNFL: retinal nerve fibre layer; GCL: ganglion cell layer; GCC: ganglion cell complex; IPL: inner plexiform layer; MA: migraineurs with aura; MO: migraineurs without aura; HC: healthy controls; VD: vessel density; WMH: white-matter hyperintensity; NA: data not available (full text unavailable). Chronic migraine classification: 15/+ attacks/month (frequency) or 15/+ days/month (duration).

APPENDIX 12. NUMBER OF STUDIES BY TIME COURSE, AURA PRESENCE AND STUDY DESIGN



Flowchart showing the number of studies per time course (episodic vs. chronic), presence or absence of aura, and study design (cross-sectional vs. longitudinal).

APPENDIX 13. OCT/OCTA FINDINGS FOR EACH MIGRAINE SUBTYPE

	Episodic migraine	**Chronic migraine**
Migraine without aura	• Thinner RNFL, GCL and choroid (149-151, 212, 222, 223, 228, 230, 231) • More oxidative stress markers, less antioxidative molecular markers, especially in temporal quadrant (236) • Reduced retinal vessel and perfusion densities (142, 145, 202, 260) • Larger FAZ (145)	• Thinner RNFL, choroid and GCL (148, 149, 222, 234, 243) • Thinner RNFL and GCL than episodic migraineurs (149, 220, 231, 235, 254) • Thicker choroid ictally (234) • Reduced macular retinal vessel and perfusion densities (254, 260) • Retinal artery diameters increased and choroidal thicknesses decreased ipsilaterally to headache (147)
Migraine with aura	• Even thinner RNFL and choroid than without aura (148, 150, 151, 222, 223, 230, 231, 239, 252, 264) • Reduced vessel and perfusion densities (142, 209, 260, 265), VD more so than without aura (269) • Larger FAZ (209, 269) • Thinner GCL (230)	• Thinner RNFL, choroid and GCL, more so than without aura (149, 220, 222, 231, 234, 235, 243, 257) • Larger FAZ (269) • Lower VD than without aura (269) • Thinner choroid, RNFL and GCL than without aura (148, 243) • Thicker choroid ictally (234) • Retinal artery diameters increased and choroidal thicknesses decreased ipsilaterally to headache (147) • Reduced retinal vessel and perfusion densities (260) • Reduced RNFL and GCL than episodic migraineurs (149)

Carroll diagram showing characteristic OCT/OCTA findings for each migraine subtype, compared to healthy controls.

APPENDIX 14. DEMOGRAPHICS FORM

1. **Date of Birth**: ___/___/___
2. **Sex**: ☐ Male ☐ Female ☐ Declined to say ☐ Other: _____
3. **Ethnicity** (Select ONLY one with which you MOST CLOSELY identify):

☐ Native American ☐ Caucasian ☐ Asian ☐ Pacific Islander☐ Black or African American

☐ Aboriginal or Torres Strait Islander ☐ Hispanic or Latino ☐ Other: _____

4. **Race** (choose all that apply):

☐ Caucasian ☐ Asian ☐ African ☐ Aboriginal or Torres Strait Islander

5. What is your **country of birth**? _____
6. What is the **highest level of school you have completed**, or the highest degree you have received?

☐ Primary school ☐ High school or equivalent ☐ Technical/apprenticeship

☐ Undergraduate university ☐ Postgraduate university

7. Do you have a **family history of migraine**? ☐ Yes ☐ No

If yes, which relatives? _____

8. Do you take **medications** for reasons **other than migraine**? ☐ Yes ☐ No

If yes, what conditions/reasons? _____

9. Do you take vitamins/supplements/nutraceuticals? ☐ Yes ☐ No

10. Have you been **diagnosed with any significant medical conditions**? ☐ Yes ☐ No

If yes, tick as many as apply:

☐ Migraines with Aura (e.g. premonitions, zig-zag vision) ☐ Menstrual migraine

☐ Complex regional pain syndrome ☐ Other:

_____

11. **Medication History:**

What medications (including vitamins/supplements/nutraceuticals) do you currently take?	*Dosage (mg)*	*Number of tablets*	*Number of times per day*	*Reason*
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				

12. Do you have any other **relevant medical and/or surgical history**? ☐ Yes ☐ No

If yes, provide detail: _____

13. Have you **smoked in the last 6 months**? ☐ Yes ☐ No

If yes, on average how many **cigarettes** do you smoke **per day**? _____

14. How many times, on average, do you **drink** per week?

☐ I don't drink ☐ Once a week ☐ 3-5 times a week ☐ 6+ times a week ☐ Once a month

If you drink, how many standard drinks would you have on average per 24 hrs.? _____

15. If female, please answer the following questions (if you are not sure, please take your best guess):

- Do you menstruate? ☐ Yes ☐ No
- Are you on hormone-replacement therapy? ☐ Yes ☐ No
- Age of Menarche (first period): _____ years old
- Age of Menopause (if relevant): _____ years old
- What was the date of your last period? _____/_____/_____

APPENDIX 15. ADVERSE-EFFECTS FORM

Participant ID: _____

SEVERITY/INTENSITY	**1 – MILD**	**2 – MODERATE**	**3 – SEVERE**			
**** Action taken with IP**	1 – Dose not changed	2 – Dose reduced	3 – Drug interrupted	4 – Drug withdrawn		
***** Relationship to IP**	1 – Unrelated	2 – Unlikely	3 – Possibly	4 – Probably		
****** Outcome**	1 – Not recovered	2 – Recovered	3 – Recovered with sequelae	4 – Recovering	5 – Unknown	6 – Fatal

Adverse Event		Start Date/Time (if known)	Meds Taken	SAE?	Severity *	Action with IP **	Related to IP ***	Investigator Review	Outcome ****	Stop Date / Time (if known)	Investigator Review *(once stopped)*
1		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	
2		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	
3		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	
4		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	
5		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	
6		Init. Date	☐ Y ☐ N	☐ Y ☐ N				Date		Date	

ABOUT THE AUTHOR

Debs (“Rhia”) Chaliha is just finishing off her Neuroscience PhD, and can't wait to finally learn how to be a normal human. So she's learning how to adult, which now includes lugging along the postdoc extension of her project like a clingy child. Between gleefully experimenting on humans, she loves historical detective novels and logic puzzles. Her occasional escape tactics involve singing, casting, graphic design, charity work and literally flying away (on a plane). She watches horror movies for a laugh, and is equally merciless towards violators of human rights.

AUTHORSHIP DECLARATIONS

I, Devahuti Chaliha, wrote all first-author papers, undertook the clinical trial and developed this thesis.

_____

I, Professor John Mamo, conceived the study and confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha’s thesis.

_____

I, Associate Professor Ryu Takechi, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha’s thesis.

_____

I, Dr. Virginie Lam, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha’s thesis.

_____

I, Professor Mauro Vaccarezza, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha’s thesis.

_____

I, Professor Satvinder Dhaliwal, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha's thesis.

_____

I, Professor Peter Drummond, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha's thesis.

_____

I, Professor Eric Visser, confirm that I made the contributions to the publications as indicated, and the level of contribution detailed by the candidate is appropriate. I agree to give permission to use these publications as a part of Devahuti Chaliha's thesis.

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