Migraine as a cardiovascular risk factor for women

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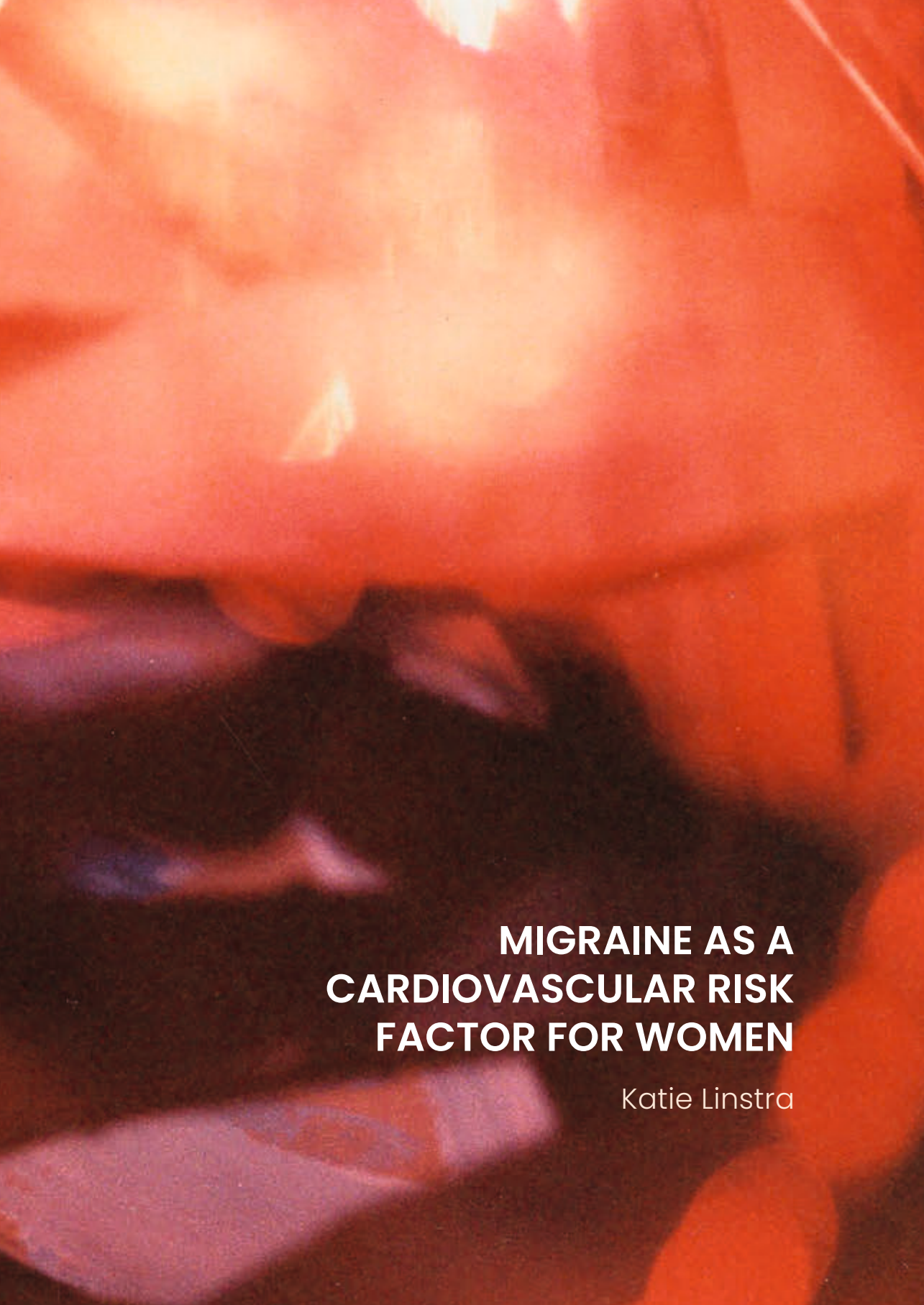

MIGRAINE AS A CARDIOVASCULAR RISK FACTOR FOR WOMEN

Katie Linstra

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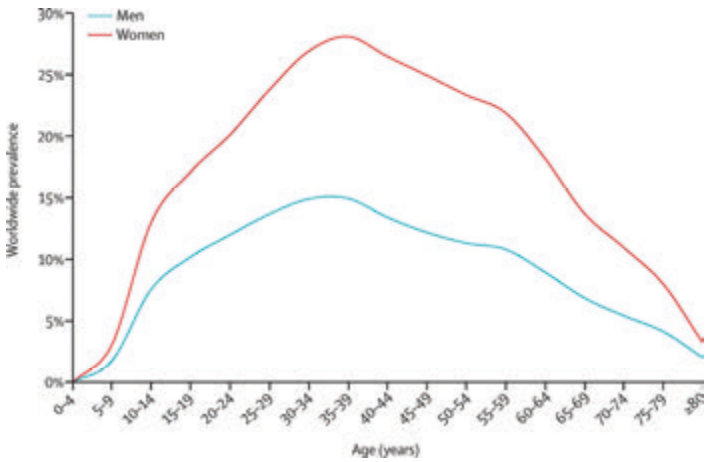
CHAPTER 1

General introduction and outline

MIGRAINE IN WOMEN

Migraine is a chronic disabling neurological disorder characterized by recurring severe headache attacks. The life time prevalence of migraine is 15% in the general population and globally more than 1 billion persons are affected.[1,2] Migraine occurs two to three times more often in women compared to men. Migraine prevalence fluctuates with age, with a peak occurring between age 30 and 40, when prevalence rises up to 15% in men and 27% in women (figure 1).[3] Migraine is the second largest cause of disability worldwide and is responsible for around 5.6% of total years lived in disability.[2] Because mainly a young working population is affected, migraine has a huge impact on individual and societal level.

Figure 1. migraine prevalence in different age groups, stratified by sex.



Source: Vetvik et al., 2017[3]

Migraine attacks are characterized by a severe pulsating one-sided headache that may last up to 72 hours, often accompanied by severe nausea and/or photo- and phonophobia.[4] Though any individual can experience a migraine attack once in a lifetime, the diagnosis migraine is made after 5 or more attacks.[4] One third of migraine patients experiences migraine auras preceding the headache phase. Migraine auras are transient, reversible positive neurological symptoms, most commonly visual but sometimes including aphasia, sensory or motor features.

Migraine headache pathophysiology is attributed to a yet incompletely understood mechanism originating in activation of the trigeminovascular system. This subsequently leads to the release of neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P, which are associated with vasodilation, inflammation and pain. It is yet unclear what lowers the threshold for this cascade to be set in motion. Most likely, a combination of factors is involved in migraine susceptibility. Next to a genetic component and external (lifestyle) factors, fluctuations in sex hormone levels are associated with increased sensitivity to attacks.

The prevalence of migraine, but also the attack frequency and severity are influenced by fluctuations in levels of gonadal hormones, especially estrogen. In women, before puberty and after menopause, migraine is far less common than during the fertile years. In times of instable hormone levels, many women experience a surge in migraine activity. This is often observed around pregnancy, perimenopause and during the early follicular phase of the menstrual cycle, when estradiol levels drop quickly.[5] Approximately 55% of women with migraine suffer from menstrually related migraine (MRM).[6] Apart from being more frequent, perimenstrual migraine attacks are reported to be longer and more severe.[6,7] Evidence on altered absolute hormone levels in any stage of the menstrual cycle in women with migraine is conflicting.[8] The use of exogenous hormones, for example in the form of oral contraceptives (OC) by migraine patients is a matter of debate. Though several guidelines argue against the use of OC by women with migraine because of the increased cardiovascular risk, this advice seems to be based on very limited evidence.[9,10] Also, it is unknown whether low dose OC carry the same risk as high dose OC. There is some evidence for OC use as prophylactic migraine treatment in women with MRM. The estrogen withdrawal during the pill free interval may trigger headache in around 70% of women, which is why a shortened pill free interval regimen is suggested to be of benefit.[8]

CARDIOVASCULAR DISEASE IN WOMEN

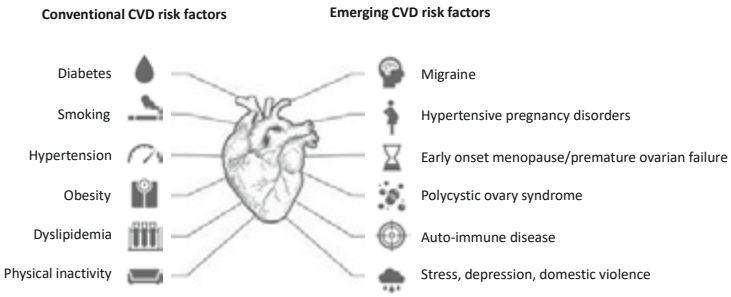
Cardiovascular disease is the most common cause of mortality in both men and women, accounting for one third of all deaths. An estimated 275 million women suffer from cardiovascular disease globally.[11] In the past few decades, a positive change has been made in the awareness of the impact of cardiovascular disease in women and the importance of acknowledging sex differences. However, there remain large steps to be made to close the gap in scientific knowledge and to improve recognition by patient and health professional.[11] Women with cardiovascular disease are still less likely to be treated correctly or to be treated at all.[12]

Sex differences in cardiovascular disease are present in etiology, risk factors and outcome. Cardiovascular disease etiology in women seems to involve a wider spectrum than atherosclerotic large vessel disease which is common etiology in men. In ischemic heart disease, an increased focus on female type pathophysiology has revealed a large role for microvascular, endothelial and vasomotor dysfunction as opposed to the obstructive atherogenic pathophysiology more often observed in men.[13,14] Instead of pathology in the large distal parts of the vasculature, female type vascular pathology more often presents in the distal, microvascular beds.[15,16]

The established traditional risk factors for cardiovascular disease such as diabetes, smoking and hypertension may have different influences depending on sex. For example, women with type 2 diabetes mellitus have a 50% higher risk of fatal coronary heart disease compared to men.[17] Moreover, besides the traditional risk factors, women are burdened by additional risk factors that are much more common for or even unique to their sex such as autoimmune disease,

obstetric complications or sex hormone disorders. Migraine is possibly a cardiovascular risk factor that is both more prevalent and induces a higher cardiovascular risk in women than in men.

■ **Figure 2.** Tradional and non-traditional cardiovascular risk factors in women



Adapted from Garcia et al., 2016[12]

REPRODUCTIVE DISORDERS AND CARDIOVASCULAR RISK

Reproductive disorders, such as preeclampsia and polycystic ovary syndrome (PCOS) are female-specific risk factors for cardiovascular disease. Preeclampsia is a syndrome presenting as gestational hypertension in combination with one or more new onset conditions which are proteinuria, organ dysfunction or placental dysfunction.[18] It occurs in 3-5% of all pregnancies and is the second cause of maternal mortality wordwide.[19] Preeclampsia is associated with an increased risk for cardiovascular disease. For women with a history of preeclampsia, the risk for stroke and myocardial infarction is twice as high, and the risk for hypertension is increased almost four times.[20] The risk of cardiovascular disease is even increased 7-8 times for women with the severe early-onset type preeclampsia.[20,21] Pregnancy is considered to be a stress test for cardiovascular disease later in life.[22] However, whether preeclampsia can be seen as a marker for future risk or as a part of the causal pathway, remains unclear. PCOS is the most common hormonal disorder in women of reproductive age, affecting 8-15% of women.[23,24] It presents as a combination of at least two criteria including oligo- or anovulation, hyperandrogenism and polycystic ovaries on ultrasonography. Women with PCOS have an increased risk for unfavourable cardiometabolic and cardiovascular profiles including increased obesity and dyslipidemia, insulin resistance, type 2 diabetes and hypertension.[25,26] However, the long term effect of these increased risk factors, as well as the role of increased testosterone in women with PCOS remains uncertain, as no evidence for increased atherosclerosis, CVD risk or mortality has been found.[27,28]

STROKE IN WOMEN

Yearly, approximately 12 million people are affected by stroke worldwide.[29] Stroke is the second most common cause of mortality and accounts for 11.5% of all deaths.[29] Both age-adjusted incidence and mortality are lower in women compared to men between the age 45-75 years, but higher at the ages below and above this range. However, because of their longer life expectancy, this amounts to a larger absolute number of women than men experiencing stroke incidence and mortality.[30,31]

Stroke is an acute restriction of blood supply to part of the central nervous system, caused by either an arterial occlusion (ischemic stroke) or rupture (hemorrhagic stroke). The resulting deprivation of oxygen and nutrients can lead to irreversible tissue damage within minutes. Approximately 85% of all strokes is ischemic in nature. Ischemic stroke etiology subtypes are described according to the 'Trial of Org 10172 in Acute Stroke Treatment' (TOAST) criteria: large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined etiology and stroke of undetermined etiology.

Similarly to cardiovascular disease in general, sex differences in stroke have been increasingly acknowledged over the past few decades. Women are at a higher risk of young stroke (at an age under 50 years) and stroke at an older age (above 75 years) compared to men. The latter may be due to the longer life expectancy for women and the loss of the protective mechanisms of estrogen after menopause.[32,33] Women more often suffer from stroke of cardio-embolic etiology whereas stroke in men more often has large vessel etiology.[34] Also, sex differences in hemostatic activation have been found in stroke patients, with increased levels of procoagulatory factors found in women.[35] Also, women with stroke deal with more debilitating consequences in outcome compared to men.[30] This may be due to the higher age at onset for women and the resulting lower pre-stroke ability.[30] Risk factor profiles are likely different between sexes, with men more often having a history of smoking and coronary artery disease and women more often atrial fibrillation and hypertension.[36] Risk factors unique to women, such as reproductive and hormonal disorders, may be accountable for sex differences as well.

MIGRAINE AND CARDIOVASCULAR DISEASE IN WOMEN

Migraine is an important risk factor for cardiovascular disease, including stroke.[37] Migraine with aura increases the risk of ischemic stroke twofold. A twofold increased risk for hemorrhagic stroke is also described for migraine with aura.[38,39] Compared to established risk factors, migraine with aura is more strongly associated with the risk for a major cardiovascular event than obesity or dyslipidemia.[40] Evidence that migraine without aura is a risk factor for stroke is less convincing.[41] Whereas some meta-analyses describe an increased risk of stroke of 1.2 for patients with migraine without aura, others do not find an increased risk at all.[42] Besides stroke, migraine is associated with an increased risk for myocardial infarction. Cardiovascular mortality was increased around 1.5 fold in women with migraine and 2.5 fold in patients with migraine with aura.[43] A young age (<45 years), smoking and the use of oral contraceptives

increase the risk of cardiovascular disease even further.[41] Although an increased cardiovascular risk has also been found in men with migraine, it is markedly more pronounced in young women.[41,44] Whereas the risk for a major CVD event was increased twofold in women it was only increased by a factor 1.2 in men.[45] Whether the lower prevalence of migraine in men has caused bias in these observations, is uncertain.[46] The matter remains unresolved as some of few studies comparing sexes find no difference between men and women at all.[47]

UNDERLYING MECHANISMS FOR THE ASSOCIATION BETWEEN MIGRAINE AND CARDIOVASCULAR DISEASE IN WOMEN

MIGRAINE AND STROKE

The pathophysiological mechanisms behind the association between migraine and stroke are incompletely understood and likely multifactorial.[45] Extensive MRI studies have shown that migraine patients (and again women in particular) have an increased risk of white matter lesions.[48] These lesions are a subclinical sign probably caused by small vessel damage. There is of yet no conclusive evidence that these lesions lead to cognitive or cerebellar symptoms or that increased frequency of headache attacks leads to more white matter lesions or faster lesion progression.[49] In contrast, increased white matter lesions were found even in patients who no longer suffered from active migraine.[50] This observation seems to indicate that there is a common pathophysiological aspect that leads to both migraine and stroke, rather than migraine attacks causing progressive brain lesions. Interestingly, the lesions are more prominent in patients with migraine with aura compared to migraine without aura.[51] Similarly, the association with stroke and other cardiovascular disease is higher in migraine patients with aura than in those without aura.[52] The occurrence of migraine auras has been linked to cortical spreading depolarizations (CSD). CSDs are slowly propagating waves of membrane depolarization, moving from the visual cortex towards the frontal cortex. Similar spreading depolarization is observed in ischemic areas during stroke. Whether and how cortical phenomena and deep tissue subclinical damage in migraine patients are related remains unclear. There are several conditions that imply the co-morbidity of migraine and stroke, including migrainous infarction, persistent foramen ovale and carotid dissection, however these do not account for the majority of cases.[39,53] There is genetic evidence linking stroke and migraine in several hereditary disorders such as spreading familial hemiplegic migraine (FHM), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), hereditary cerebral hemorrhage with amyloidosis – Dutch type (HCHWA-D) and retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S).[54–58] In genome-wide assocation (GWAS) studies, a common locus was found implicated to migraine and four other cardiovascular disorders (coronary artery disease, cervical artery dissection, fibromuscular dysplasia and hypertension). The SNP thought to be responsible was revealed to have a regulatory effect on expression of the EDN1 gene, which codes for the protein endothelin 1 (ET-1).[59] Higher levels of ET-1 have been found in migraine patients, especially in the interictal period. Moreover ET-1 has been found to trigger CSDs in

rats, providing an interesting link between the substrate of the migraine aura and the vascular mechanims of migraine.[60] However, it is difficult to comprehend how these genetic findings can be translated to the clinical context. For example, the effect on coronary artery disease was contradictory to the effect on migraine, hypertension and cervical artery dissection.

MIGRAINE AND CARDIOVASCULAR DISEASE

Together, the observations above suggest that migraine is linked to stroke because of local vascular phenomena. However, migraine is associated with vascular disease outside the brain as well, which calls for a broader explanation.[61] One such explanation is a possible increased presence of established cardiovascular risk factors such as hypertension and hypercholesterolemia in migraine patients.[62] However, it is unlikely that a single traditional cardiovascular risk factor will be the sole driving force behind the migraine – cardiovascular disease association. Moreover, the association between migraine and stroke is most prominent in women with a lower Framingham Risk Score, which suggests that there are other factors involved besides the asessed conventional vascular risk factors.[63] Although migraine is associated with an increase of vascular markers such a prothrombin, von Willebrand factors and endothelin, there are no signs of enhanced atherosclerosis.[64,65] In women with migraine with symptoms of angina, lower scores for coronary artery stenosis are found on angiography.[66,67] It is hypothesized that a systemic vascular vulnerability predominantly present in the proximal microvasculature underlies the association and overlap between migraine and cardiovascular events.[39,68,69] Existence of comorbidities involving disturbed peripheral vasoregulation such as Raynaud's syndrome in migraine patients seem to support this view. Women with migraine often report cold extremities which could suggest impaired vascular regulation. However, it has never been investigated whether cold extremities actually more often occur in women with migraine. Among the proposed mechanisms behind the vascular vulnerability are increased platelet aggregation and prothrombotic state, vasospasm and endothelial dysfunction.[68] Endothelial dysfunction occurs when the endothelial vascular lining loses its ability to balance blood vessel tone and blood fluidity. It is an early, and sometimes still reversible, step in the pathological process towards atherosclerosis. An increased pro-inflammatory and procoagulatory state of the endothelium is combined with reduced vasodilatory properties, particulary by a decreased release of nitric oxide (NO). Several parameters for endothelial dysfunction have shown to be associated with migraine, including reduced flow-mediated dilation (FMD) in the brachial artery and increased serum levels of endothelial microparticles and endothelial precursor cells (EPC).[70,71] However, evidence is contradictory and based on a heterogeneity of biomarkers and techniques. Several new and uninvasive techniques exist which can be used to determine endothelial response in the microvasculature after provocation. The EndoPAT (peripheral artery tonometry) technique measures plethysmographic changes in the finger after occlusive hyperemia. Another technique uses laser Doppler imaging to measure skin blood flow changes after local thermal hyperemia (LTH), induced by dermal application of warm water. This method has excellent reproducibility and can be combined with application of chemical blockades to allow for distinction of NO-dependent and axon reflex-related components.

Why the cardiovascular risk for migraine patients is more pronounced in women remains unknown. The sex-specificity of migraine and the associated increased cardiovascular risk is likely indicating pathophysiological dissimilarities between men and women. Possibly, the proposed systemic vascular vulnerability is sex-specific. Also, gonadal hormone levels may account for these sex differences. Moreover, there may be a role for female-specific cardiovascular risk factors, such as pregnancy-related complications, hormonal disorders such as PCOS and the use of exogenous hormones including oral contraceptives. Besides the existence of risk factors that are unique to women, it is likely that conventional risk factors, such as smoking and hypertension, act differently in the female context.[32] The risk for cardiovascular disease in migraine patients is increased more than multiplicative by smoking, oral contraceptive use, young age and female sex.[53] Whether female-specific risk factors, including PE and PCOS, have a further supra-additive effect on cardiovascular risk in migraine patients is currently unknown.

THESIS OUTLINE

Although insights in sex differences in cardiovascular disease have increased over the last years, the role of migraine is still largely unknown. In this thesis I aim to add to this knowledge by further unravelling the role of migraine in cardiovascular disease in women.

Chapter 2 provides an overview of the knowledge at the time of the start of this thesis on (pathophysiological) mechanisms behind the association between migraine and cardiovascular disease in women. Also, this chapter summarizes the current clinical recommendations for young women with migraine, including recommendations on oral contraceptive use.

In **Chapter 3** I use the extensive Leiden University Medical Center Migraine Neuro Analysis programme (LUMINA) database to investigate whether women with migraine more often suffer from cold extremities than men, as a possible indication of a sex-specific systemic vascular dysregulation.

In **Chapter 4** I focus on the female-specific type of migraine that is related to the menstrual cycle. More specifically, I investigate whether pain sensation across the menstrual cycle is different in women suffering from menstrually related migraine (MRM) compared to women without migraine and postmenopausal women.

Chapter 5 describes a cross-sectional study aimed to determine and compare microvascular function in women suffering from the endocrine disorder PCOS with and without migraine with two techniques for the assessment of endothelial dysfunction: 1) laser Doppler assessment of blood flow changes induced by local thermal hyperemia (LTH) and 2) EndoPAT plethysmography to assess vascular reactivity after occlusion-derived hyperemia.

Chapter 6 describes the association between a history of pregnancy-related disorders (preeclampsia, HELLP syndrome and placental abruption), migraine and the occurrence of ischemic stroke in a large Dutch acute stroke cohort.

Chapter 7 presents a case control study and meta-analysis based on the current literature on the effect of combined oral contraceptives use on stroke risk in women with migraine.

In **Chapter 8**, sex-related differences in cardiovascular risk profile, stroke cause and outcome in ischemic stroke patients with and without migraine are investigated in a large prospective dataset of the collaborating university hospitals involved in the "Dutch Parelsnoer Institute Cerebrovascular Accident PSI-CVA Initiative".

Finally in **Chapter 9** I discuss the implications of my findings as well as possible directions for future research.

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CHAPTER 2

Migraine and cardiovascular disease in women

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ABSTRACT

Migraine is responsible for high rates of disability. In addition, it is associated with an increased risk of cardiovascular disease. This association is not limited to the brain in the form of stroke, but includes cardiac ischemia. The increased risk is most consistently described in the female population and in particular for migraine with aura. This article reviews the current knowledge on migraine and the associated risk of cardiovascular disease, with a focus on female-specific factors.

1.EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF MIGRAINE

Migraine is a chronic neurovascular disorder characterized by attacks of severe pulsating one-sided headache, often accompanied by nausea and photophobia, that may last up to 72 h. One third of migraine patients experiences migraine auras; most commonly transient visual or sensory neurological disturbances. Due to the frequent, debilitating attacks and the high prevalence of 11% in the general population and up to 25% in young women[1] migraine is firmly placed as the seventh cause of years lost to disability worldwide.[2] Migraine pathophysiology involves an incompletely understood mechanism originating from neural activation in the brainstem and subsequent release of neuropeptides associated with vasodilation, inflammation and pain. Interestingly, it appears that migraine pathophysiology may be linked to long term cardiovascular complications.

2. MIGRAINE INCREASES CARDIOVASCULAR RISK

2.1 EPIDEMIOLOGY

There is increasing evidence that migraine, especially migraine with aura, increases the risk of cardiovascular disease (CVD) (HR 1.5, CI 1.33–1.69) and cardiovascular mortality (HR 1.37, 1.02–1.83).[3] The increased risk of stroke (RR ~2.0) has been demonstrated in a considerable amount of literature.[4] Especially when combined with other cardiovascular risk factors, such as use of oral contraceptives and smoking, the risk of ischemic stroke may be increased over 30 times in women.[5] Besides ischemic stroke there is also an increased risk for hemorrhagic stroke.[6] The involvement of cardiac disease such as myocardial infarction (HR 1.39, 1.18–1.64) has not yet been reported extensively and with inconsistent outcomes as can be seen in Table 1.[3] The association of CVD (including stroke) and migraine is described more often in women than in men.[4] Those studies that reportincreased risks in both genders, mostly describe a higher risk in women. [4] Partly, this difference may be a result of bias caused by the higher prevalence of migraine in women. Currently, there is no explanation for the gender difference in migraine prevalence, severity and the consequences in the form of CVD risk. It is important to specify that despite the increased relative risk, the absolute risk for CVD in young women remains low. However, the high prevalence of migraine in this population in combination with the increased awareness of the impact of CVD in women make understanding the pathophysiological mechanisms behind migraine and other (female specific) risk factors and their interaction all the more urgent. A good example of female specific CVD related factors are gestational hypertension and preeclampsia, which interestingly occur more often in women with migraine.[7] Whether a history of both migraine and preeclampsia gives rise to a multiplicative increase in CVD risk is yet unknown, but certainly worthwhile to explore because of the high prevalence of both conditions.

2.2. PATHOPHYSIOLOGY

The mechanism behind the association between migraine and cerebrovascular damage is a widely explored topic. Dysregulation of the neurovascular system is triggered during a migraine attack and may be a manifestation, or a cause, of cerebrovascular damage. Subclinical damage, presented as white matter hyperintensities and silent brain lesions on MRI, is found to be more prevalent in female migraineurs when compared with healthy controls and male migraineurs.[8] There is no clear proof that an increased frequency of migraine attacks gives rise to more extensive damage.[9] Interestingly, the risk for strokes seems to be especially increased for migraine with aura (OR 2.16, CI 1.53–3.03).[4] It is suggested that cortical spreading depression (CSD) is involved in this interaction. This wave of depolarization and neurovascular uncoupling spreads over the posterior cortex during migraine aura and is involved in stroke where it is connected to the extent of ischemic tissue damage.[10] In mouse

Table 1: Cohort studies reporting association migraine – CVD other than stroke

Author	Study type	Population (N=)	Age	Migraine type (diagnosis)	CVD specification	Associated risk (95 %CI)
Kurth 2016	Prospective cohort (FU >20 years)	Women (115,541)	25-42	All migraine (Self reported physisican's diagnosis)	Major CVD (MI, stroke, fatal CVD) MI CV mortality	HR 1.50 (1.33-1.69) HR 1.39 (1.18-1.64) HR 1.37 (1.02-1.83)
Wang 2014	Retrospective cohort	Both (23,082)	18-45	All migraine (Medical records)	IHD	HR 2.50 (1.78-3.52)
Bigal 2010	Case-control	Both (11,345)		All migraine MA MO (Validated questionnaire IHS2 criteria)	MI MI MI	OR 2.19 (1.73-2.77) OR 2.99 (2.27-3.95) OR 1.80 (1.39-2.34)
Gudmundsson 2010	Prospective cohort	Both (18,725)	33-81	All MA MO (Interview IHS 2 criteria)	CV mortality CV mortality CV mortality	HR 1.19 (1.07-1.32) HR 1.27 (1.13-1.43) HR 1.10 (0.91-1.34)
Schürks 2009	Meta-analysis	Both	Any	Heterogenous	MI CV mortality	Pooled 1.12 (0.95-1.32) Pooled 1.03 (0.79-1.34)

Table 1: Continued

Author	Study type	Population (N=)	Age	Migraine type (diagnosis)	CVD specification	Associated risk (95 %CI)
Kurth 2007	Prospective cohort (FU 16 years)	Men (20,084)	40-84	All migraine (Self report migraine attack)	Major CVD (MI, stroke, fatal CVD) CV mortality	HR 1.12 (0.84-1.50) HR 1.07 (0.80-1.43)
Ahmed 2006	Retrospective cohort	Women (873)	Any	All migraine (Self report questionnaire)	CV event CV mortality	HR 1.21 (0.93-1.58) HR 1.16 (0.20-6.7)
Velentgas 2004	Retrospective cohort	Both (260,822)	Any	All migraine (Triptan use or based on medical record)	MI CV mortality	RR 0.96 (0.80-1.15) RR 0.60 (0.33-1.09)
Hall 2004	Retrospective cohort	Both (140,814)	Any	All migraine (Medical record)	MI CV mortality	HR 1.15 (0.96-1.38) HR 0.93 (0.76-1.13)
Sternfeld 1995	Retrospective cohort	Both (79,588) Men Women Men Women	Any	All migraine Frequent unilateral headaches with nausea or affected vision Self reported physician's diagnosis or treatment	MI MI MI MI	RR 0.8 (0.5-1.2) RR 0.7 (0.4-1.0) RR 1.2 (0.7-1.9) RR 1.4 (0.9-2.1)
		Men Women Men Women	<40	Frequent unilateral headaches with nausea or affected vision Self reported physician's diagnosis or treatment	MI MI MI MI	RR 0.3 (0.1-2.4) RR 1.5 (0.5-5.1) RR 0.6 (0.1-4.4) RR 2.1 (0.5-9.5)

models with CADASIL (a severe monogenic stroke type with migraine with aura), increased vulnerability for CSD has been shown.[11]The connection of migraine with non-cerebral vascular damage, such as coronary heart disease, is more difficult to comprehend. It is suggested that a certain systemic 'vascular vulnerability' may be involved.[10] Migraine could be regarded as an expression of this underlying condition which, when combined with other modifiers of vascular health, may lead to a synergistic increase in CVD risk. This hypothesis could explain the multiplicative risk seen in young female migraineurs who smoke and use oral contraceptives.

Ongoing research is aiming to define this vascular vulnerability. Migraine is associated with classical cardiovascular risk factors, such as hypertension and hypercholesterolemia, as well as increased levels of factors associated with CVD risk such as prothrombin, von Willebrand factor and endothelin.[12,13] However, migraine is not associated with enhanced atherosclerosis, which illustrates the complexity.[14] Increased thrombogenicity, inflammation and altered vasodilatory reaction are found in migraineurs and can be seen as markers of dysfunction of the endothelium, the inner lining of the blood vessels that normally has vasoprotective properties.[15] Endothelial dysfunction might be the vascular vulnerability of which both migraine and CVD are expressions, but high quality studies taking into account endothelium-dependent as well as – independent vascular function measurements are lacking. Interestingly, in preeclampsia similar abnormal vasoreactivity, altered platelet activity and other signs of endothelial dysfunction are found.[7] A search for shared mechanisms between preeclampsia and migraine may lead to interesting discoveries. For the time being, the pathological basis behind the migraine - CVD association remains puzzling, especially when regarded in the context of female health.

3. HORMONES AS PART OF PATHOPHYSIOLOGY

Sex hormone levels are closely linked to both migraine and cardiovascular disease and could account for gender differences. In the case of migraine, a steady rise in prevalence and attack frequency occurs right after menarche and a decline is seen after menopause. Throughout the fertile years, migraine may be partly linked to the menstrual cycle (menstrually related migraine, in this case migraine attacks also occur outside the perimenstrual period) or may occur exclusively during the menstruation (pure menstrual migraine). Especially around fluctuations in hormone levels the sensitivity for migraine seems to increase. Possibly, this marks the influence of estrogen on factors involved in pain transmission and migraine pathophysiology. Indeed, recently the peak estradiol levels in women with menstrually related migraine were reported to be lower than those in healthy women.[16] In another study, women with migraine had faster late luteal estrogen withdrawal rates compared to healthy controls although this did not correlate with occurrence of perimenstrual headache.[17] Additionally, vasoreactivity mediated by the trigeminal nerve, which is part of the neurovascular system involved in migraine attacks, responds differently to hormone fluctuations in women with menstrually related migraine.[16] Estrogen, in relation to progesterone and low leveltestosterone, has a major influence on vascular health in a multitude of ways. Estrogen is involved in different modalities such as thrombotic propensity, vasodilatory response and is thought to act protective against CVD risk until menopause, when hormone balance shifts and CVD prevalence surges. This may partly explain the higher CVD risk of migraine observed in younger women. After menopause, major CVD risk factors such as hypertension and hypercholesterolemia may outweigh migraine as a risk factor. However, because of the complexity of the effects and interactions of estrogen in the vascular system, its role in the migraine – CVD connection remains ambiguous.

4. CLINICAL ADVICE BASED ON THE EXISTING LITERATURE

Clinical trials on management to decrease CVD risk in migraine patients are nonexistent. There is no evidence that prophylactic treatment in the form of statins or antiplatelets is of benefit for otherwise healthy female migraine patients, especially when considering side-effects, costs and burden. Caution is warranted with the use of triptans after a cardiovascular event because of their vasoconstrictive properties, however, there is no real evidence that the use of this medication in migraineurs is in itself related to CVD risk.[18] This might be the case for 'older generation' migraine drugs such as ergotamines.[19] Whereas the AHA stroke association recommends caution when prescribing oral contraceptives (OC) to women with migraine, it is also stated that this recommendation is based on very limited evidence.[20] More targeted research on this topic would be of great use in the clinical setting, especially since quitting OC use can be unfavorable for migraine frequency and severity. To conclude, there is currently no evidence for changes in medication use that may be beneficial to the CVD risk in women with migraine. However, the importance of awareness and lifestyle advice is evident. Smoking should be quitted, as the already increased risk of stroke shows a dramatic rise, especially in those suffering from migraine with aura.

5. SUMMARY

Migraine is associated with an increased risk of CVD. This association is described especially in young women suffering from migraine with aura. Though the precise mechanisms behind this association remain unknown, it is hypothesized that a vascular vulnerability is at the basis of both migraine and CVD. Limited knowledge exists on why this risk is possibly gender specific, and how it relates to sex hormones and to other female specific cardiovascular risk factors. How much caution is warranted in women with migraine in combination with other female specific events associated with CVD, such as preeclampsia, is yet unknown. Clinical recommendations are currently limited to raising awareness and giving lifestyle advice.

PRACTICE POINTS

- Although overall cardiovascular risk in young women remains low, migraine with aura should be regarded as an important risk factor in clinical assessment.
- Careful lifestyle advise is warranted, with a focus on the importance of quitting smoking.
- As of yet, there is not enough evidence to support changes in medication use, such as quitting OC, prescribing antiplatelets, or preventing migraine attacks, in order to decrease CVD risk in young women with migraine

RESEARCH AGENDA

- Physiological basis for gender differences in CVD risk in migraine patients.
- Further explore the interaction between migraine and other, female specific, risk factors.
- Useful preventative measures, benefits of altering prescription of migraine specific medication, oral contraceptives and statins/anticoagulation.

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CHAPTER 3

Cold extremities in migraine: a marker for vascular dysfunction in women

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ABSTRACT

Objective: Migraine is recognized as a vascular risk factor, especially in women. Presumably, migraine, stroke and cardiovascular events share pathophysiological mechanisms. We investigated self-reported cold extremities as a marker for vascular dysfunction in migraine. Secondly, we hypothesized that suffering from cold extremities affects sleep quality, possibly exacerbating migraine attack frequency.

Methods: In this case control study, a random sample of 1084 migraine patients and 348 controls (aged 22-65 years) from the LUMINA migraine cohort were asked to complete questionnaires concerning cold extremities, sleep quality and migraine.

Results: A total of 594 migraine patients and 199 controls completed the questionnaires. In women, thermal discomfort and cold extremities (TDCE) was more often reported by migraineurs versus controls (OR 2.3; 95%CI: 1.4-3.7; $p<0.001$), but not significantly so in men (OR 2.5; 95%CI: 0.9-6.9; $p=0.09$). There was no difference in TDCE when comparing migraine with or without aura. Female migraineurs who reported TDCE had higher attack frequencies compared to female migraineurs without TDCE (4 versus 3 attacks per month; $p=0.003$). The association between TDCE and attack frequency was mediated by presence of difficulty initiating sleep (DIS) ($p=0.02$).

Conclusion: Women with migraine more often reported cold extremities compared with controls, possibly indicating a sex-specific vascular vulnerability. Female migraineurs with cold extremities had higher attack frequencies, partly resulting from sleep disturbances. Future studies need to demonstrate whether cold extremities in female migraineurs are a predictor for cardio- and cerebrovascular events.

INTRODUCTION

Migraine is three times more prevalent in women than in men.[1] Patients with migraine have an increased risk for cerebro- and cardiovascular disease, which is even more pronounced in young women, especially those with migraine with aura.[2] A systemic vascular vulnerability, possibly influenced by female sex hormones, is hypothesized to underlie this association.[3] Comorbidities of migraine include small vessel disease such as coronary artery vasospasm and Raynaud's phenomenon.[2,3] These syndromes are more often reported in women, similar to increased thermal discomfort and cold extremities (TDCE).[4] Difficulties in regulating body temperature are associated with a delay in sleep onset, termed difficulty initiating sleep (DIS). Normally, peripheral vasodilation causes redistribution of heat, enabling the reduction of core body temperature that is essential for rapid sleep induction.[5] Experiencing cold extremities may indicate an autonomic or peripheral microvascular dysfunction and may interfere with falling asleep. For patients with migraine, insufficient sleep may be a trigger factor for attacks.[6] Based on these observations, the present study aimed to investigate self-reported cold extremities as a marker for vascular dysfunction in migraine. Additionally, we hypothesized that suffering from cold extremities may be associated with difficulties initiating sleep, and therefore, with an increased migraine attack frequency.

METHODS

SUBJECTS

This study was conducted as part of the Leiden University Migraine Neuro-Analysis (LUMINA) programme.[7] Participants of the LUMINA project are Dutch adults aged 18–80 years who fulfil the criteria for migraine with or without aura according to the International Classification of Headache Disorders (previous ICHD-2, now ICHD-3).[8] Healthy controls were free of any known neurological or psychiatric disorders and did not have any primary or secondary headaches apart from an occasional episodic tension type headache. Further description of the LUMINA cohort is found as supplement (Supplement 1). The study was approved by the medical ethics committee of Leiden University Medical Centre. All subjects provided written informed consent prior to participation. A random selection of 1084 migraine patients and 348 controls (aged 22-65 years) was made from the LUMINA cohort for this present study.

QUESTIONNAIRES

Participants were invited to fill out questionnaires concerning Thermal Discomfort and Cold Extremities (TDCE) and Difficulties Initiating Sleep (DIS), between December 2016 and January 2017. For TDCE, questions included whether and to what extent participants suffered from cold hands and/or feet over the past month. For DIS, questions concerned to what extent participants experienced problems with falling asleep and/or a sleep onset latency of more than 30 minutes in the last month. The questionnaires and ratings are presented in detail as supplement (Supplement 2). The questionnaires were developed and externally validated with objective finger skin temperature measurements in a large population based study.[4] Recent information on migraine characteristics and medication use was obtained through

extended questionnaires. Vasoactive medication use was defined as chronic daily use of triptans, ergotamines, beta-blockers, amphetamines and/or SSRI's.

DATA ANALYSIS

In general, non-parametric statistical tests were used. Chi-square statistics were used to calculate prevalence rates of TDCE and DIS in migraineurs and controls. Odds Ratios (OR) were calculated for the association between TDCE and DIS and migraine for each sex separately. The association of migraine subtype and attack frequency to TDCE and DIS was calculated for each sex. Multiple linear regression was performed to investigate association of TDCE and migraine in women, correcting for age, BMI, smoking and vasoactive medication use, followed by correction for multiple comparisons. Sobel test for mediation was applied to investigate whether DIS was a mediator in the association between TDCE and increased migraine attack frequency. All analyses were performed using SPSS 24.0 (SPSS Inc., IBM, USA).

DATA AVAILABILITY

Anonymized data will be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with European Union legislation on the general data protection regulation.

RESULTS

Questionnaires were completed by 594 (55%) migraine patients and 199 (57%) controls. Women were overrepresented in this study and among migraineurs compared to controls (88% vs 61%). Vasoactive medication use was higher among migraineurs compared to controls (15% vs 5%). There were no significant differences in the distribution of age, BMI or smoking (Table 1).

Table 1. Clinical and headache characteristics [a]

Variable	Migraine (n = 594)	Controls (n = 199)
Age, y	45 (13)	46 (10)
Sex		
Female	520 (87.5)	123 (61.8)
Male	74 (12.5)	76 (38.2)
BMI[b]	25.0 (6.8)	24.1 (3.6)
Current smoker	77 (13.0)	22 (11.0)
Vasoactive medication[c]	90 (15.2)	9 (4.5)
Age at onset, y	19 (10)	n.a.
Migraine with aura	212 (35.7)	n.a.
Attack frequency[d]	3.0 (3.4)	n.a.

Abbreviations: BMI=body mass index, n.a.= not applicable.
[a] Data is represented as mean ± SD or number of subjects (%)
[b] Calculated as weight in kilograms divided by height in meters squared
[c] Daily use of triptans, ergotamines, beta-blockers, amphetamines and/or SSRI's.
[d] Number of attacks per month

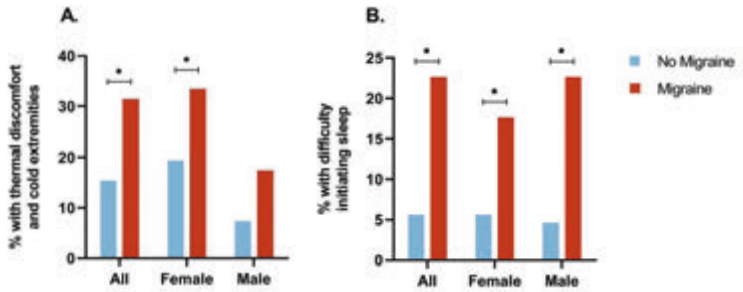
THERMAL DISCOMFORT AND COLD EXTREMITIES (TDCE)

In women, TDCE was more often reported by migraine patients versus controls (OR 2.1; 95%CI: 1.3-3.3) (34% vs 20%; p<0.01) (Figure 1). The difference remained statistically significant after adjustment for age, BMI, smoking and vasoactive medication (OR 2.3; 95%CI: 1.4-3.7; p<0.001). In men, TDCE was reported more often by migraineurs versus controls (OR 2.5; 95%CI: 0.9-6.9) (18% vs 8%) however this difference was not statistically significant (p=0.09). No difference in TDCE was found comparing migraine subtypes (migraine with aura versus migraine without aura) (OR 1.2; 95%CI: 0.8-1.7; p=0.43).

DIFFICULTIES INITIATING SLEEP (DIS)

In general, positive outcome of TDCE was associated with DIS (OR: 2.4; 95%CI: 1.7-3.5; p<0.001). DIS was reported more often in both women (OR: 5.2; 95%CI: 2.4-11.4; p<0.0001) and men (OR: 3.8; 95%CI: 1.2-12,4; p=0.02) suffering from migraine compared to healthy controls (Figure 1).

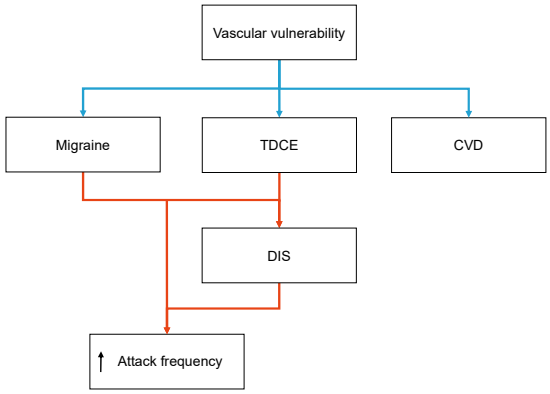
Figure 1. Thermal discomfort and cold extremities and difficulty initiating sleep in migraine patients



(A) Thermal discomfort and cold extremities (TDCE) and (B) difficulties initiating sleep (DIS). Showing patients with migraine (red) and controls (blue) separately in percentages, within all participants (n=793), female participants (n=520) and male participants (n=273), * p<0.05.

Both female and male migraineurs who reported TDCE had significantly higher migraine attack frequencies compared to migraineurs who did not report TDCE, increasing from an average of 3 to 4 attacks per month (p=0.02) for women and 6 attacks per month for men (p=0.002). Sobel test for mediation indicated that this association between TDCE and migraine attack frequency was mediated by presence of DIS (p=0.02) (Figure 2).

Figure 2. Schematic representation of proposed mechanism and Sobel analysis.



Proposed mechanism of sex-specific vascular vulnerability underlying the association between cerebro- and cardiovascular disease (CVD), Thermal discomfort and cold extremities (TDCE) and migraine (blue) and direction of Sobel analysis for mediation of association between migraine attack frequency as dependent variable and relevant TDCE (yes/no) as independent variable and relevant difficulties initiating sleep (DIS) (yes/no) as mediator (red).

DISCUSSION

We were able to substantiate an association between migraine and cold extremities in our large, well defined cohort of migraine patients. We found that this observation was significant only in women. Previous studies showed decreased peripheral skin temperature in migraineurs. However, these studies had severe limitations with small sample sizes, poor migraine diagnosis, illogical findings (showing an association with right-sided headache only), and all lacked appraisal of sex differences.[9,10] Our findings coincide with the current views concerning the sex-specificity of the vascular risk for migraine patients, and with suggestions that the underlying vascular vulnerability may be a systemic female type pathophysiology, possibly involving sex hormones.[3] It has been debated whether the increased vascular risk for migraineurs is in fact exclusive to women or is less apparent in men due to underpowered analyses for male migraineurs.[2] This limitation might also have influenced our findings as the majority of our participants were women and therefore the confidence intervals around the OR in men were relatively wide. Larger studies are needed to further clarify the role of TDCE in men with migraine. Based on the higher increased risk of vascular disease for migraine with aura[2] we expected to find a difference in TDCE between migraine types, but found no evidence for that although the percentage of migraine with aura patients (36%) is what is generally expected in population-based studies.[1] It is noteworthy that our findings are still significant after adjustment for age, since the association of migraine with cardiovascular disease is especially prominent in young women (>45 years).[2] Although age was present as a co-variate in our regression analysis we did not take pre- or post-menopausal state into account. However, when we divided our groups in ≤45 years (presumably premenopausal) and >45 years (presumably peri- or postmenopausal) we did find that especially the younger female

migraineurs contributed to our findings on TDCE as the OR in this group was 2.3 (95% CI 1.2-4.5; p= 0.012) comparing migraineurs with controls, whereas for the >45 years groups the OR was 1.9 (95% CI 0.9-3.7; p=0.067). Further studies investigating the mechanisms of hormonal contribution could provide interesting insights. Another finding worth mentioning, is the increased attack frequency for migraineurs who reported TDCE. This association was mediated by difficulties falling asleep (DIS). This is comprehensible since the disability to decrease core temperature through peripheral vasodilation may lead to delayed sleep onset and this in turn may be detrimental for migraine sensitivity.[4–6] It can be hypothesized that cold extremities are associated with more severe migraine because the underlying vascular vulnerability is likely to be more prominent in these patients. This would concur with suggestions that a higher attack frequency is associated with an even further increased cerebro- and cardiovascular risk in migraine patients.[2] Using clinical markers such as TDCE might thus provide useful clinical tools to assess vascular vulnerability, but also migraine treatment response in the future.

CONCLUSION

Women with migraine report cold extremities more often than women without migraine, which may indicate the presence of a systemic vascular vulnerability in migraine. Migraineurs with cold extremities experience higher migraine attack frequencies, which is partly due to difficulties initiating sleep. If cold extremities in female migraineurs are proven to be associated with development of vascular complications later in life, this may present an easily assessed marker for vascular vulnerability.

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RESPONSE TO LETTER TO THE EDITOR

Dear Editor,

We would like to take the opportunity to respond to the letter to the Editor of dr. Celikbilek in response to our recent article *Cold extremities in migraine: a marker for vascular dysfunction in women,* which was recently published in your journal.[1]

Dr. Celikbilek points out that temperature regulation and sleep onset are both under the influence of the autonomic nervous system. The onset of sleep follows a cascade of events, including vasodilatory responses in the peripheral microvasculature and a subsequent decrease of body temperature. Dr. Celikbilek reasons that both cold extremities and delayed sleep onset are attributable to an autonomic imbalance and suggests that no sex specific related mechanisms play a role. In response, we would like to clarify that the sex specific vascular vulnerability we propose, can indeed (in part) be dysfunction of the autonomic nervous system. The correspondent suggests that we interpreted the cold extremities and delayed sleep onset to be causative factors of each other, which we did not. What we did observe was that female migraineurs with cold extremities have a higher attack frequency, and that this higher attack frequency is partly explained by the presence of 'difficulty initiating sleep'.

Dysfunction of the autonomic nervous system in migraineurs is widely described.[2,3] Not only do migraineurs deviate in heart rate variability and autonomic reflex testing, they also more often experience clinical symptoms such as syncope.[4] Autonomic thermo- and baroregulation are greatly influenced by gonadal hormones, specifically oestradiol, resulting in striking sex differences.[5] Thus, there is evidence that the autonomic nervous system is influenced by sex.

The correspondent further states that an increased inflammatory state, rather than sleep problems, explain the increased migraine attack frequency. We do not argue that this might also play a role. We do not propose delayed sleep onset as the only pathophysiological mechanism behind an increased attack frequency in migraine, let alone migraine chronification. In the latter, many other important facts are implicated, such as depression, cutaneous allodynia and medication overuse. To conclude, however, we did show a significant role for delayed sleep onset in the association between cold extremities and an increased attack frequency in women with migraine.

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CHAPTER 4

Pain perception in menstrually related migraine

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ABSTRACT

Background - Cyclic hormonal fluctuations influence migraine incidence and severity. Previously, we described reduced menstrual cyclicity in estradiol levels and dermal blood flow (DBF) reaction to capsaicin in female migraineurs. It is unclear whether pain perception in women with migraine is influenced by the menstrual cycle.

Methods - Women with menstrually-related migraine (MRM) (n=14), healthy age-matched controls (n=10) and postmenopausal women (n=15) were asked to grade trigeminal and non-trigeminal painful stimuli on a numeric pain rating scale (NPRS) on menstrual cycle day 19-21 (mid-luteal) and day 1-2 (early follicular).

Results - In women with MRM, trigeminal pain remained low throughout the cycle. Controls showed increased trigeminal pain during mid-luteal phase compared to early follicular phase. Changes throughout the cycle were significantly different between women with MRM and controls.

Conclusion - The compromised menstrual cyclicity of pain perception in women with MRM parallels our earlier findings on estradiol levels and DBF.

INTRODUCTION

Migraine manifestation is closely associated with fluctuations of sex hormone levels.[1] Migraine onset typically occurs around menarche and prevalence is highest among women in their fertile period, decreasing again after menopause. Migraine attack frequency and severity change during pregnancy and lactation, the use of hormonal oral contraceptives as well as the menstrual cycle, resulting in frequent perimenstrual attacks and the menstrually-related migraine (MRM) subtype.[1,2] One of the most important fluctuating sex hormones affecting migraine is estradiol.[1] Perimenstrual migraine attacks that occur immediately following the natural drop in estradiol levels are reported to be more painful and disabling, and are more often accompanied by allodynia.[2,3] We previously reported that menstrual cyclicity of estradiol levels and forehead dermal blood flow (DBF) response to capsaicin, a measure for trigeminal nerve-mediated microvascular reactivity, are compromised in women with MRM.[4] Besides this neurovascular mechanism, also pain perception may be influenced by changes during the menstrual cycle. Therefore, with this case-control study, we aim to explore differences in pain perception in women with MRM after peak estradiol levels drop in the menstrual cycle (day 1-2; early follicular phase) as compared to an interval with relatively steady, high estradiol levels (day 19-21; mid-luteal phase). We will include healthy women without migraine as controls and postmenopausal women as a reference group without menstrual cyclicity. Since the sensation of pain during migraine attacks is attributed to activation of the trigeminovascular system, we will further distinguish between pain perception in the trigeminal and non-trigeminal dermatomes. The results will indicate whether, apart from trigeminal nerve-mediated microvascular reactivity, also pain perception is affected by the menstrual cycle.

METHODS

SUBJECTS

Premenopausal women with MRM (n=14), age-matched healthy female controls (n=10) and postmenopausal women (n=15) were recruited between March 2011 and August 2012. Women with MRM were recruited through the Leiden University Migraine Neuro-Analysis (LUMINA) headache database (see supplement).[5] MRM was diagnosed according to the International Classification of Headache Disorders, (ICHD-3-b) criteria.[6] MRM patients with prophylactic antimigraine treatment were excluded, and included participants refrained from using acutely acting antimigraine therapy 48 hours before visits. Women with known cyclical irregularities or comorbidity and women using hormonal contraceptives were excluded. Basic parameters, such as length, weight and blood pressure and migraine characteristics were assessed. The recruitment and selection design is described in further detail in our previous publication.[4]

ASSESSMENT OF PAIN

Women with MRM and healthy women were invited for visit 1 at day 19-21 after the first day of menstruation (mid-luteal phase) and for visit 2 at day 1-2 of their following menstruation (early follicular phase). Postmenopausal women were invited for two visits with a 7-10 day interval.

All participants followed a study protocol including venipuncture and forehead and forearm dermal blood flow measurements. During both visits, participants were asked to assign pain scores according to the numeric pain rating scale (0-10 NPRS) when certain painful stimuli were applied. Since the forehead skin is innervated by the trigeminal nerve, stimuli that were applied there were considered as stimuli to the trigeminal nervous system. These included electrical stimulation and topical application of different concentrations (0.06 mg/mL and 6.0 mg/mL) of capsaicin, a TRPV1 receptor agonist and the active component of chili peppers. As stimuli to the non-trigeminal nervous system, pain scores were assessed at electrical stimulation to the skin in the neck, application of high pressures with a sphygmomanometer around the upper arm for 5 minutes and during venipuncture.[4]

STATISTICAL ANALYSES

For each subject, trigeminal and non-trigeminal pain scores were calculated as the average of all measurements per visit. Differences within groups were analysed using Wilcoxon matched pair signed rank test and differences between groups per visit and across the menstrual cycle (Δ) were analysed using Mann Whitney and Kruskal-Wallis test, separately for trigeminal and non-trigeminal pain scores. Statistical analyses were performed using SPSS 25.0 and GraphPad Prism 8. P values < 0.05 were considered to indicate significant differences.

RESULTS

Body mass index, blood pressure and heart rate were not statistically different between groups and within the normal range (Table 1). Mean age was highest in postmenopausal women (60 ± 5 years), and there was no statistically significant difference between women with MRM (33 ± 7 years) and controls (38 ± 7 years) (P = 0.446).

Table 1. Clinical and headache characteristics[a]

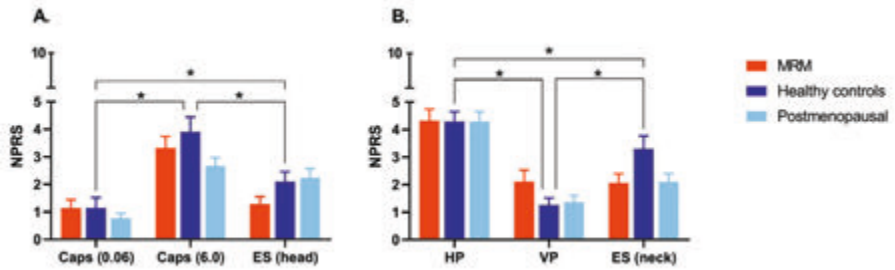
Variable	Migraine patients (n = 14)	Controls (n = 10)	Postmenopausal women (n = 15)
Age, y	33 ± 7 (21-44)	38 ± 7 (26-45)	60 ± 5 (50-68)
BMI, kg/m^2	22.7 ± 2.8	23.7 ± 1.5	23.5 ± 2.4
BP, mmHg			
Systolic	110 ± 8	109 ± 8	117 ± 9
Diastolic	67 ± 7	65 ± 6	70 ± 7
HR, bpm	62 ± 9	65 ± 8	62 ± 9
Age at migraine onset, y	19 ± 7 (8-36)	-	-
Disease duration, y	15 ± 7(6-31)	-	-
Attack frequency[b]			
1-2	0	-	-
3-6	2	-	-

Table 1. Continued

Variable	Migraine patients (n = 14)	Controls (n = 10)	Postmenopausal women (n = 15)
7-12	3	-	-
13-54	9	-	-
Ictal during visit			
Visit 1	7 (50)	-	-
Visit 2	7 (50)	-	-
Both	4 (29)	-	-
Pain scores[c]			
Trigeminal			
Visit 1	1.9 ± 0.3	2.9 ± 0.4	2.0 ± 0.3
Visit 2	2.0 ± 0.3	1.9 ± 0.4	1.7 ± 0.2
Non-Trigeminal			
Visit 1	2.8 ± 0.4	3.1 ± 0.5	2.7 ± 0.3
Visit 2	2.8 ± 0.4	3.4 ± 0.4	2.4 ± 0.3

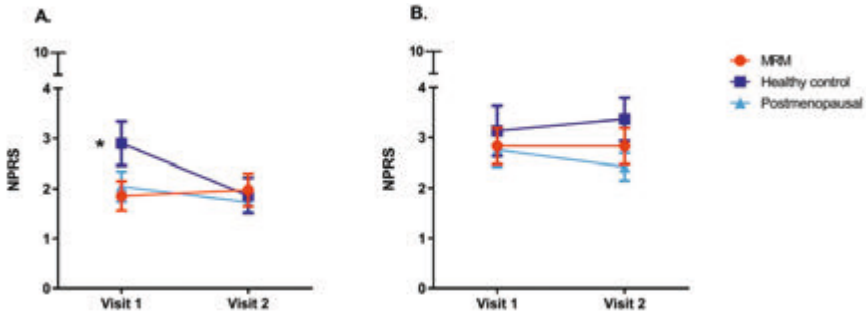
Abbreviations: BMI = body mass index, BP = blood pressure, HR = heart rate, bpm = beats per minute
[a] Data are represented as mean ± SD or number of subjects (%)
[b] Number of attacks per year
[c] Mean ± SEM

Figure 1. Comparison of trigeminal and non-trigeminal pain stimuli



Pain scores in numeric pain rating scale (0-10 NPRS) for trigeminal (A. left panel) and non-trigeminal (B. right panel) pain stimuli: Capsaicin 0.06 mg/ml, capsaicin 6.0 mg/ml, electrical stimulation to the forehead (ES head), high pressures applied with sphygmomanometer (HP), venipuncture (VP) and electrical stimulation to the neck (ES neck). Controls (dark blue) and patients with MRM (red) and postmenopausal women (light blue). * Significant difference in NPRS between painful stimuli ($P < 0.05$).

■ **Figure 2.** Comparison of pain between groups and visits.



Pain scores in numeric pain rating scale (0-10 NPRS) for trigeminal (A. left panel) and non-trigeminal (B. right panel). For controls (dark blue ■) and patients with MRM (red ●): visit 1 = days 19-21 of the menstrual cycle and visit 2 = days 1-2 of menstruation. For postmenopausal women (light blue ▲): visit 1 and visit 2 planned randomly with 7-10 days in between. # Significant difference in NPRS between visit 1 and visit 2 for healthy controls (P=0.003).

Most women with MRM suffered from 13-54 migraine attacks per year (1-4.5 attacks per month), with an average disease duration of 15 years. Seven women with MRM were in the ictal phase during either visit 1 or visit 2, of which four women were ictal during both visits. Comparing the different pain stimuli, capsaicin (6.0 mg/ml) and high pressures with a sphygmomanometer induced the highest pain grades, followed by electrical stimulation, venipuncture and capsaicin (0.06 mg/ml) (Figure 1). When discriminating between study visits to assess changes during the menstrual cycle, healthy women scored trigeminal pain significantly higher on visit 1 (day 19-21, mid-luteal phase) compared to visit 2 (day 1-2, start menstruation) (P = 0.003) (Figure 2, Table 1). The increase in trigeminal pain score of healthy controls between visits (Δ: 1.0±0.3, (mean ± SEM)) was significantly different from the stable responses in MRM patients (Δ: -0.1±0.3) (P = 0.011), but did not differ significantly from postmenopausal women or for non-trigeminal pain. Trigeminal pain scores were equally low on both visits for women with MRM. This was also found in post-menopausal women. No significant differences in pain perception were observed, either between visits or groups, for the non-trigeminal pain stimuli. (Figure 2)

DISCUSSION

In this study, we assessed trigeminal and non-trigeminal pain perception in women with MRM, healthy controls, as well as postmenopausal women. Women with MRM reported equally low pain grades throughout the menstrual cycle, in both the trigeminal and non-trigeminal dermatome. Results were similar for post-menopausal women. In healthy controls, reported pain for the trigeminal dermatome, but not for non-trigeminal pain, showed a significant decrease from the mid-luteal phase to the start of menstruation, which was significantly different from the stable responses in women with MRM over these phases. Literature on pain perception throughout the menstrual cycle is conflicting. A small study using laser-evoked pain sensations reported cyclical changes, but differently from our study, this was found in both

trigeminal and non-trigeminal dermatomes and in women with and without migraine.[7] A direct comparison with our study is hampered by the fact that different phases of the menstrual cycle were investigated. In line with our findings on non-trigeminal pain, another study reported that non-trigeminal pain thresholds upon electrical stimulation did not differ between migraineurs and non-migraineurs and did not vary throughout the menstrual cycle.[8] However, a recent review reported no association of the menstrual cycle phase to either trigeminal or non-trigeminal pain perception in healthy women, although imaging studies showed menstrual cycle-sensitive activation of brain regions associated with pain.[9] Though previous data may seem conflicting, our observation of a compromised menstrual cyclicity in pain perception in women with MRM does substantiate our earlier findings of reduced cyclicity in estradiol levels and forehead DBF responses to capsaicin.[4] Both pain perception and estradiol levels in women with MRM are relatively low during the mid-luteal phase compared to those of healthy women, and steadily remain so throughout the cycle. Our findings may indicate a decreased sensitivity of one or more factors in the trigeminovascular system to cyclic changes in women with MRM. The mechanisms behind the influence of the menstrual cycle on the trigeminal system remain uncharted, but it is likely that sex hormones, specifically estradiol, play a major role.[10,11] Decreased pain thresholds are generally associated with a withdrawal of estradiol, not with an absolute high level of the sex hormone in itself.[1,9] Thus, a reduction in fluctuating estradiol levels in MRM patients as compared to women without migraine[4] could be responsible for the reduced cyclicity in trigeminal pain sensation. Trigemino-specific menstrual cycle dependence may be explained by estrogenic modulation through a myriad of candidate receptors and pathways, including serotonergic and GABA-ergic pathways, and trigeminovascular CGRP, TRPV1 and P2X3 receptors.[10,11] The exact physiological mechanisms remain elusive, as estradiol has a complex genomic and non-genomic influence on both pro- and anti-nociception. A noteworthy limitation to our study is the potential bias in women with MRM, whose experience with severe migraine pain may have compromised their assessment of the painful stimuli. Moreover, decreased pain thresholds and allodynia have been reported during the ictal phase[12], and some of the MRM patients were ictal during the study visits (Table 1). However, since our results show no increased pain scores in women with MRM in either phase compared to controls, and the number of ictal patients was identical during both study visits, it is unlikely that this has affected our study. Additionally, pain was not measured using specifically designated methods, possibly resulting in relatively low overall scores and an indirect comparison between trigeminal and non-trigeminal pain. Besides, it could be argued that monitoring more than one menstrual cycle using more frequent intervals and urinary LH measurements to determine phase would improve the ability to assess the cyclicity of our endpoints. Finally, our sample size was limited, which means our results should be interpreted accordingly. Notwithstanding the shortcomings of our study, our results seem to indicate that trigeminal, but not non-trigeminal pain perception fluctuates throughout the menstrual cycle, and is affected in women with MRM. This may provide additional evidence for an altered cyclic sensitivity of the trigeminovascular system in these women. Whether our findings are part of the pathophysiological basis of MRM and could lead to altered therapy responses in these women, remains to be seen.

CLINICAL IMPLICATIONS

- Women with menstrually related migraine appear to have a compromised menstrual cyclicity in trigeminal pain perception.

- This is in accordance with earlier findings describing a compromised menstrual cyclicity in estradiol levels and forehead dermal blood flow response to capsaicin in women with MRM compared to controls.

- Our finding of an altered trigeminal pain sensitivity in women with MRM may aid in our understanding of MRM pathophysiology and could be of influence on response to therapy in these women.

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CHAPTER 5

Decreased role of neuropeptides in the microvascular function in migraine patients with polycystic ovary syndrome

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Submitted

ABSTRACT

Aims - To better understand the pathophysiological mechanisms underlying migraine as a cardiovascular risk factor, we studied neuropeptide action and endothelial function as measures of peripheral microvascular function in middle-aged women with or without migraine.

Methods and Results - We included women with the endocrine disorder Polycystic Ovary Syndrome (PCOS), a population with supposed elevated cardiovascular risk, with and without comorbid migraine. In 26 women without and 23 women with migraine in the interictal phase (mean age 50.8 years (SD=2.9)) Local Thermal Hyperemia (LTH) of the skin of the volar forearm was measured coss-sectionally under control conditions, after inhibition of neuronal axon reflex and neuropeptide release by 5% lidocaine/prilocaine (EMLA) cream application, and after inhibition of nitric oxide formation by iontophoresis of NG-Monomethyl-L-arginine (L-NMMA). Hereafter, changes in the natural logarithm of the Reactive Hyperemia Index (lnRHI) and Augmentation Index (AI) during reperfusion after occlusion-derived ischemia were measured, using the EndoPAT device. While mean values under control conditions and L-NMMA conditions were similar, women with migraine had a significantly higher mean Area of the Curve (AUC) of the total LTH response after EMLA application than those without (86.7% (SD=26.5) versus 67.9% (SD=24.2); p=0.014). This was also reflected by a higher median AUC of the plateau phase under similar conditions in women with migraine compared to those without (83.2% (IQR [73.2–109.5]) versus 73.2% (IQR [54.3–92.0]); p=0.039). Mean changes in lnRHI (0.73 (SD=0.25) versus 0.67 (SD = 0.29)) and AI (11.53 (SD=14.57) versus 8.68 (SD=15.17)) scores were similar in both groups.

Conclusion - In women with PCOS neuropeptide action was lower in those with comorbid migraine compared with those without migraine. These findings have consequences for the development of preventive measures against the elevated cardiovascular risk in migraine patients. Indeed, it provides a potential mechanism supporting previous findings that migraine is not associated with traditional risk factors, including atherosclerosis.

TRANSLATIONAL PERSPECTIVE

It is currently unknown which pathophysiological mechanisms are related to the increased cardiovascular risk in migraine patients. By comparing the microvascular status in women with PCOS, and with or without migraine, using a sophisticated technique, we identified a putative mechanism underlying the increased cardiovascular risk of migraine patients with comorbid PCOS. This association of migraine with an affected peripheral neuropeptide activity is not reflected by traditional cardiovascular risk scores and, therefore, challenges our current ideas on how to assess and mitigate this risk in migraine patients.

1. INTRODUCTION

Migraine is a chronic neurovascular headache disorder, typically characterised by attacks of debilitating unilateral headache, accompanied by photo- and phonophobia, nausea and emesis (1). About one-third of migraine patients experience migraine aura and have attacks that are preceded by spreading, reversible neurological disturbances, often consisting of visual symptoms (1). Migraine is classified as the top cause of disability in persons under fifty years (2, 3), and is two to three times more prevalent in women (global age-standardised prevalence 19%) than in men (4, 5). In addition to the disabling nature of its headache attacks, migraine – particularly with aura – has been associated with significant cardiovascular endpoints, including cardiac mortality, especially in women. More specifically, population-based cohort studies report increased risks of myocardial infarction, coronary heart disease, ischemic and haemorrhagic stroke, and venous thromboembolism in migraine patients (6-14). Recently, a cohort study by Kurth et al. confirmed the importance of migraine with aura as a cardiovascular risk factor, compared to other traditional risk factors, like obesity or unfavourable lipid levels (10).

Despite its high prevalence, the exact pathophysiological mechanism of migraine has not yet been unravelled. There is growing evidence that, besides the involvement of neuronal factors, this neurovascular disorder is driven by small artery disease or dysfunction of the microvasculature (15), which can be distinguished into (i) endothelial function (i.e. endothelium-dependent responses) and (ii) smooth muscle cell function as a response to neuropeptide release from sensory nerves mediated by an axon reflex (i.e. endothelium-independent responses). Endothelial dysfunction involves decreased bioavailability of (potent) vasodilatory factors that are released by the endothelium, including the gaseous signalling molecule nitric oxide (NO) – which is believed to be one of the mediators in migraine initiation and maintenance (16, 17). The second mechanism is thought to originate from brainstem neural activation and involves the simultaneous trigeminovascular release of neuropeptides, including calcitonin gene-related peptide (CGRP), substance P, neurokinin A and pituitary adenylate cyclase-activating peptide (PACAP-38) (18-23). This release causes dilation of cranial (dural and pial) blood vessels and pain sensation (24-27).

Both mechanisms are also involved in (peripheral) cardiovascular disease. Endothelial dysfunction has been described as an early indicator of cardiovascular risk and a reversible phase of atherosclerosis (28), while neuropeptides contribute to vasodilation in the peripheral cardiovascular system and are implicated in cardiovascular health and disease pathogenesis. For instance, neuropeptide Y is increasingly implicated in a variety of cardiovascular diseases, including atherosclerosis, myocardial ischemia and infarction, as well as hypertension, as reviewed by Tan et al. (29). In contrast, the potent vasodilator CGRP is considered to possess cardioprotective characteristics, including a protective role in vessel remodelling and atherosclerosis, which highlights the need for further research regarding the long-term effects of anti-CGRP monoclonal antibodies for migraine treatment (30-32). The contribution of the endothelium to vasodilatory responses induced by CGRP is, however, debated (31).

This is the first study using an innovative method to study the microvasculature patients with migraine. Therefore, we set out to study these techniques in a population with a possibly challenged microvascular system. Specifically, we studied middle-aged women with Polycystic Ovary Syndrome (PCOS). PCOS is a common endocrine disorder affecting 8-15% of women of reproductive age (33). Women with PCOS may present with ovulatory dysfunction, clinical or biochemical signs of hyperandrogenism and polycystic ovarian morphology (35). Contradictory results have been reported regarding the association of PCOS with cardiovascular disease. Some studies found an association of PCOS with an increased cardiovascular risk profile (36, 37), including a twofold increase in stroke prevalence compared to controls, and with cardiovascular risk factors, such as metabolic syndrome, multivessel coronary artery disease, obesity and hypertension (38, 39). This is in contrast to results from other cohorts, showing no evidence of increased all-cause mortality, cardiovascular disease (risk) or more severe atherosclerosis in women with PCOS (40, 41). Several studies describe increased endothelial dysfunction in women with PCOS; however, contrasting views also exist on whether this association is dependent on more general risk factors, like obesity (35, 42, 43).

As ageing is one of the determinants of NO bioavailability in the human body, as well as cardiovascular health (45, 46), we incorporated the influence of aging by investigating middle-aged women.

In the current cross-sectional study, the primary objective was to determine and compare the microvascular status as well as the cardiovascular risk as traditionally determined by the Framingham Risk Score (FRS) in middle-aged women with PCOS with or without migraine in a non-invasive manner. To understand the role of microvascular dysfunction and the increased risk of cardiovascular disease in these patients, we differentiated between endothelium-dependent responses (the NO bioavailability or endothelial dysfunction) and endothelium-independent responses (the contribution of neuropeptide activity). Therefore, middle-aged women with or without migraine, all with PCOS, underwent two different measurements to determine their (micro)vascular health, including their endothelial function. The secondary objective was to compare these different measurement techniques of the microvasculature.

2. METHODS

Our study is part of CREW-MIST, an acronym for: “Cardiovascular RiskprofilE in Women: MIcrovascular Status”, which is a consortium aimed at elucidating the female-specific cardiovascular risk profile. The CREW study has been approved by the Medical Research Ethics Committee (institutional review board) of Leiden University Medical Center, Leiden, the Netherlands (P.15.384). Local approval was obtained from the Medical Research Ethics Committee of Erasmus Medical Center, Rotterdam, the Netherlands. Written informed consent was obtained from all participants after written and verbal explanation of the study. The study was conducted according to the principles of the declaration of Helsinki (Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General

Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

2.1 STUDY POPULATION

The study population consisted of women who were previously diagnosed with PCOS (approximately 3-5 years prior to inclusion for the current study), as defined by the (revised) Rotterdam consensus criteria (47). According to these criteria, women must fulfil at least two of the following criteria to be diagnosed with PCOS: (i) oligo- or anovulation, which includes irregular menses, (ii) characteristics of hyperandrogenism, including clinical symptoms and high plasma levels of testosterone, and (iii) polycystic ovaries, observed using on ultrasonography.

The original study population consisted of 200 women with PCOS who had been invited in an earlier stage of the CREW consortium for extensive endocrine and cardiovascular assessments; these outcome measures have been described in detail previously (41). Eligible subjects were selected and invited for the current study. The assessment of PCOS had taken place at an earlier stage in the context of a previous study (41). Women aged between 40 and 60 years who fulfilled the criteria for the diagnosis PCOS and who were capable and willing to provide informed consent were included. Patients with any of the following conditions were excluded: the presence of dermal diseases, scars or tattoos on the ventral sides of lower arm that could interfere with the measurements, current pregnancy, insufficient mastery of the Dutch language, any serious illness which obstructed study participation or an allergy for EMLA cream or the compound NG-monomethyl-L-arginine (L-NMMA).

2.2 MIGRAINE CLASSIFICATION

All participants were asked to fill in the Leiden University Migraine Neuro-Analysis (LUMINA) questionnaire to diagnose lifetime migraine. This validated web-based questionnaire aids in diagnosing migraine, including further subdivisions: migraine with aura (MA), migraine without aura (MO), aura without headache and subjects without migraine. Migraine auras are transient focal neurological symptoms, that manifest as visual, sensory, motor or verbal disturbances. The division was based on the third edition of the International Classification of Headache Disorders (ICHD) criteria (48). Both women with a history of migraine – i.e. women who had migraine in the past, but no migraine headache (attacks) and/or aura symptoms in the past 12 months – and women with active migraine were included. The investigator (LA-H) verified the migraine diagnosis during the visit by conducting a semi-structured interview. Moreover, a random sample of these diagnoses – including doubtful cases – has been judged independently by a specialized headache neurologist (GMT). We assured that none of the migraine patients experienced migraine on the day of the assessment, as our aim was to measure the microvascular status in the interictal period.

2.3 ADDITIONAL DATA

In addition, the questionnaire included baseline characteristics as displayed in Table 1 and the Supplementary Material, consisting of questions regarding health, lifestyle, intoxications, medication use, and gynaecological/obstetric as well as cardiovascular history. Self-reported amenorrhea (at least 12 months) was used to classify participants according to their reproductive stage.

Additional data for every participant were collected, including the weight and length (the latter was asked) for calculations of the Body Mass Index (BMI)), and the circumference of the waist and hip for the Waist-to-Hip ratio (WHR). The blood pressure was measured three times in total at different time points, in a sitting position after at least five minutes of rest with an automated oscillometric device. Information regarding intoxications, use of medications as well as menstruation or menopausal status was obtained. Lastly, we collected blood samples for the assessment of hormone levels and lipid profiles. Total cholesterol levels, HDL-cholesterol levels, sex, age, current smoking (yes/no), use of antihypertensive medication(s), systolic blood pressure, and diabetes (yes/no) were used as variables to calculate the FRS that assess the 10-year risk of developing overall cardiovascular disease (49). Former smokers were considered as non-smokers when calculating this score.

2.4 MEASUREMENT TECHNIQUES

Participants were restricted from food and drinks (except for water and necessary medications due to ethical reasons) three hours before the start of the experiments. In addition, consumption of caffeine and heavy meals was not allowed after midnight on the day before the experiments. All measurements were performed consistently by the same investigator in a silent and temperature-controlled room (21.6°C ± 1.3). Subjects were comfortably seated with the right arm in supine position supported by the armrest and with uncrossed legs. During the measurements, subjects were not allowed to talk or sleep.

We performed measurements of the peripheral microvascular function by using the following techniques: (i) measurement of changes in dermal blood flow (DBF) after Local Thermal Hyperemia (LTH) by using the Laser Doppler Perfusion Imager (LDPI, PeriScan Pim 3 system, Perimed, Järfälla, Sweden) and (ii) measurement of the post-occlusive reaction of the forearm by using the EndoPAT device (Endo-PAT2000, Itamar Medical, Caesarea, Israel).

2.4.1 LTH measurements

The first method is based on a model described by Roustit and Cracowski (50). We aimed to study the LTH response of the skin at the volar site of the right forearm. DBF was measured in the superficial dermal layers by using the LDPI. Heating probes (Moor Instruments Ltd, Devon, UK) filled with water were used for the induction of local heating. The skin was heated up to 40°C after a five-minute baseline measurement at 33°C to standardize the temperature of the skin. Within ten minutes after the start of the skin heating generally an initial peak in DBF appears, caused by a neuronal axon reflex, which is largely mediated by neuropeptides (51, 52).

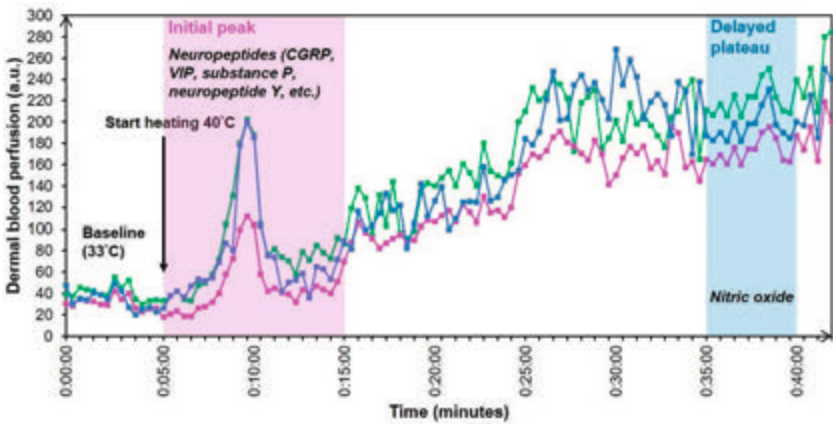
The peak phase is followed by the plateau phase 30-35 minutes after heating. NO is the key contributor to this plateau phase, responsible for approximately 60% of the response (50, 52-54).

To unravel the influence and attribution of endothelium-independent and endothelium-dependent pathways responsible for the reactive vasodilation after heating, we blocked these selectively. Therefore, we applied 5 grams lidocaine/prilocaine cream (both 25 mg/g, EMLA®, Aspen Netherlands b.v., Gorinchem, The Netherlands) at least one hour prior to the experiment to inhibit neuropeptide release, similar to the protocol applied by Minson et al. (52). Additionally, iontophoresis at 60 µA during 7 minutes as previously validated in pilot experiments (data not shown, Perilont LI611, Järfälla, Perimed Sweden (55)) of the NO-synthase inhibitor L-NMMA (Bachem, Clinalfa®, Bubendorf, Switzerland) was used to deliver L-NMMA to the upper dermal layers to inhibit NO formation, allowing a study of smooth muscle cell dysfunction. We used a 2% solution of L-NMMA, which was dissolved in hypotonic water (Fresenius Kabi).

A so-called "iontophoresis artefact", or current-induced vasodilation, was present in several participants. This artefact presents itself as an increased DBF during baseline (thus, before heating) only on the site where iontophoresis of L-NMMA was applied. Therefore, we extended the baseline period of 5 minutes during the LTH measurements, in case an artefact was visually observed to obtain a stable baseline and to avoid possible biased outcomes before heating the skin to 40°C as much as possible.

Figure 1 provides a chronological illustration of a single LTH experiment including the different phases of the vasodilatory response under three conditions (control, EMLA and L-NMMA).

Figure 1. An example of a LTH response



Induction of LTH on the skin of the forearm under control conditions (green line), application of EMLA (lidocaine/prilocaine) (pink line) and L-NMMA (NO synthase inhibitor) (blue line). Abbreviations: a.u.: arbitrary units; CGRP: calcitonin gene-related peptide; VIP: vasoactive intestinal polypeptide.

To answer our primary objective, we calculated and compared the Area Under the Curve (AUC) (expressed in ms*a.u.) for the entire curve, including the five minute baseline period, as the main outcome measure for all three conditions (control conditions, after application of EMLA cream, and after iontophoresis of L-NMMA) by using the trapezoidal rule. DBF responses after EMLA application and iontophoresis of L-NMMA were expressed as a percentage relative to DBF responses under control conditions for each participant. DBF responses under control conditions only have been expressed as such. Additional outcome measures included the AUC of the LTH peak phase (defined as the ten-minute phase after heating) and plateau phase (defined as the five-minute phase starting 30 minutes after heating) separately, for all three conditions (control conditions, after application of EMLA cream, and after iontophoresis of L-NMMA).

Anomalies in individual measurements caused by movement and motion of the subject were visually detected and reported by the investigator for later adjustment. Additionally, possible outliers were detected in case the standard deviation (SD) of a single image deviated 1.5 times from the mean SD of all images of the entire LDPI measurement.

We only corrected for outliers, if consensus was reached with an independent investigator. Both the independent investigator and principal investigator were blinded for the migraine diagnosis. We handled these outliers by imputation of the concerning value, using the average of one image before and after, as calculated by the LDPI software.

In case of an "iontophoresis artefact", we systematically corrected for the increased baseline. If the LTH response inhibited by L-NMMA (blue line, Figure 1) had a 1.5-fold higher baseline of 5 minutes before heating to 40°C compared to the LTH response without pretreatment (green line, Figure 1), we took the average of the DBF measurements of approximately 1.5 minutes before until 1.5 minutes after start heating.

2.4.2. EndoPAT measurements

Approximately five minutes after completion of the LTH measurements, EndoPAT measurements were performed using the index fingers of both arms. The EndoPAT device measures plethysmographic changes during reperfusion after a five-minute period of occlusion-derived ischemia of the brachial artery. This occlusion, which we applied on the left arm (in contrast to the LTH method), and which was obtained by rapidly inflating a blood pressure cuff to a pressure of 200 mmHg, reflects a downstream hyperemic response and is a measure of peripheral arterial endothelial function (56, 57). The ratio of the post-to-pre occlusion peripheral arterial tone (PAT) amplitude of the tested finger (on the left arm), divided by the post-to-pre occlusion ratio of the control finger (on the right arm) was automatically calculated by the device to obtain the Reactive Hyperemia Index (RHI). The lnRHI is a monotonic natural log transformation of this same index in arbitrary units (a.u.), which provides a better double-sided (Gaussian) distribution than the RHI, and which has been described to be a measure of the peripheral vascular endothelial function (57, 58). A normal lnRHI (or EndoScore) is defined as an outcome above 0.51 – i.e. approximately a 1.665-fold

higher ratio of the occluded arm compared to the control arm – whereas an abnormal score is an outcome of less than or equal to 0.51 and is associated with endothelial dysfunction (59). In addition, we used the augmentation index (AI), normalized to heart rate of 75 bpm (AI@75), as a measure for arterial stiffness (56).

Both measures were calculated automatically by the EndoPAT software (version 3.6.2.) that uses computerized algorithms.

A previous study of the Framingham Cohort found a significant inverse relation between cross-sectional measurements of the endothelial function (LnRHI) and multiple traditional cardiovascular risk factors (including male sex, BMI, total/HDL cholesterol, diabetes, (current) smoking, and use of lipid-lowering treatment) (60). Therefore, we performed secondary analyses to investigate the external validity of the relationship between the EndoScore and FRS as a measure of cardiovascular risk in women with PCOS. To investigate potential effect modification by migraine, we stratified the results according to the migraine status.

2.5 STATISTICAL ANALYSIS

The distribution of values, whether normally distributed or not, was determined with the Shapiro-Wilk test. Various statistical tests have been applied to compare the gynaecological/obstetric, endocrine and cardiovascular outcomes as well as the LTH and EndoPAT measurement outcomes between patients with and without migraine: Student T-tests for continuous variables with a normal distribution, Mann–Whitney U tests for non-normally distributed continuous data, and Fisher's exact tests for categorical variables.

Secondary analyses were performed in all participants to investigate the association between both techniques, the EndoPAT results (including the lnRHI and AI@75) and LTH results. In addition, to study the relationship between traditionally determined cardiovascular risk and both techniques, we investigated the association between the EndoPAT results and FRS and between the LTH results under control conditions and FRS. The Pearson correlation coefficient (r) was used to assess the strength of these associations. We performed a natural log transformation of the FRS to ensure a normal distribution of these data.

Two-tailed P-values of ≤ 0.05 were considered to be statistically significant. No corrections for multiple testing of LTH results of the primary objective have been made, as we considered each individual condition (control, EMLA, and L-NMMA) as independent variables. All data were handled and analysed blinded for migraine diagnosis using GraphPad Prism software version 8.0.1 and SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. RESULTS

Forty-nine women with PCOS of the invited 200 PCOS patients fulfilled the inclusion criteria and were willing to participate, comprising 23 individuals with migraine (16 with MA and 7 with MO) and 26 individuals without migraine. In total, 6 women experienced migraine in the past and 17 women experienced a migraine headache and/or aura symptoms recently (no longer than 12 months prior to their visit).

Table 1 and the Supplementary Material present the baseline characteristics, various cardiometabolic, endocrine and gynaecological/obstetric parameters for all participants and for both women with or without migraine separately. No differences were observed between women with or without migraine. Blood collection and the assessment of lipid levels were unsuccessful in one woman without migraine.

On average, migraine patients experienced their first migraine headache attack at the age of 24 years (SD=11). Triptans were used by 7 participants, namely sumatriptan (5 patients) and rizatriptan (2 patients). None of the migraine patients used triptans on the day of the assessments. Moreover, no use of ergotamine was reported.

Table 1. Study population characteristics

	All subjects (N = 49)	**Women with migraine (N = 23)**	**Women without migraine (N = 26)**
General parameters and intoxications[1]			
Age (years)	50.4 (48.7–52.9)	50.2 (49.4–53.5)	50.7 (48.3–52.7)
Ethnicity (race)			
Black	2 (4.1%)	0 (0%)	2 (7.7%)
Mediterranean	3 (6.1%)	2 (8.7%)	1 (3.8%)
Multi-ethnic	2 (4.1%)	2 (8.7%)	0 (0%)
White	42 (85.7%)	19 (82.6%)	23 (88.5%)
Alcohol consumers[2]	32 (65.3%)	16 (69.6%)	16 (61.5%)
Caffeine intake (units/week)	38.5 (23.3–52.5)	38.5 (28.0–52.5)	36.9 (14.0–55.1)
Illicit drugs use	1 (2.0%)	0 (0%)	1 (3.8%)
Current smoker	4 (8.2%)	1 (4.3%)	3 (11.5%)
Pack years of previous and current smokers[3]	7.5 (3.2–16.2)	8.7 (2.8–18.1)	7.5 (3.2–21.0)
Anthropometrics			
BMI (kg/m^2)	30.3 (6.7)	30.1 (7.7)	30.4 (5.9)
Waist/Hip ratio[3,4]	0.9 (0.8–0.9)	0.9 (0.8–0.9)	0.9 (0.8–0.9)
Cardiometabolic parameters			
Systolic BP (mm Hg)	128.7 (16.6)	130.5 (17.9)	127.1 (15.5)
Diastolic BP (mm Hg)	80.4 (10.4)	80.8 (10.0)	80.0 (10.9)
(Previous) myocardial infarction[1,4]	2 (4.2%)	1 (4.5%)	1 (3.8%)

Table 1. Continued

	All subjects (N = 49)	Women with migraine (N = 23)	Women without migraine (N = 26)
Diabetes Mellitus[1,5]	6 (12.2%)	3 (13.0%)	3 (11.5%)
Framingham Risk Score (10 year Cardiovascular Disease Risk %)[4,5]	4.9% (3.7–8.5)	4.8% (3.4–8.8)	5.6% (3.7–7.8)
Framingham Risk Score categories[4,5]			
Low risk (<10%)	41 (85.4%)	19 (82.6%)	22 (88.0%)
Intermediate risk (10–20%)	5 (10.4%)	3 (13.0%)	2 (8.0%)
High risk (>20%)	2 (4.2%)	1 (4.3%)	1 (4.0%)
Antihypertensive drug use	14 (28.6%)	8 (34.8%)	6 (23.1%)
Gynaecological/obstetric parameters			
Current use of hormones[1,6]	15 (30.6%)	7 (30.4%)	8 (30.8%)
Reproductive stage			
Pre-/perimenopausal stage	23 (46.9%)	11 (47.8%)	12 (46.2%)
Postmenopausal stage	6 (12.2%)	2 (8.7%)	4 (15.2%)
Unclear[7]	20 (40.8%)	10 (43.5%)	10 (38.5%)
Endocrine parameters[3]			
Estradiol (pmol/L)[8]	58.4 (55.1–168.8)	70.1 (55.1–232.1)	55.1 (55.1–130.9)
SHBG (nmol/L)	50.0 (37.0–79.8)	48.3 (39.3–86.2)	50.4 (32.4–73.6)
Testosterone (nmol/L)	0.7 (0.5–1.0)	0.9 (0.6–1.1)	0.6 (0.4–0.9)
Dihydrotestosterone (nmol/L)[9]	0.2 (0.2–0.2)	0.2 (0.2–0.2)	0.2 (0.2–0.2)
FAI	1.3 (0.9–2.2)	1.5 (0.8–2.4)	1.3 (1.0–1.9)

Abbreviations: BMI: Body Mass Index; BP: blood pressure; FAI: Free Androgen Index; SHBG: sex hormone-binding globulin

Values of all subjects are displayed as means (standard deviation) or medians (interquartile range, $Q_3 - Q_1$), or as numbers (percentage). Differences between participants with or without migraine were tested with Student's T-test for variables with a normal distribution, while the Mann-Whitney U test was used for variables with a skewed distribution and for variables with a non-normal distribution in one group. The Fisher's exact test was used for categorical data.

[1] Self-reported; [2] This includes both frequent and (very) infrequent alcohol consumers; [3] Data are missing in one migraine patient, that has been excluded from the analyses; [4] Data are missing in one patient without migraine, that has been excluded from the analyses; [5] Four participants did not know whether they are diabetic. These patients have been considered as non-diabetics, based on their medication use; [6] This includes OCP use, having a hormone spiral, but also use of hormone therapy during menopause or hormonal treatments for breast cancer; [7] Unclear, for example due to hormone use, which masks their natural menstrual cycle, or due to a hysterectomy; [8] Hormone levels below the detection limit have been considered to be at the detection limit, which is 55.1. This was the case in 10 migraine patients and 14 patients without migraine; [9] Hormone levels below the detection limit have been considered to be at the detection limit, which is 0.2. This was the case in 15 migraine patients and 20 patients without migraine.

3.1 LTH MEASUREMENTS

LTH measurements were performed in all 49 participants. The measurement of one participant was shorter than 40 minutes. Therefore, only data on the AUC of the peak phase have been included for this particular participant, while the AUC of the plateau phase as well as the total AUC have not been taken into account. Furthermore, the "iontophoresis artefact" was observed in 12 subjects in total and was not related to the migraine diagnosis ($p = 0.748$), as this subgroup consisted of 5 women with migraine and 7 women without migraine. As we observed no significant differences in the LTH baseline between individuals with or without migraine under all three conditions (control conditions (95% CI of the difference [-5.70–2.62]; $p = 0.460$), iontophoresis of L-NMMA ($p = 0.444$), and application of EMLA cream ($p = 0.866$)), results are presented without baseline corrections. Overall and stratified LTH results of the three conditions are presented in Table 2.

Firstly, we analysed differences under control conditions between participants with or without migraine, as expressed by the AUC. We did not detect any significant difference in the AUC of the total LTH response under control conditions between both groups, which was also reflected by similar responses between subjects with or without migraine in the AUC of the ten-minute peak phase or in the mean AUC of the plateau phase (Table 2).

LTH RESPONSES UNDER EMLA CREAM CONDITIONS

Secondly, we analysed LTH responses of the total response, peak phase, and plateau phase after EMLA application relative to control conditions in the total study population. Overall, application of EMLA cream led a total LTH response of 76.53% (SD=26.70) compared to control conditions, indicating an inhibition of 23.47%. More specifically, we observed a median AUC of the peak phase of 55.35% (IQR [41.45–77.31]) compared to control conditions in all participants, which implies an inhibition of 44.65%. Also, under similar circumstances, it reduced the median AUC of the plateau phase to 78.07% (IQR [62.25–97.77]), implying an inhibition of 21.93%.

We further studied differences of LTH responses between women with or without migraine after EMLA application. We observed a higher AUC of the total LTH response in participants with migraine after inhibition by EMLA cream relative to control conditions expressed as a percentage, see Figure 2A. Although inhibition of neuropeptides by EMLA cream did not lead to significant differences in the AUC of the peak phase compared to control conditions, we found a consistent difference in the AUC after inhibition in the plateau phase, see Figure 2B.

Figure 2A. Box plot showing the significant difference of the AUC of the total LTH response between subjects without migraine and with migraine after inhibition by EMLA cream (pink area). This is expressed as a percentage relative to the responses under control conditions (green area) (error bars: 95% CI). The right-sided graph represents an example of a single LTH response under EMLA conditions.

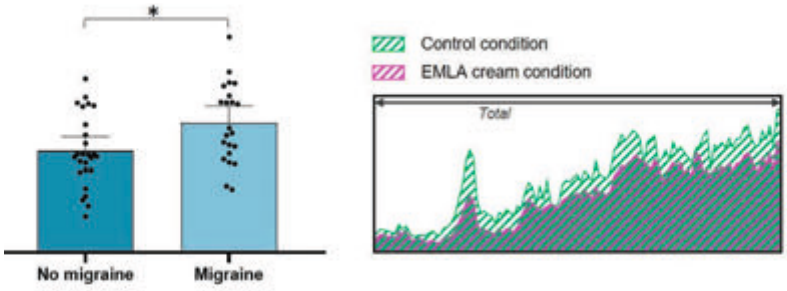


Figure 2B. Box plot that depicts the significant difference of the AUC in the plateau phase of the LTH response between subjects without migraine and with migraine after inhibition by EMLA cream (pink area). This is expressed as a percentage relative to the responses under control conditions (green area) (error bars: 25th and 75th percentiles). The right-sided graph represents an example of a single LTH response under EMLA conditions.

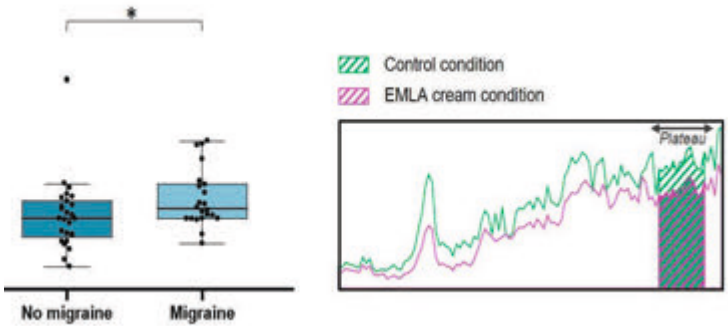


Table 2. LTH responses under different conditions

	All subjects	Women with migraine	Women without migraine	*P-value*
CONTROL CONDITIONS				
Total LTH response in ms*a.u. [IQR]	$2.00*10^8$ [$1.47*10^8$– $2.48*10^8$]	$1.80*10^8$ [$1.37*10^8$– $2.47*10^8$]	$2.10*10^8$ [$1.59*10^8$– $2.55*10^8$]	0.280
Peak phase in ms*a.u. [IQR]	$3.78*10^7$ [$3.01*10^7$–$4.89*10^7$]	$3.64*10^7$ [$2.78*10^7$–$4.46*10^7$]	$4.08*10^7$ [$3.15*10^7$–$5.06*10^7$]	0.421
Plateau phase in ms*a.u. (SD)	$3.71*10^7$ ($1.29*10^7$)	$3.41*10^7$ ($1.09*10^7$)	$3.97*10^7$ ($1.42*10^7$)	0.141
EMLA CREAM CONDITIONS				
Total LTH response in % (SD)	76.53 (26.70)	86.69 (26.48)	67.94 (24.15)	0.014*
Peak phase in % [IQR]	55.35 [41.45–77.31]	61.16 [40.48–79.44]	54.07 [44.13–72.24]	0.655
Plateau phase in % [IQR]	78.07 [62.25–97.77]	83.16 [73.17–109.51]	73.17 [54.31–92.01]	0.039*
L-NMMA CONDITIONS				
Total LTH response in % [IQR]	98.31 [72.97–117.20]	91.50 [66.39–128.30]	98.31 [78.54–110.48]	0.720
Peak phase in % [IQR]	107.66 [81.02–128.68]	107.66 [74.42–131.48])	105.70 [81.18–116.53]	0.613
Plateau phase in % [IQR]	88.46 [76.47–109.75]	87.63 [63.04–125.33]	88.46 [79.48–101.90]	0.927

Median AUC (with the corresponding IQR) or mean AUC (with the corresponding SD) of LTH responses of all subjects and stratified for women with or without migraine under control conditions, under EMLA conditions compared to control conditions (expressed as a percentage, %), and under L-NMMA conditions compared to control conditions (expressed as a percentage, %). LTH responses of women with or without migraine have been analyzed, as displayed by the P-values.

LTH RESPONSES UNDER L-NMMA CONDITIONS

Thirdly, we analysed LTH responses of the total graph, peak phase, and plateau phase after iontophoresis of L-NMMA relative to control conditions. Overall, iontophoresis of L-NMMA led to a median AUC of the peak phase of 107.66% (IQR [81.02–128.68]) and a median AUC of the plateau phase of 88.46% (IQR [76.47–109.75]) compared to control conditions in all participants.

We also further studied differences of LTH responses between women with or without migraine after iontophoresis of L-NMMA. No statistically significant differences between both groups were found after inhibition of NO by application of L-NMMA in the AUC of the total LTH response, peak phase or plateau phase during 35 minutes after start heating relative to control conditions (Table 2).

3.2 ENDOPAT MEASUREMENTS

EndoPAT measurements were performed in 43 participants in total: 21 women with migraine and 22 women without migraine. The occlusion period was shorter than 5 minutes in one participant (approximately 2:15 minutes), due to uncomfortable pain sensations. In another participant, the blood pressure cuff was inflated to a pressure of 180 mmHg, instead of 200 mmHg, due to pain sensations.

The EndoPAT measurements showed no statistically significant difference in the mean lnRHI scores between individuals with or without migraine ($p = 0.475$). Women with migraine had a mean lnRHI of 0.73 (SD=0.25), while women without migraine had a mean lnRHI of 0.67 (SD=0.29). In addition, no differences were observed in the AI@75 ($p = 0.534$) between both groups. Women with migraine had a mean AI@75 of 11.53 (SD=14.57), while women without migraine had a mean AI@75 of 8.68 (SD=15.17).

3.3 ASSOCIATIONS BETWEEN LTH AND ENDOPAT MEASUREMENTS

Based on the aforementioned hypothesis that endothelial dysfunction is characterized by decreased NO bioavailability, secondary analyses were performed in 42 participants to investigate whether the EndoPAT score shows an agreement with the (NO-mediated part of the) LTH measurements. The AUC of the plateau phase under control conditions, corrected for their baselines, did not show any positive correlations with the lnRHI score (Pearson's $r = 0.07$ (95% CI [-0.24–0.37]; $p = 0.641$). In addition, the AUC of the plateau phase under L-NMMA conditions relative to control conditions (square root transformed in order to obtain normally distributed data), also corrected for their baselines, did not show any positive correlations with the lnRHI score either (Pearson's $r = 0.03$ (95% CI [-0.28–0.33]; $p = 0.855$).

The FRS was used as a traditional measure of cardiovascular risk and did not show statistically significant differences between participants with or without migraine (Table 1). The supplementary materials contain additional analyses on cardiovascular risk score in relation to LTH measurements and Endopat measurements (see Supplementary Material).

4 DISCUSSION

In this cross-sectional study, in a population at supposed elevated CVD risk, namely patients with PCOS, we investigated the microvascular status in women with and without migraine.

A variety of studies have reported measurements of the endothelial function as a measure of cardiovascular risk by using and validating the EndoPAT method in different populations (61, 62). This device measures the flow-mediated vasodilation after brachial occlusion and does not specifically target the microvasculature.

This is in contrast with our novel method that involves measurements of the skin perfusion during local heating after applying EMLA cream and L-NMMA. Blockade of the two main components involved in response to heating, neuropeptides and NO, allows a more detailed evaluation of their influences in our study population. This method has not been described before.

4.1. SUMMARY OF LTH MEASUREMENTS

We did not find differences in LTH responses under control conditions. Also, we did not detect differences in the NO bioavailability, as assessed by iontophoresis of L-NMMA, between patients with or without migraine. However, EMLA inhibited neuropeptide action in women without migraine to a larger extent than in women with migraine. This was reflected by the AUC of both the total response and the plateau phase after application of EMLA cream.

These results indicate a significantly decreased neuropeptide action in migraine patients in the interictal period, which may either be due to a reduced release or due to a diminished postsynaptic response in comparison with women without migraine. Our finding contributes to the hypothesis that an altered action of neuropeptides in the periphery is associated with the pathophysiology of migraine. This finding is in accordance with a previous study conducted by Tuka et al., who found decreased levels of PACAP-38 in peripheral blood plasma in the interictal period in migraine patients as compared to healthy subjects (64). Considering other studies that have found elevated CGRP levels in the peripheral vascular system outside migraine attacks, our results might also contribute to the hypothesis of receptor desensitization in patients with migraine (65). Hypothetically, this receptor desensitization could be caused by a higher release in female migraine patients at a younger age, as suggested by results of a human in vivo model by Ibrahimi et al. (66).

The absence of differences in LTH responses under control conditions might indicate that certain mechanisms are “masked” under control conditions, with the result that the detection of subtle differences is only possible when applying a more sophisticated model. Also, the presence of other compensatory mechanisms – systems beyond the neuropeptide release induced by neuronal axon reflexes and NO-dependent vasodilation – might mask the presence of LTH differences under control conditions. These compensatory mechanisms are more likely to be present in the overall LTH response, and might be reflected by percentages above 100% in some individuals after blockade by L-NMMA or EMLA cream. One of these possible

mechanisms might be related to a class of factors called endothelium-derived hyperpolarizing factors (EDHFs), whose effects cannot be investigated using the current method and protocol. EDHFs cause vasodilation mainly through calcium-activated potassium channels present on both smooth muscle cells and the endothelial cell membrane, and are shown to be upregulated when endothelium derived NO is suppressed (54, 67). EDHFs have been described to compensate the loss of NO synthesis in humans (68).

It has been generally accepted that NO accounts for approximately 60% of the plateau phase of LTH (52-54), and in our previous study, in which we applied a similar technique using the LDPI device in healthy men (mean age 40 years (SD=12)), we also confirmed a robust decrease of the LTH peak and plateau by 31% (SD=21) and 65% (SD=14), respectively, after iontophoresis of the NO-synthase inhibitor L-NMMA. However, in the current study, we found no profound decrease of the AUC of the plateau phase after iontophoresis of L-NMMA. Compared to our previous study, we modified the iontophoresis protocol and validated it in pilot studies. Indeed, the protocol that we applied in our previous study mentioned above, in which we included healthy men, increased the occurrence of an iontophoresis artefact in the current study population of middle-aged women. Therefore, the presence of PCOS (and related comorbidities) and/or age and sex differences are most likely to be responsible for the absence of effects after NO synthase blockade. Although we assume that sufficient L-NMMA was properly delivered in the upper dermal layers, we cannot entirely exclude influences of our enhanced iontophoresis protocol that was applied in this specific study population for the first time, that further hampered proper analyses (55).

We also observed no differences of NO bioavailability between individuals with and without migraine. This is in line with a previous study, which showed no differences in endothelium-dependent vasodilatation, basal endothelial NO production and stimulated release of tissue plasminogen activator in forearm resistance vessels between migraine patients and healthy controls (70). In addition, this observation could again support the predominant effects of EDHF in the LTH response. A study performed by Brunt and Minson in a younger study population (18–30 years of age) showed that NO and EDHF have an equally important role in the plateau phase, which leads to the additional hypothesis that the effects of EDHF are larger than NO in middle-aged women with PCOS (54). Another explanation for the low NO bioavailability in these women with PCOS might be related to their low estrogen levels (71), masking potential differences between women with or without migraine, that might be present in women with a normal hormone profile.

The decreased action of neuropeptides, observed in the current study, may be part of the explanation for the increased cardiovascular risk in female migraine patients (6, 9), as certain neuropeptides, for example CGRP, are proposed to exhibit cardioprotective effects in cardiovascular disease (72).

4.2. SUMMARY OF ENDOPAT MEASUREMENTS AND FRAMINGHAM RISK SCORES

Outcomes of the EndoPAT measurements of patients with or without migraine and comorbid PCOS did not differ and fell within the normal range (56, 59). This value indicates a normal endothelial function or arterial stiffness for this population, which is in line with previous research in these study participants, that indicated a similar (low) cardiovascular risk profile compared to age-matched controls (41). This was also reflected by the relatively low FRS in the participants, similar to our current study. We did not detect any significant differences of the FRS between women with or without migraine and comorbid PCOS. The possibility exists that these women with PCOS already received proper preventive treatments and were counselled at an early age to avoid developing diabetes and cardiovascular problems in relation to their disease. Importantly, the FRS is mostly based on traditional risk factors. Despite the increased cardiovascular risk in migraine patients, previous studies found no relationship between migraine and coronary artery calcification scores or atherosclerosis – which are generally viewed as traditional markers for cardiovascular disease (73, 74). In combination with our findings, the increased cardiovascular risk in migraine patients might be explained through non-traditional, non-atherosclerotic pathways, including a subtle difference in the peripheral activity of neuropeptides. Our finding concerning the decreased action of neuropeptides in migraine patients might, therefore, be a possible non-traditional risk factor for the increased cardiovascular risk in these individuals. This hypothesis that alternative pathways might be implicated in migraine is in line with findings of a recent population based study, where migraine patients, compared to individuals without migraine, appear to have less arterial calcification in the intracranial carotid artery (75). In addition, our findings could be relevant in view of the cardiovascular safety of novel anti-migraine medications that may interfere with neuropeptide function (31).

As may be expected with our relatively small and homogenous study population, we did not find a correlation between the FRS and the outcome measures of microvascular function as assessed by the different techniques. The absence of a correlation between the FRS and EndoPAT outcomes in accordance with a previous study which found that the predictive value of the reactive hyperemia signal on late cardiovascular adverse events went beyond the traditional FRS in a similar age group (76). Besides, we found no correlations between outcome measures of microvascular function assessed by the EndoPAT and the LTH method. This might be related to the evident differences between both techniques, as the LTH technique assesses the microvasculature locally on the forearm, while the EndoPAT provides a composite outcome of the entire forearm.

4.3 LIMITATIONS AND STRENGTHS

There are several limitations to our study. Firstly, while the cross-sectional design of the study allows a direct comparison of the results, a disadvantage of this design is that both methods were performed only once and that no inferences about causality can be made. Also, restricted by our sample size, we could not assess the influence of all confounding variables in the association between the increased cardiovascular risk in PCOS and migraine patients.

Secondly, our measurements of endothelial dysfunction could be biased by the iontophoresis artefact we found, a phenomenon and possible confounding factor which deserves further attention as (the combination of) an electric current and NO might be involved. This current-induced vasodilation has been demonstrated to be triggered by C-fiber stimulation, and downstream pathways mainly involve cyclooxygenase-1 (COX-1) in healthy rats (77). Axon reflex responses via activation of mechano-insensitive C-nociceptors have also been hypothesized to be responsible, as EMLA cream results in elimination of this artefact (78). Yet, a clear-cut explanation of the occurrence of this artefact in only a selection of human participants is still lacking, but influences of the pH value of the skin, the skin thickness and vehicle, among others, have been described in detail elsewhere (78, 79).

We did not exclude participants exhibiting this artefact, as this could have led to potential selection bias related to the microvascular function. Thirdly, as we had no complete data on the exact timing of the previous and next migraine attack in migraine patients, we could not investigate the temporal association between our microvascular measurements and the prodromal or postdromal phases of a migraine attack. Lastly, we found a migraine prevalence of 47% in our study population, which approaches previous reports on the lifetime migraine prevalence of approximately 32% in these women (80, 81). This high prevalence is a surprising finding, as a previous case-control study by Pourabolghasem et al. (82) showed no significant increase in the migraine prevalence in women with PCOS compared to a control group. In contrast, a study in a nationwide Danish population of patients with PCOS described a significantly increased and two-fold higher risk of migraine (headache) in patients with PCOS compared to controls (83). While we did not include participants actively based on their migraine diagnosis, (self-) selection bias might have played a role in our study. Yet, the observed relatively high prevalence deserves further attention for additional investigation. Limited by our small sample size, we were unable to distinguish microvascular responses between MO and MA patients in this study, whereas it is the migraine type with aura that is especially associated with an increased cardiovascular risk (10, 84). Interestingly, the prevalence of MA in our population of women with PCOS far exceeded that of the general migraine population (70% versus 33%). One of the strengths of this study is that, to the best of our knowledge, this is the first study which assesses and compares endothelial dysfunction and neuropeptide activity using the LTH method in women suffering from PCOS with or without migraine. Blockade of the two main components involved in response to heating, being neuropeptides and NO, allows a more detailed evaluation of their influences in our study population. Using Laser Doppler Imaging to assess dermal blood flow allowed for a focus on the microvascular function. Secondly, we were able to detect subtle differences in the microvascular function using a more sophisticated and high-tech LTH technique, which were underexposed when using the already established, but more simple EndoPAT method. Therefore, the EndoPAT device provides a composite measure of the functioning of the inner lining of the blood vessels, but does not allow a closer study of the relative contribution of the various mediators involved in the vasodilatory response, in contrast to the LTH method.

Thirdly, migraine diagnoses were established through our well-validated questionnaire and subsequent confirmation by a headache specialist.

4.4 FUTURE WORK

Our study points to a difference in the blockade of neuropeptide action between women with or without migraine. However, our methods do not allow us to distinguish which neuropeptides are responsible for this difference, highlighting the need for basic and translational studies to disentangle their relative contribution and effects. Besides, research on the role of additional mechanisms involved in the vasodilatory response, including EDHFs, is warranted to better understand the mechanisms underlying the increased cardiovascular risk of PCOS and migraine patients. External validation of our findings in endocrinologically healthy and/or younger female migraine patients is needed to further investigate the effects of age and disease (PCOS in this case) on the microvasculature. It is yet also unknown to what extent these results are generalizable to other (middle-aged) women with migraine. Also, further studies to replicate our findings in larger settings are warranted.

Further investigation of the prevalence of migraine (with aura) in women with PCOS could be interesting. The different hormone profile in these women may be associated with an altered susceptibility to migraine and their cardiovascular risk profile may pose an ultimately supra-additive risk for cardiovascular disease in women with migraine. Therefore, incorporating such common pathways between migraine and PCOS in further (follow-up) studies might allow a more detailed measurement of the microvascular function to disentangle the contribution of different components and to understand the heterogeneity of LTH responses after EMLA and L-NMMA application between individuals. These longitudinal studies might also take the relationship between the microvascular function and the timing of migraine attacks into account.

As the endothelium and peripheral neuropeptides play a pivotal role in the development of cardiovascular disease, more knowledge on its functioning is needed to understand the underlying pathophysiology and to develop more targeted (and cardiovascularly safe) therapies in patients with migraine.

CONCLUSION

In conclusion, we determined the contribution of neuropeptide activity and NO bioavailability to peripheral microvascular function in women with or without migraine and comorbid PCOS using a novel set-up, namely the LTH technique. Despite no differences in the FRS – which is a traditional measure for the cardiovascular risk – we found a significantly decreased neuropeptide action in women with migraine and comorbid PCOS in the interictal period, compared to women with PCOS and without migraine. Although further studies are warranted to clarify the role of neuropeptides in younger as well as endocrinologically healthy female and male individuals with migraine, this decreased neuropeptide activity might serve as an explanation for the increased cardiovascular risk in migraine patients, which might be related to non-traditional cardiovascular risk factors. Lastly, it indicates that yet underexposed peripheral systems could serve as compensatory mechanisms for the decreased peripheral neuropeptide activity in patients with migraine.

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CHAPTER 6

| Stroke after pregnancy disorders

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Dear Editor,

Women with hypertensive pregnancy disorders are at risk of developing stroke, possibly mediated by female-specific risk factors. Pregnancy is considered to be a stress test for cardiovascular health later in life.[1] In the current study we assessed the occurrence of pregnancy disorders, among women with a history of ischemic stroke who participated in the Dutch acute stroke study (DUST) and related these risk factors to migraine, age of stroke onset, stroke subtype, radiological characteristics and clinical outcome. Details and results of the prospective, multicenter DUST study have previously been published elsewhere.[2] Out of the 429 living female participants in DUST with ischemic stroke and clinical outcome at 3 months follow-up, 166 women consented to participate in the current questionnaire follow-up study, assessing female-specific risk factors for cardiovascular disease.

In total, 144 participants reported one or more pregnancies (86.7%). The most common reported pregnancy disorders were miscarriage (31 participants, 22.5%) and hypertensive pregnancy disorders (49 participants, 35.3%,). No differences were observed for age of stroke onset, stroke subtype, or clinical outcome when comparing the separate groups of patients with a history of miscarriage, preterm delivery, hypertensive pregnancy disorders or placental abruption with patients without these disorders. However, a difference was observed for age of stroke onset (mean difference 10.2 years, 95% CI 2.6–17.8 years, see Table 1) when comparing participants with and without a history of pregnancy complications associated with vascular disease (preeclampsia, HELLP syndrome and placental abruption). Six (50%) of these participants with a history of these pregnancy complications had a stroke before the age of 50 years. Logistic regression resulted in an odds-ratio for having stroke onset <50 years of 4.71 (95% CI 1.38–16.06) for women with a history of pregnancy complications associated with vascular disease compared with women without a history of such pregnancy complications. Moreover, the median age of stroke onset was also earlier in women with migraine compared to women without migraine with a median of 9 years. (Unpublished data: see supplements) No differences were observed for age of stroke onset, stroke subtype, radiological characteristics or clinical outcome when comparing the separate groups of patients with a history of miscarriage, preterm delivery, hypertensive pregnancy disorders, placental abruption or migraine with patients without these disorders.

Table 1. Stroke characteristics in patients with and without pregnancy complications.

	Pregnancy complications (n = 12)[a]	No pregnancy complications (n = 119)[a]	p-value
Age at stroke, mean (SD)	53.9 (13.4)	64.1 (12.8)	0.009
BMI at stroke, mean (SD)	29.8 (9.1)	26.0 (4.3)	0.057
Migraine*	4 (36.4)	17 (14.9)	NS
Stroke classification (TOAST)			0.795
Large-artery atherosclerosis	3 (25.0 %)	29 (24.4 %)	
Cardioembolism	1 (8.3 %)	18 (15.1%)	
Small-vessel occlusion	3 (25.0 %)	22 (18.5%)	
Stroke of other determined etiology	2 (16.7%)	9 (7.6%)	
Stroke of undetermined etiology	3 (25.0%)	41 (34.5%)	
Stroke classification (Oxfordshire)			0.497
PACI	5 (41.7%)	48 (40.3%)	
TACI	0 (0.0%)	11 (9.2%)	
LACI	3 (25.0%)	25 (21.0%)	
POCI	2 (16.7%)	23 (19.3%)	
Unclear	1 (8.3%)	12 (10.0%)	
Pre-stroke disability			
mRS ≥ 2	0 (0.0%)	9 (7.6%)	0.602
NIHSS score admission ≥ 5	6 (50.0%)	60 (50.4%)	1.000
Follow up at 3 months			
mRS ≥ 2	4 (33.3%)	53 (44.5%)	0.549
EQ5D ≥ 6	6 (50.0%)	75 (63.0%)	0.681
Barthel index ≤ 17	0 (0.0%)	11 (9.2%)	0.594

BMI, body-mass index; PACI, partial anterior circulation infarcts; TACI, total anterior circulation infarcts; LACI, lacunar infarcts; POCI, posterior circulation infarcts; mRs, modified Rankin scale; NIHSS, National Institutes of Health Stroke Severity Scale, EQ5D, EuroQol five dimensions questionnaire. *Unpublished data
[a] Total number of women, not all variables were available for each participant.

An increased risk of ischemic stroke in women with a history of pregnancy complications has been described in large retrospective and prospective cohort studies.[3,4] The relation of age at stroke onset and history of pregnancy disorders has not been previously reported. The 10 years earlier age of stroke onset in participants with a history of pregnancy complications is consistent with our previous observations of an earlier onset of hypertension and type 2 diabetes mellitus in women with adverse pregnancy outcome.[5] Pregnancy complications and ischemic stroke share common risk factors, such as obesity and hypertension. This might explain the accelerated stroke development after pregnancy complications. Our finding of a younger age at stroke onset for women with migraine is in line with existing literature.[6,7]

Some limitations of our study need to be addressed. Firstly, our conclusions are based on relatively few women who experienced pregnancy complications within the cohort and information on pregnancy complications was based on self-reporting and could not be checked in medical records. Therefore, our findings need to be confirmed in a larger study sample. Secondly, selection bias may have occurred due to the retrospective nature of our sub-study and the use of self-reporting.

In conclusion, pregnancy disorders are common among women who experience ischemic stroke. We found that the mean age of stroke onset was about 10 years earlier in participants with a history of pregnancy complications associated with vascular disease (preeclampsia, HELLP syndrome and placental abruption) and 9 years earlier in women with migraine compared with women without such a history. Whether a combination of pregnancy complications and migraine leads to an even further increased risk of younger age at stroke onset should be addressed by future research.

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CHAPTER 7

Ischemic stroke risk in women with migraine and hormonal contraception: new case-control study and extended meta-analysis

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ABSTRACT

Objective - To investigate whether migraine and the use of oral contraceptives (COCs) have a supra-additive risk-increasing effect on ischemic stroke.

Methods - We performed an interaction analysis of migraine, COC use and risk of ischemic stroke in a population-based, nested case-control study of women aged 18–49 years with no history of ischemic stroke. In addition we did a systematic review of the extant literature as well as an extended meta-analysis. We included cohort or case-control studies in premenopausal women and first-ever ischemic stroke as clinical outcome. We extracted adjusted odds ratios (aORs) and performed a subanalysis based on a COC estrogen dose of <50 µg.

Results - Our nested case-control study included 617 women with first-ever ischemic stroke. Mean age was 37 years. Comparing women with migraine who used COCs to women with neither of the two risk factors, we found a substantial increase in the risk of ischemic stroke (aOR: 6.83; 95% CI:3.95–11.7). Women with migraine who used COC and also smoked compared with women without these risk factors had an even higher risk of stroke (aOR: 30.2; 95% CI:4.22–610). In our meta-analysis of seven studies, including our nested case-control study, the risk of ischemic stroke in women who had migraine and used COCs compared with women who did not have either risk factor ranged from an aOR of 2.04 to 16.9 (pooled aOR: 4.95; 95%CI:2.13–11.5, I2 = 84.7%). In a subanalysis based on estrogen dose, the risk of ischemic stroke in women who had migraine and used COCs containing <50 µg estrogen ranged from an aOR of 1.80 to 13.9 (pooled aOR: 3.14; 95%CI:1.75–5.62; I2 = 86.6%).

Conclusion - In women aged 18–49 years, the co-occurrence of migraine and use of COCs, even of low estrogen dose, results in a substantially increased risk of ischemic stroke. The additive effect of smoking appears to be large.

INTRODUCTION

Migraine, especially with aura, increases the risk of ischemic stroke approximately two times.[1] This risk increase appears to be strongest in women of reproductive age.[2] In women in this age group the use of hormonal contraceptives, especially combined oral contraceptives (COCs), is another common risk factor for ischemic stroke.[3] COCs are the most commonly prescribed form of hormonal contraceptives, and are used by approximately 20% of women of childbearing age in developed countries.[4] The absolute risk of ischemic stroke remains low because COC-users are usually young and healthy.[3, 5] Moreover, the risk of ischemic stroke associated with COC use depends on the estrogen dose, and has decreased significantly in recent decades as estrogen doses have fallen to <50 µg.[6,7]

However, the use of COCs by women with migraine may lead to an increase in the risk of ischemic stroke, which seems supra-additive compared with the effect of the two risk factors alone.[8, 9] Smoking may have a further additive effect on the risk of ischemic stroke.[10] Consequently, the World Health Organization (WHO) and the American Congress of Obstetricians and Gynecologists (ACOG) have advised against the use of COCs in women with migraine, particularly with aura.[11, 12] However, this advice has been questioned due to the limited availability and quality of the evidence.[13, 14] Women with migraine represent up to 33% of the female population, of whom one third has migraine with aura.[15] Defining migraine as a contraindication to the use of COCs may therefore impose a significant burden on society, given the contraceptive and non-contraceptive importance of COCs.[16]

The evidence on the risk of ischemic stroke in women with migraine using COCs, including those with a low (<50 µg) estrogen dose, has been extensively reviewed. The prevailing conclusion is that too little data are available to draw strong conclusions about the safety of prescribing COCs in women with migraine.[17, 18, 19] Therefore, we firstly present data from a nested case-control study based on a prospective population-based cohort, in which we assessed the risk of ischemic stroke in women with migraine using COCs and the potential additive effect of smoking. We then integrated our results with previously published evidence using a systematic review and meta-analysis.

METHODS

NESTED CASE-CONTROL STUDY

Study population

We used data from the STIZON database, which directly retrieves data from electronic patient records of a large number of healthcare providers throughout the Netherlands. From the STIZON general practitioner (GP) database we selected women from general practice centers which, based on their location, were in the catchment area of hospitals participating in the STIZON network. This enabled us to link information on hospital diagnoses with primary care

data. The STIZON GP database contains International Classification of Primary Care (ICPC) diagnosis codes for clinical entities and Anatomical Therapeutic Chemical (ATC) medication prescriptions from primary care pharmacies.[20,21] ICD-9 and ICD-10 codes are present for all in-hospital diagnoses during follow-up, and ICPC diagnosis codes are in principle available from birth. The inclusion criteria for both cases and controls were women who were registered in a STIZON general practice between 1st of January 2007 and 31st of December 2020 for at least one year, and were aged between 18–49 years within this time window. We used a nested case-control design in which cases were defined as patients with a first-ever ischemic stroke based on either one ICD-9 or ICD-10 hospital or ICPC diagnosis code registered during follow-up. The date of the first-ever ischemic stroke was used as the index date. For each case we then randomly sampled ten controls who had the exact same age as the case on the index date, without replacement. The index date was used to define the baseline characteristics for cases and age-matched controls. The ascertainment of a history of migraine was clinic-based, and was defined using registrations in the electronic patient record of an ICD-9, ICD-10 or ICPC diagnosis code for migraine, or migraine-specific drugs (ATC-code: N02C* with * indicating all registration subcodes) before the index date. Migraine-specific drugs included ergot alkaloids, flumedroxone, triptans, and monoclonal antibodies against calcitonin gene-related peptide (CGRP) or its receptor. We defined current COC use based on the registration of one or more ATC medication prescription codes: current COC use (ATC: G03AA*, G03AB*), within 180 days before the index date. Further, we examined other risk factors for ischemic stroke including age, smoking, diabetes mellitus, hypertension, a history of hemorrhagic stroke, TIA, subarachnoid hemorrhage, myocardial infarction, angina pectoris, and peripheral artery disease. We could not sufficiently distinguish between past and present smoking status based on our data. The local medical research and ethics committee declared that this study was not within the scope of the Dutch Medical Research Involving Human Subjects Act.

Statistical analysis

To assess the interaction effect between COC use and migraine, we performed an analysis of additive interaction in a standard case-control comparison.[22] We used logistic regression analysis to estimate odds ratios (ORs) and associated 95% confidence intervals (CIs) as a measure of the relative risk of ischemic stroke for COC use alone, migraine only, and the presence of both compared with the reference category with neither risk factor. All ORs from the logistic regression models were adjusted for age, hypertension, hypercholesterolemia, diabetes and smoking (aORs). In addition, we performed an analysis of additive interaction for COC use, migraine, and smoking, for which we compared women who had all three risk factors with women without any of the three risk factors.

SYSTEMATIC REVIEW AND META-ANALYSIS

Inclusion criteria

The following inclusion criteria were used for the systematic review. First, we only included studies with a cohort or (nested) case-control design. Second, participants were women of reproductive age. Studies focusing on (peri)menopause were excluded. Third, studies had to contain information on both migraine and use of any form of hormonal contraception (i.e. COC as well as progestogen-only preparations in all available types of administration [oral, transdermal, vaginal ring, injection, intra-uterine device]). Hormonal contraceptive use was compared with non-use, defined as either never having been exposed to a hormonal contraceptive or being a former hormonal contraceptive user. To perform a meta-analysis of the combined effect of hormonal contraception and migraine on the risk of ischemic stroke compared with women without migraine who did not use hormonal contraception, we included only studies that reported data on the combined effect. COCs were classified according to the estrogen dose, as this is the likely thrombogenic component. Studies on emergency contraception were excluded. Fourth, we chose first-ever ischemic stroke as the clinical outcome, which was defined in the original publications. The outcome was measured at the end of the follow-up period of the study. Finally, Two authors (HO, KL) independently reviewed titles and abstracts of the records obtained from the electronic searches and excluded irrelevant studies. Of the remaining records, full copies were obtained to identify studies suitable for inclusion. We settled disagreements by discussion with an independent third review author (MW). We searched in the following databases: PubMed, Embase, Web of Science, Cochrane Database of Systematic Reviews, CENTRAL, CINAHL, PsycINFO, Academic Search Premier, ScienceDirect, LWW, and Wiley. The search strategy was amended for each database. We have not set a language restriction on the study search, and searched for meeting abstracts in Embase and Web of Science to find additional studies. Databases were searched on February 5th 2022, from the date of their inception.

Statistical analysis

We extracted adjusted odds ratios (aORs) or adjusted risk ratios (aRRs) depending on what was reported in the original publications. To assess the influence of both migraine and hormonal contraceptive use on risk of ischemic stroke, we pooled effect estimates for migraine versus no migraine, contraceptive use versus no contraceptive use, and for both factors combined versus neither of both factors present. For the pooling of effect estimates of the included studies, we used random-effects models and pooled by weighing the log of the odds ratios or hazard ratios by the inverse of their variance. Cochran's Q and Higgin's I^2 statistic were reported to assess heterogeneity across studies. Since differences in estrogen dose of COCs across studies may be an important source of heterogeneity, a subanalysis based on use of COCs with <50 µg estrogen was performed. R version 4.1.0 was used for all analyses.

Risk of bias assessment

We used a version of the Newcastle-Ottawa tool that was customized for risk of bias assessment in case-control studies.[5] The following risk of bias assessment criteria were customized: 1. Selection of participants (low risk of bias: study with controls/ unexposed sampled from source population or same community as cases/ exposed; high risk of bias: controls not representing the study population). 2. Adjustments for confounding (low risk of bias: adjustment for age or adjustments by design such as matching; high risk of bias: no adjustments in analyses). 3. Hormonal contraceptive exposure evaluation (low risk of bias: database record selection or written self-report, type and dosage reported, differentiation made between current and past; high risk of bias: no description). 4. Migraine exposure evaluation (low risk of bias: migraine diagnosis according to International Headache Society-criteria (version I, II or III); high risk of bias: self-report without diagnostic criteria). 5. Outcome (low risk of bias: (pre)defined outcome assessment, objectively confirmed stroke in all cases by MRI or CT, and distinction between ischemic and hemorrhagic stroke; high risk of bias: no (pre)defined outcome assessment, or not objectively confirmed in in all cases or unclear).

RESULTS

NESTED CASE-CONTROL STUDY

From the 1st of January 2007 to the 31st of December 2020, 617 of all 258,828 women aged between 18–49 years had a first ischemic stroke. This corresponded to an average annual cumulative incidence of 26 strokes per 100,000 women. We included these 617 cases and 6170 age-matched controls. The mean age was 37 years. Of all cases, 115 (18.6%) women fulfilled our defined criteria for clinic-based migraine, versus 556 (9.0%) women in the control group, resulting in an increased risk of ischemic stroke of aOR: 1.67 (95% CI: 1.31–2.61). Women who currently used COCs also had an increased risk of ischemic stroke compared with those who did not currently use COC (20.6% versus 9.4%; aOR: 2.40; 95% CI: 1.91–2.65).

For combined migraine and COC use versus neither factor we found a significant increase in the risk of ischemic stroke (3.1% versus 0.4%; aOR: 6.83; 95% CI: 3.95–11.7), which was supra-additive to what would be expected from the presence of migraine (aOR: 1.52; 95% CI: 1.16–1.97) or COC use alone (aOR: 2.19; 95% CI: 1.71–2.79, Table 2a). Women with migraine who both smoked and used COCs versus women without migraine who neither smoked nor used COCs, had a clearly increased risk of stroke (aOR: 30.2 95% CI: 4.22–610, Table 2b).

Table 1. Baseline characteristics of ischemic stroke cases and controls of ages 18–49 years

Baseline characteristics	Cases (n = 617)	Controls (n = 6170)
Age (mean ± SD)	37.3 (6.4)	37.3 (6.4)
Cardiovascular risk factors, *n (%)*		
Smoking (ever)	91 (14.7)	382 (6.2)
Hyperlipidemia	80 (13.0)	158 (2.6)
Hypertension	199 (32.3)	723 (11.7)
Diabetes mellitus	27 (4.4)	120 (1.9)
Hemorrhagic stroke	7 (1.1)	0 (0.0)
TIA	13 (2.1)	5 (0.1)
Subarachnoid hemorrhage	4 (0.6)	0 (0.0)
Myocardial infarction	6 (1.0)	11 (0.2)
Angina pectoris	4 (0.6)	8 (0.1)
Peripheral artery disease	9 (1.5)	48 (0.8)
Migraine	115 (18.6)	556 (9.0)
Preeclampsia	23 (3.7)	119 (1.9)
Hormonal contraceptive use, *n (%)*	127.0 (20.6)	583.0 (9.4)

Table 2a. Risk of ischemic stroke: interaction analysis of migraine and combined oral contraceptive use

	COC* use	Cases	Controls	OR (95% CI)	aOR** (95% CI)
Migraine	Yes	19 (3.1)	23 (0.4)	9.78 (5.86 - 16.2)	6.83 (3.95 - 11.7)
	No	52 (8.4)	311 (5.0)	2.1 (1.63 - 2.69)	1.52 (1.16 - 1.97)
No migraine	Yes	108 (17.5)	560 (9.1)	2.28 (1.79 - 2.87)	2.19 (1.71 - 2.79)
	No	438 (71.0)	5276 (85.5)	Ref.	Ref.

*COC = combined oral contraceptive; aOR = adjusted odds ratio
**Odds ratio adjusted for age, smoking, diabetes, hypertension, hyperlipidemia

Table 2b. Risk of ischemic stroke: interaction analysis of migraine, combined oral contraceptive use, and smoking

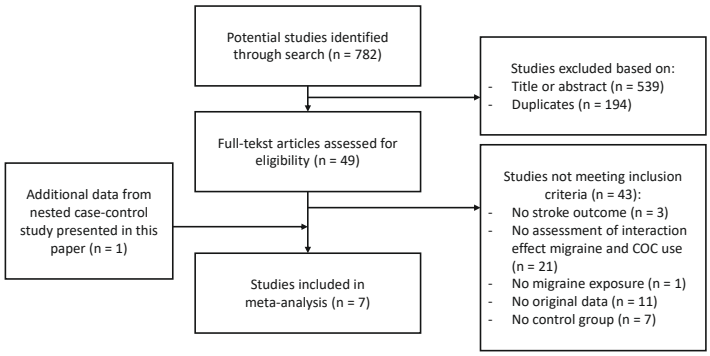
	COC* use + smoking	Cases	Controls	OR (95% CI)	aOR** (95% CI)
Migraine	Yes	5 (0.8)	1 (0.0)	68.2 (11.0 – 13.8)	30.2 (4.22 – 6.10)
No migraine	Neither	68 (11.0)	473 (7.7)	Ref.	Ref.

*COC = combined oral contraceptive; aOR = adjusted odds ratio
**Odds ratio adjusted for age, smoking, diabetes, hypertension, hyperlipidemia

SYSTEMATIC REVIEW

Our systematic review identified 782 potentially eligible articles through an electronic search, of which 194 were duplicates. We excluded 534 articles based on title and abstract assessment. In addition, 49 articles were excluded after a full review. Seven studies, including the case-control study presented in this paper, met the inclusion criteria[9, 10, 23-25] (Figure 1).

Figure 1. PRISMA study flow diagram



Authors of one included study shared additional data on the joint effect of migraine and COC use, the joint effect of migraine, COC use and smoking on risk of ischemic stroke, and on the subanalysis based on low estrogen dose.[24] All seven included studies had a case-control design, with ischemic stroke as the outcome measure.[9, 10, 23-25] Two included studies also performed a subanalysis for hemorrhagic stroke as outcome measure.[10, 25] Hormonal contraception consisted mainly of COC use, and no studies reported isolated effect estimates for other hormonal contraceptives. Two studies distinguished between migraine with and without aura[23, 24], and two studies assessed the joint effect of migraine, hormonal contraceptives and smoking on the risk of ischemic stroke.[9, 24] Characteristics of the seven included studies are listed in Table 3. One study reported only the aOR for migraine with and without aura separately, while all other studies reported the aOR for migraine with and without aura combined. To include this study in the quantitative analysis, we combined the OR from migraine with and without aura.[23]

Table 3. Characteristics of studies included in the systematic review

Author, Year, Setting	Design	Population	Age	Migraine	Contraceptive use	Outcome	Adjustments
Collaborative Group 1975, hospitals in 12 cities, US	Case-control, inclusion period 1969-1971	598 cases, 429 hospital controls matched for age, race and geographical area, 451 neighbourhood controls matched for race and age	15-44	Self-reported during structured interview not based on IHS-criteria. No subspecification with/ without aura	Data from participant questionnaire. Dose: 100 µg and 50 µg, distribution of dose not further specified*	Ischemic and hemorrhagic stroke based on discharge diagnosis, confirmed by neurologist	None
Tzourio et al. 1995, five hospitals in France	Case-control, inclusion period 1990-1993	72 cases, 173 hospital controls with rheumatologic of orthopedic diagnoses	18-44	Neurologist interview based on IHS criteria. No subspecification with/without aura	Data from participant questionnaire. Distribution estrogen dose: 30-40 µg (73%), 50 µg (15%), 20 µg (7%), progestin only (5%)	Ischemic stroke, defined clinically using WHO criteria, confirmed by imaging	None
Schwartz et al. 1998, from Kaiser Permanente (KP) Medical Care Plans and University of Washington Study	Case-control, inclusion period 1991-1995	175 cases, 1191 population controls, for Kaiser Permanente study matched on exact year of birth and facility of usual care	18-44	Self-reported: migraine diagnosis from clinician or having visited clinical for migraine	Self-reported. Distribution estrogen dose: <50 µg for all patients	Ischemic stroke, 2 physicians reviewed medical records or single board-certified neurologist reviewed the records	Treated hypertension, treated diabetes, smoking (current, not current), ethnicity, body mass index, and menopausal status

Table 3. Continued

Author, Year, Setting	Design	Population	Age	Migraine	Contraceptive use	Outcome	Adjustments
Chang et al. 1999, hospitals in eight cities in Europe	Case-control, inclusion period 1990-1993	291 cases, 736 hospital controls from same hospital as matched cases, matched for age and time of admission	20-44	Neurologist interview based on IHS criteria, stratified for migraine with and without aura	Data from patient questionnaire, distribution estrogen dose: ≥50 µg (31%), <50 µg (69%)	Ischemic and hemorrhagic stroke based on clinical diagnosis, confirmed by review medical records	High blood pressure, education, smoking categories, family history of migraine, alcohol consumption, and social class;
MacClellan et al. 2007, 59 hospitals in Baltimore, US	Case-control, inclusion period 1992-2003	386 cases, 614 population controls matched for geographic area, race and age	15-49	Standardized questionnaire using IHS-criteria, differentiated between migraine with probable visual aura and migraine without	Data from patient questionnaire, estrogen dose specified in 75% of participants, in whom: ≥50 µg (3%), <50 µg (97%)	Ischemic stroke discharge diagnosis, confirmed by review medical records and imaging (CT/MRI)	Age, race, geographic region, study period
Champaloux et al. 2016, National Healthcare claims database, US	Case-control, inclusion period 2006-2012	1884 cases, 7536 age-matched controls from database	15-49	ICD-9 codes in database, recorded prior to stroke	Data from pharmaceutical claims database, estrogen dose not specified	Ischemic stroke, ICD-9 codes in database	Hypertension, diabetes, obesity, smoking, ischemic heart disease, and valvular heart disease;
Van Os et al. 2022, population-based open cohort in The Netherlands	Nested case-control, inclusion period 2007-2020	617 cases, 6170 age-matched controls	18-49	ICPC-, ICD-9 and ICD-10 codes including migraine specific drugs (ATC N09C)	Data from pharmaceutical database (ATC codes), estrogen dose not specified	Ischemic stroke, ICD-9 and ICD-10 codes in database	Ischemic stroke, ICD-9 and ICD-10 codes in database

*23 of the 25 women who suffered thrombotic stroke while taking the mestranol-containing formulation took 100-µg pills, and all 20 women who had strokes while taking the ethinyl estradiol formulation took 50-µg pills

RISK OF ISCHEMIC STROKE IN WOMEN WITH MIGRAINE, USE OF COCS, OR BOTH

In the seven included studies, the aORs for ischemic stroke in women without migraine who used COCs compared with women without migraine who did not use COC ranged from 1.11–4.90 (pooled aOR: 1.85; 95% CI: 1.24–2.74; Q: 30.2, $p < 0.001$; I^2: 87.7%). The risk of ischemic stroke in women with migraine compared with women without migraine, the aORs ranged from 1.03–3.70 (pooled aOR: 1.57; 95% CI: 1.22–2.01; Q: 10.9, $p = 0.03$; I^2: 72.1%). The risk of ischemic stroke in women with migraine and use of COCs compared with women who did not have migraine and did not use COCs, the aORs ranged from 2.04–16**.**9 (pooled aOR: 4.44; 95% CI: 2.40–8.21; Q: 22.6, $p < 0.001$; I^2: 84.7%, Figure 2).

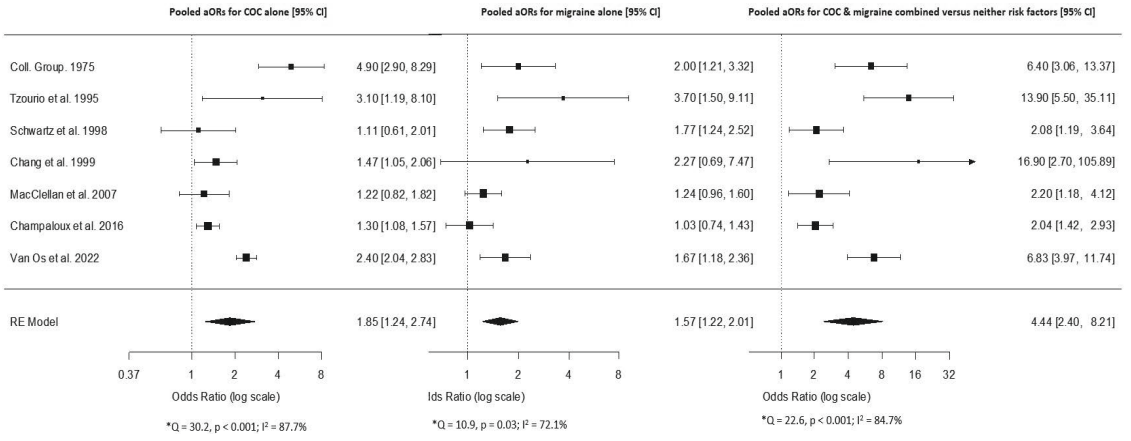
RISK OF ISCHEMIC STROKE IN WOMEN WITH MIGRAINE WITH AURA VERSUS THOSE WITHOUT AURA

Two studies distinguished between migraine with and without aura. One study found an aOR of 6.08 (95% CI: 3.07–12.1) for the risk of ischemic stroke in women with migraine with aura and using COCs compared with women without migraine and using COCs. In women with migraine without aura and using COCs compared with women without migraine and using COCs, the aOR was 1.77 (95% CI: 1.09 – 1.88).[23] The second study found an aOR of 2.34 (95% CI: 1.09–5.00) for risk of ischemic stroke in women with migraine with probable visual aura who used COC compared with women without migraine and using COCs. In the same study, the risk of ischemic stroke in women with migraine with and without aura combined who used COC versus women with no migraine who did not use COCs resulted in an aOR of 2.21 (95% CI: 1.16–4.21).[24]

THREE-WAY INTERACTION BETWEEN MIGRAINE, USE OF COCS, AND SMOKING

One study found an aOR of 34.4 (95% CI: 3.27–361) for the risk of ischemic stroke in women with migraine who used COCs and were current smokers (nine in the cases and two in the controls) versus women without any of these three risk factors.[10] In another study, a similar comparison resulted in an aOR of 5.33 (95% CI: 1.81–15.7) (unpublished data, see supplement).**[24]** In our nested case-control study, we found an aOR of 30.2 (95% CI: 4.22–610), which was also based on a very small number of exposed cases and controls (five cases and one control with migraine who used COC and smoked).

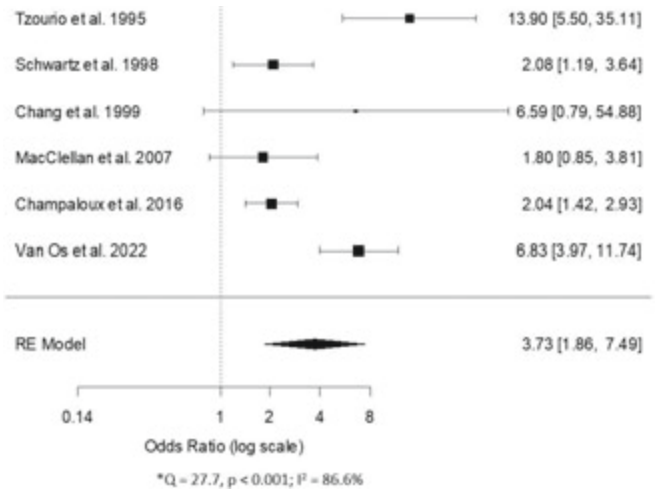
Figure 2. Association of hormonal contraceptives and migraine alone, and both combined with risk for stroke



RISK OF ISCHEMIC STROKE IN WOMEN WITH MIGRAINE AND USE OF LOW DOSE ESTROGEN COCS

One study reported the subanalysis based on use of COC with <50 µg estrogen in the original publication,[10] and authors of another study shared data on this subanalysis with us.[24] For one case control study from 2017 and our nested case-control study no information on COC estrogen dose was available. However, it can be assumed that during this follow-up period more than 75% of all women used low estrogen dose.[23] In the six studies included in the subanalysis of low dose estrogen, aORs for ischemic stroke in women with migraine with aura and use of COCs ranged from 1.80 – 13.9 (aOR: 3.14; 95%CI: 1.75–5.62; Q = 27.7, p < 0.001; I^2 = 86.6%, Figure 3).

Figure 3. Association of hormonal contraceptives with estrogen dose of <50µg and migraine combined with risk for stroke



RISK OF BIAS IN INCLUDED STUDIES

Four studies sampled controls from the general population, two studies used hospital-based controls and in one study controls came from the US National Health Claims database, and the exact origin of controls was unknown. All studies adjusted for confounding either with matching or through multivariate analysis, or both. Three studies reported distribution of estrogen dose of COCs across the study population. Three studies collected migraine data with International Headache Society-criteria, and two studies explicitly reported ascertainment of stroke diagnosis with imaging (Table 4).

Table 4. Risk of bias assessment of included studies

Author, Year	Selection of participants	Adjustments for confounding	OC exposure evaluation	Migraine exposure evaluation	Outcome
Collaborative Group 1975	Low	Low	High	High	High
Tzourio et al. 1995	High	Possible	Low	Low	Low
Schwartz et al. 1998	High	Low	Low	High	Low
Chang et al. 1999	High	Low	Low	Low	Low
MacClellan et al. 2007	Low	Low	Low	Low	Low
Champaloux et al. 2016	Low	Low	High	High	High
Van Os et al. 2022	Low	Low	High	High	High

DISCUSSION

We first conducted a nested case-control study in women aged 18–49 years and found that migraine, use of COCs, and smoking were independent and supra-additive risk factors for first-ever ischemic stroke. The additive relative effect of smoking was substantial, but remained small in absolute terms. We then conducted a systematic review of the literature and identified six studies that reported on the joint effect of migraine and use of COCs on the risk of ischemic stroke. A pooled analysis of these six previous studies together with our novel nested case-control study showed that migraine and the use of COCs have an supra-additive increasing effect on the risk of first-ever ischemic stroke in women. Pooled estimates for migraine and COCs use together resulted in high heterogeneity (I^2 = 84.7%), indicating that there was a large discrepancy between reported odds ratios. Importantly, all studies showed that the risk of ischemic stroke for migraine and COC use was positively associated, meaning that the studies are in agreement that the combination of migraine and COC increases the risk of ischemic stroke. In a subanalysis in women with migraine who used COCs containing <50 µg of estrogen, the pooled effect estimate for the risk of ischemic stroke remained supra-additive, although the aOR was lower (pooled aOR: 3.14; 95%CI: 1.75–5.62) compared with total COCs use and migraine (pooled aOR: 4.44; 95% CI: 2.40–8.21). This analysis also suffered from a high heterogeneity (I^2 = 86.6%).

Compared with the extant literature[17, 18], the present study adds unpublished data from a previously published case-control study[24], our nested case-control study, as well as a meta-analysis of all six “old” studies plus our “seventh new” study, meeting the critical inclusion criteria. The findings of our nested case-control study provide an additional argument that migraine and the use of COCs – even those with low-dose estrogen – have a supra-additive increasing effect on the risk of ischemic stroke, consistent with the findings of other studies.[9, 10] Our nested case-control study and meta-analysis, however, could not distinguish between migraine with and without aura. This distinction is important, because based on the literature it seems to be specifically migraine with aura that is associated with an increased risk of

ischemic stroke.[26] Further, one previous study found a clearly supra-additive effect of migraine with aura and concomitant COC use on the risk of ischemic stroke[23], while this could not be confirmed in another study.[24]

STRENGTHS AND LIMITATIONS

Strengths of our nested case-control study include the linkage of multiple data sources (primary care data, hospital diagnosis codes, and pharmacy registrations) and the prospective collection of data. A strong point of our systematic review and meta-analysis is that we were able to retrieve previously unpublished data from one study.[24]

Our study has several potential limitations. First, the migraine definition in our nested case-control study was derived from electronic patient record registrations, and may have suffered from underreporting.[27] The cumulative lifetime incidence of migraine in our nested case-control study was 18.6% in cases and 9% in controls, which is substantially higher compared with a previous report on Dutch primary care electronic patient record registrations of migraine (2.5%).[28] This was potentially because we used multiple sources of electronic patient record registrations for our migraine definition (primary care, hospital, and medication registrations) and we included women of reproductive age in whom active migraine prevalence is highest.[29] However, the lifetime incidence of migraine in our nested case-control study was still lower than the estimated cumulative lifetime incidence of migraine according to population based studies, in which the migraine diagnosis was verified using the International Headache Society criteria (up to 33%).[15, 29] The underreporting of migraine may be due to the fact that a substantial proportion of migraine patients do not visit the general practitioner for their migraine[29] and when they do, migraine may not be accurately reported by the GP in the electronic patient record.[27] However, differential misclassification of migraine by case-control status was unlikely, as all data were routinely and prospectively recorded and problems such as recall bias were absent. Therefore, the underreporting of migraine may have led to an underestimation of the association between migraine and the risk of ischemic stroke which we found in this study.[30] Because we used a clinic-based definition of migraine in our nested case-control study, our migraine group likely consists of women who have searched for help in primary or hospital care or are treated for migraine. In this subgroup of migraine patients, the risk of ischemic stroke may be relatively more increased compared with the overall migraine population.[31] Second, in Dutch electronic patient records, often overall stroke classification codes are used instead of codes specific to ischemic or hemorrhagic stroke. Therefore, we included the codes for overall stroke in our definition of ischemic stroke. Of all events in our nested case-control study, only 23% were based on codes for overall stroke. Since about 80% of strokes are ischemic, it is likely that less 5% of all stroke registrations in our nested case-control study represents hemorrhagic stroke. Misclassification of hemorrhagic as ischemic strokes may have caused a small dilution of the observed effects. Third, we found significant heterogeneities in the random-effect analyses, which complicates interpretation of the pooled aORs. Multiple potential sources of bias for the included studies could be identified in our risk

of bias assessment, which made it difficult to identify a primary cause for the heterogeneities and to perform meta-regression or sensitivity analyses.

IMPLICATIONS FOR CLINICAL PRACTICE

Recently, the European Headache Federation (EHF) and the European Society of Contraception and Reproductive Health (ESC) published a consensus statement in which authors suggested against prescription of COCs in women with migraine with aura. The supra-additive increase in the risk of stroke in the migraine patients who use COCs, which was found in our nested case-control study, likely constitutes a lower limit of the risk increase in migraine patients with aura because of the following. Although we could not distinguish between migraine with or without aura based on our data, we do know that migraine with aura constitutes only 30% of all migraine cases. Moreover, since more than a decade the Dutch national primary care guideline for hormonal contraceptive use mentions migraine with aura as a risk factor for ischemic stroke, and states migraine with aura in combination with smoking as a contraindication for the prescription of COC.[32] It is, therefore, likely that a substantial number of GPs have refrained from prescribing COC to women with migraine with aura, which would result in a relatively smaller fraction of women with migraine with aura in our overall migraine group. Because the effect of migraine with aura on the risk of ischemic stroke appears to be much stronger than that of migraine without aura, the effect we found in our nested case-control study could have been diluted compared with the true effect of migraine with aura. However, if the supra-additive increase in the risk of ischemic stroke would have only been caused by the smaller migraine with aura subgroup, this implies that the effect of migraine with aura would be many times higher than the effect that we found in our study. Compared with findings from previous studies this is unlikely[23, 24], and, therefore, we cannot exclude the possibility of a supra-additive effect on the risk of ischemic stroke in migraine patients who use COCs. Regarding women without aura who have additional cardiovascular risk factors, authors of the EHF and ESC consensus statement suggest non-hormonal contraception or progestogen-only contraceptives as the preferential option.[33] Given the supra-additive effect of smoking in addition to migraine and the use of COCs in our case-control study (aOR: 30.2), our study supports this suggestion. Although the absolute risk of ischemic stroke in young women is low (11–25 per 100.000[23]), migraine, COCs use and smoking can still significantly increase the risks and stroke at young age will often result in many years of disability.[34] Based on our results, we advise healthcare professionals – and in particular general practitioners – to (i) be careful in prescribing COCs to women with migraine without aura who also smoke, (ii) to actively ask about migraine including aura status in this context, and (iii) to invest in the quality of routine care registrations of migraine diagnoses.

Future research should focus on more personalized advice on the use of COCs in women with migraine. This includes explaining relative and absolute risks of ischemic stroke for different doses of estrogen and taking into account migraine attack frequency and aura status[2, 31] and the presence of traditional cardiovascular female-specific and psychosocial risk factors.[35, 36] For many women with migraine, the contraceptive and non-contraceptive benefits of COCs

(e.g. reduction in the risk of ovarian and endometrial cancer[37]may outweigh the relatively small increase in absolute risk of ischemic stroke.

CONCLUSION

In young women, migraine with aura and possibly also without aura, COC use and smoking have supra-additive effects on the risk of ischemic stroke. This effect may be slightly lower but still significant for COCs containing low doses of estrogen.

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CHAPTER 8

Sex differences in risk profile, stroke cause and outcome in ischemic stroke patients with and without migraine

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ABSTRACT

Background: An increased risk of stroke in patients with migraine has been primarily found for women. The sex-dependent mechanisms underlying the migraine–stroke association, however, remain unknown. This study aims to explore these sex differences to improve our understanding of pathophysiological mechanisms behind the migraine–stroke association.

Methods: We included 2,492 patients with ischemic stroke from the prospective multicenter Dutch Parelsnoer Institute Initiative study, 425 (17%) of whom had a history of migraine. Cardiovascular risk profile, stroke cause (TOAST classification), and outcome [modified Rankin scale (mRS) at 3 months] were compared with both sexes between patients with and without migraine.

Results: A history of migraine was not associated with sex differences in the prevalence of conventional cardiovascular risk factors. Women with migraine had an increased risk of stroke at young age (onset < 50 years) compared with women without migraine (RR: 1.7; 95% CI: 1.3–2.3). Men with migraine tended to have more often stroke in the TOAST category other determined etiology (RR: 1.7; 95% CI: 1.0–2.7) in comparison with men without migraine, whereas this increase was not found in women with migraine. Stroke outcome was similar for women with or without migraine (mRS ≥ 3 RR 1.1; 95% CI 0.7–1.5), whereas men seemed to have a higher risk of poor outcome compared with their counterparts without migraine (mRS ≥ 3 RR: 1.5; 95% CI: 1.0–2.1).

Conclusion: Our results indicate possible sex differences in the pathophysiology underlying the migraine–stroke association, which are unrelated to conventional cardiovascular risk factors. Further research in larger cohorts is needed to validate these findings.

INTRODUCTION

Migraine is a prevalent brain disorder and important risk factor for cardiovascular disease (CVD), including stroke. The increased risk is especially evident in women and less clear in men.[1] In addition, sex differences in ischemic stroke are increasingly acknowledged. Women more often suffer from ischemic stroke compared with men, especially after menopause, and have an increased risk of poor outcome.[2,3] Although it has been recognized that cardiovascular pathophysiology is partly different between women and men, the role of sex in the migraine–stroke association remains poorly understood.[1,4,5] Missing gaps in the association are the role of conventional and non-conventional vascular risk factors, the relation with underlying stroke cause, and the effect of migraine susceptibility on brain tissue recovery after ischemia. Until now, it is unknown how sex affects these factors. This explorative study aims to investigate differences in cardiovascular risk profiles, stroke cause, and stroke outcome between men and women to improve our understanding of pathophysiological mechanisms underlying the migraine–stroke association.

METHODS

We selected patients with ischemic stroke for whom information on a history of migraine was available from the prospective registry and biobank "Dutch Parelsnoer Institute Cerebrovascular Accident (PSI-CVA) Initiative" in eight university hospitals in the Netherlands.[6] The PSI-CVA registry is a large cohort of stroke patients in which comprehensive clinical data, detailed phenotyping of stroke, imaging data, and biomaterials were prospectively and uniformly collected. The registry started in 2009 and ended in 2019. The Ethics Committees of all participating centers approved the PSI-CVA Initiative.

Data on cardiovascular risk profile (conventional risk factors including smoking, diabetes mellitus, hyperlipidemia, previous stroke, myocardial infarction, atrial fibrillation, BMI ≥ 25, and hypertension) and stroke classification were obtained prospectively upon hospital admission. Ischemic stroke was defined according to the WHO criteria and confirmed on CT or MRI and further specified according to the trial of ORG 10172 in acute stroke treatment (TOAST) classification in the subcategories large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined etiology, and stroke of undetermined etiology.[7] The modified Rankin Scale (mRS) was used to grade stroke outcome. A poor outcome was defined as mRS at 3 months after discharge ≥ 3.

Migraine history was prospectively obtained at hospital admission using a short, validated questionnaire that was specially developed to establish migraine diagnosis in patients with stroke (MISS questionnaire, see Supplementary Material.[8]

We performed a complete case analysis with respect to migraine status. Poisson regression analysis was performed to calculate risk ratios (RR) including 95% confidence intervals (CI) for the associations between age of stroke onset, cardiovascular risk factors, stroke subtype and

outcome, and migraine diagnosis, for all patients and for each sex separately. The analyses were adjusted for potential confounders.

RESULTS

In total 6,259 participants were included in the PSI-CVA database, of whom 4,273 had ischemic stroke and 2,492 (40% women) also with information on migraine status. A lifetime history of migraine was present in 425/2,492 (17% overall, 10% in men, and 27% in women) of the participants. Age, sex, and cardiovascular risk profile were similar between patients with or without available information about migraine status.

There were no differences in cardiovascular risk factor profile in stroke patients with vs. without migraine overall or between sexes (Table 1).

Women with migraine had their stroke on average 7 years ($p < 0.0001$) and men 5 years earlier than stroke patients without migraine ($p < 0.0001$). Stroke onset < 50 years occurred more often in women with than in women without migraine (RR: 1.7; 95% CI: 1.3–2.3, Table 1 and Figure 1). This increased risk could not be confirmed in men (RR: 1.4; 95% CI: 0.9–2.1).

Men with migraine tended to have a higher risk for stroke of other determined etiology compared with men without migraine (RR: 1.7; 95% CI: 1.0–2.7), whereas no differences in this TOAST category were found in women (RR: 0.9; 95% CI: 0.6–1.4, Table 2 and Figure 1). Other stroke subtypes were comparable with men and women with and without migraine, although the effect estimate had an opposite direction for the category small vessel occlusion.

Outcome after stroke seemed to be comparable with women regardless of migraine diagnosis (RR: 1.1; 95% CI: 0.7–1.5), whereas men tended to have a worse outcome compared with their counterparts without migraine (RR: 1.5; 95% CI: 1.0–2.1, Table 3 and Figure 1).

Table 1. Demographics and cardiovascular risk factors

	All				Women				Men			
	Migraine	No migraine	RR	aRR[a]	Migraine	No migraine	RR	aRR[b]	Migraine	No migraine	RR	aRR[b]
Demographics												
Number	425 (17)	2,067 (83)	-	-	264 (62)	730 (35)	-	-	161 (38)	1,337 (65)	-	-
Age, years	61 ± 15	67 ± 14*	-	-	61 ± 17	68 ± 15*	-	-	61 ± 13	66 ± 14*	-	-
Age of onset < 50	93 (22)	266 (13)	1.7 (1.3 - 2.1)	-	65 (25)	105 (14)	1.7 (1.3 - 2.3)	-	28 (17)	161 (12)	1.4 (0.9 - 2.1)	-
Age of onset ≥ 50	332 (78)	1,801 (87)	0.8 (0.8 – 0.9)	-	199 (75)	625 (86)	0.9 (0.7 – 1.0)	-	133 (83)	1,176 (88)	0.9 (0.8 – 1.1)	-
Pre-stroke mRS	29 (7)	164 (9)	0.8 (0.5 - 1.2)	0.9 (0.6 - 1.3)	21 (8)	82 (12)	0.7 (0.4 - 1.1)	0.8 (0.5 - 1.3)	8 (5)	82 (7)	0.8 (0.4 - 1.5)	0.9 (0.4 - 1.8)
CV risk factors												
Hypertension[c]	226 (54)	1,115 (54)	1.0 (0.9 - 1.1)	1.1 (1.0 - 1.3)	138 (53)	412 (57)	0.9 (0.8 - 1.1)	1.1 (0.9 - 1.3)	88 (55)	703 (53)	1.0 (0.8 - 1.3)	1.2 (0.9 - 1.4)
DM[d]	58 (14)	304 (15)	0.9 (0.7 - 1.2)	1.1 (0.8 - 1.4)	35 (13)	113 (16)	0.9 (0.6 - 1.2)	1.0 (0.7 - 1.5)	23 (14)	191 (14)	1.0 (0.6 - 1.5)	1.1 (0.7 - 1.7)
Hyperlipidemia[e]	145 (35)	752 (37)	0.9 (0.8 - 1.1)	1.1 (0.9 - 1.3)	89 (34)	240 (34)	1.0 (0.8 - 1.3)	1.2 (0.9 - 1.5)	56 (35)	512 (39)	0.9 (0.7 - 1.2)	1.0 (0.7 - 1.3)
Previous Stroke	115 (28)	515 (26)	1.1 (0.9 - 1.3)	1.2 (1.0 - 1.5)	69 (27)	169 (24)	1.1 (0.9 - 1.5)	1.2 (1.0 - 1.7)	46 (29)	346 (27)	1.1 (0.8 - 1.5)	1.2 (0.9 - 1.6)
History of MI	34 (8)	262 (13)	0.6 (0.4 - 0.9)	0.9 (0.6 - 1.3)	15 (6)	57 (8)	0.7 (0.4 - 1.3)	0.9 (0.5 - 1.6)	19 (12)	205 (16)	0.8 (0.5 - 1.2)	1.0 (0.6 - 1.5)
Atrial fibrillation	42 (10)	259 (13)	0.8 (0.6 - 1.1)	1.1 (0.8 - 1.6)	22 (9)	81 (11)	0.8 (0.5 - 1.2)	1.0 (0.6 - 1.7)	20 (13)	178 (14)	0.9 (0.6 - 1.5)	1.3 (0.8 - 2.0)
Smoking ever[f]	25 (6)	186 (9)	0.7 (0.4 - 1.0)	0.8 (0.5 - 1.2)	15 (6)	50 (7)	0.8 (0.5 - 1.4)	0.9 (0.5 - 1.7)	10 (6)	136 (10)	0.6 (0.3 - 1.1)	0.7 (0.3 - 1.3)
BMI ≥ 25	258 (62)	1,267 (64)	1.0 (0.8 - 1.1)	1.1 (0.9 - 1.2)	147 (57)	349 (51)	1.1 (0.9 - 1.4)	1.1 (0.9 - 1.3)	111 (69)	918 (71)	1.0 (0.8 - 1.2)	1.0 (0.8 - 1.2)

mRS, modified Rankin Scale; CV, cardiovascular; DM, diabetes mellitus; MI, Myocardial infarction; BMI, body mass index (kg/m^2).
Data are represented as mean ± SD or number of subjects (%).
*Migraine vs. no migraine: $p < 0.001$.
[a] Adjusted for age and sex.
[b] Adjusted for age.
[c] Ever or current diagnosis or treatment with antihypertensive drugs.
[d] Ever or current diagnosis or treatment with antidiabetic drugs.
[e] Total cholesterol > 3.5 mmol/L, low-density lipoprotein cholesterol > 2.5 mmol/L or treatment with lipid-lowering agents.
[f] Current smokers and smokers who stopped smoking > 6 months ago.

Table 2. Stroke subtype according to TOAST classification

	All				Women				Men			
	Migraine	No migraine	RR	RR[a]	Migraine	No migraine	RR	RR[b]	Migraine	No migraine	RR	RR[b]
LAA	84 (20)	530 (26)	0.8 (0.6 – 1.0)	0.9 (0.7 - 1.2)	45 (17)	147 (21)	0.8 (0.6 - 1.2)	0.9 (0.7 - 1.3)	39 (24)	383 (29)	0.8 (0.6 - 1.1)	0.9 (0.7 - 1.3)
Cardioembolism	50 (12)	296 (15)	0.8 (0.6 - 1.1)	0.9 (0.7 - 1.2)	32 (12)	102 (14)	0.9 (0.6 - 1.3)	1.0 (0.7 - 1.5)	18 (11)	194 (15)	0.8 (0.5 - 1.2)	0.8 (0.5 - 1.3)
SVO	72 (17)	373 (18)	0.9 (0.7 - 1.2)	0.9 (0.7 - 1.2)	43 (17)	158 (22)	0.8 (0.5 - 1.0)	0.8 (0.6 - 1.1)	29 (18)	215 (16)	1.1 (0.7 - 1.6)	1.1 (0.7 - 1.6)
Other determined	47 (11)	137 (7)	1.7 (1.2 - 2.3)	1.1 (0.8 - 1.6)	27 (10)	59 (8)	1.3 (0.8 - 2.0)	0.9 (0.6 - 1.4)	20 (12)	78 (6)	2.1 (1.3 - 3.4)	1.7 (1.0 - 2.7)
Undetermined	167 (40)	692 (34)	1.2 (1.0 - 1.4)	1.1 (0.9 - 1.3)	113 (43)	250 (35)	1.2 (1.0 - 1.6)	1.2 (0.9 - 1.5)	54 (34)	442 (34)	1.0 (0.8 - 1.3)	1.0 (0.7 - 1.3)

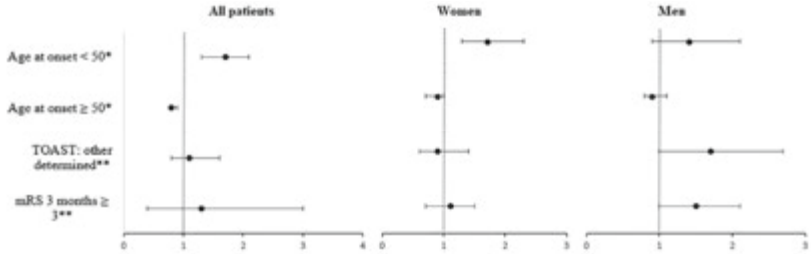
TOAST, Trial of ORG 10172 in acute stroke treatment; LAA, Large-artery atherosclerosis; SVO, Small-vessel occlusion.
Data are represented as mean ± SD or number of subjects (%)
a Adjusted for age and sex
b Adjusted for age

Table 3. Stroke severity and outcome

	All				Women				Men			
	Migraine	No migraine	RR	RR[a]	Migraine	No migraine	RR	RR[b]	Migraine	No Migraine	RR	RR[b]
NIHSS ≥ 7[c]	67 (17)	340 (18)	0.9 (0.7 - 1.2)	0.9 (0.7 - 1.2)	36 (15)	131 (20)	0.7 (0.5 - 1.1)	0.8 (0.5 - 1.1)	31 (21)	209 (17)	1.2 (0.8 - 1.1)	1.2 (0.8 - 1.8)
mRS discharge ≥ 3	102 (30)	238 (32)	1.0 (0.8 - 1.2)	1.1 (0.9 - 1.4)	59 (28)	184 (32)	0.9 (0.7 - 1.2)	1.2 (0.8 - 1.6)	43 (33)	307 (32)	1.1 (0.8 - 1.4)	1.2 (0.8 - 1.6)
mRS 3 months ≥ 3	73 (20)	368 (20)	1.0 (0.7 - 1.2)	1.3 (0.9 - 1.7)	41 (18)	142 (22)	0.8 (0.6 - 1.1)	1.1 (0.7 - 1.5)	32 (23)	226 (19)	1.2 (0.8 - 1.7)	1.5 (1.0 - 2.1)

NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin Scale.
Data are represented as mean ± SD or number of subjects (%)
a Adjusted for pre-stroke mRS, NIHSS at admission (for mRS at discharge and at 3 months), age and sex
b Adjusted for pre-stroke mRS, NIHSS at admission (for mRS at discharge and at 3 months) and age
c NIHSS on admission

Figure 1. Forest plot of the most important findings on associations between migraine and risk factors, etiology, or outcome of stroke, stratified for sex



On the X-axis the odds ratio for association with migraine is displayed. *No adjustments; **Adjustment for age and sex in all patients, and adjustment for age in analyses stratified for women and men.

DISCUSSION

Our explorative study suggests that sex differences in stroke pathophysiology in patients with migraine cannot be explained by differences in conventional vascular risk factors. Women with migraine had a higher risk for stroke under the age of 50. Men tended to more often have stroke of other determined etiology and a worse outcome compared with men without migraine.

Evidence in the literature about the relationship between conventional vascular risk factors and migraine is conflicting, and rarely, data of men and women are analyzed separately.[9] In general, the association between migraine and stroke is thought to be more prominent in patients without a traditional vascular risk profile and with a lower Framingham Risk.[9,10] Only little is known about the association between migraine and sex-specific cardiovascular risk factors. Unfortunately, our PSI-CVA database did not contain all factors needed to construct Framingham Risk score. Also, our database did not include non-conventional sex-dependent vascular risk factors such as (pre)-eclampsia, sex hormone disorders, or use of hormones. Future studies are therefore needed to investigate the effect of these non-conventional risk factors. A younger age at stroke onset in patients with migraine in general, has been reported previously.[1,10]

Previous studies on stroke etiology reported lower frequencies of large vessel and cardio-embolic stroke etiology in female migraine patients and more infarcts of unknown origin in migraine patients in general.[10,11] In a recent study, migraine with aura was strongly associated with cryptogenic stroke, whereas such association was not found in migraine without aura.[12] The association of migraine with aura with stroke was independent of vascular risk factors or patent foramen ovale. The association was present in both women and men, although the odds ratios were higher in women. We observed an increase in stroke of other determined etiology only in men with migraine (with and without aura combined). Sex differences in migraine pathophysiology are likely multifactorial and may reflect genetic and hormonal sex differences. In addition, migraine is associated with cerebral hyperexcitability and spreading depolarization (SD), the neurophysiological correlate of migraine aura. SD is associated with

neurovascular uncoupling and can also be found in the penumbra of cerebral ischemia.[13] These mechanisms may be associated with a sex-specific systemic vascular pathology in migraine patients.[9] Since the increased stroke risk in migraine patients is not associated with enhanced atherosclerosis, alternative pathology, including micro-embolisms, vasospasms in the microvasculature and endothelial dysfunction, may be involved.[13–16] These "non-conventional" mechanisms may explain the higher proportion of other determined causes in men with migraine. We have no good explanation why the higher risk was only found in men and not in women with migraine.

Existing literature on functional stroke outcome in patients with migraine is limited to the Women's Health Study, which only included female health care employees and reported a relatively favorable mRS at hospital discharge after ischemic stroke for women with migraine with aura.[9] In general, female sex has been associated with a less favorable stroke outcome in terms of disability and mortality.[4,5] Our study found no differences in outcome between women with and without migraine but did not investigate women with migraine aura separately. In men with migraine, our data cautiously suggested a worse outcome compared with their counterparts without migraine. As these are the first data on stroke outcome in men specifically, further research is needed to confirm these findings and investigate underlying causes.

Strengths of our study are the relatively large sample size, prospective design, and the use of standardized definitions of cardiovascular risk factor and stroke characteristics. Also, we compared men and women with stroke directly with their counterparts without migraine. Migraine diagnosis was established with a validated questionnaire, and migraine prevalence was as expected for this population. Our study also has limitations. First, the MISS questionnaire has only moderate positive predictive value for aura symptoms. Therefore, we did not distinguish between migraine with and without aura, although the migraine–stroke connection is particularly apparent in migraine with aura. Second, from 4,273 participants with ischemic stroke in our cohort, only 2,492 had complete data on migraine. Not all PSI-CVA study centers participated in our migraine study. We consider this selection to be random and assume that it did not result in selection bias. Third, we did not correct for multiple comparisons. Finally, although our study included almost 2,500 stroke patients, the sample size in several sub-analyses was low, and therefore, our study should be considered explorative and hypothesis generating. To confirm our findings and to study sex differences in migraine with aura patients separately, studies with far, with over 10 thousands of stroke patients will be necessary (because of the relative low prevalence of migraine with aura). Future studies are also needed to study sex-specific non-conventional cardiovascular risk factors and investigate stroke causes in more detail to enable sex-specific prevention of strokes in patients with migraine.

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CHAPTER 9

Summary, conclusions and future perspectives

In this thesis, the role of migraine as a cardiovascular risk factor is investigated in different populations with a varying medical history, including stroke and polycystic ovary syndrome (PCOS). We focused primarily on women and sex-specific features, exploring both conventional and novel measures for vascular health. This thesis was written in the context of the CREW consortium. The aim of the CREW consortium, a cooperation of major medical centers in the Netherlands and commissioned by the Dutch Heart Institute, was to study female-specific cardiovascular pathophysiology. The CREW-MIST (Cardiovascular Riskprofile in Women – MIcrovascular STatus) project, which was part of this consortium, focused on migraine in relation to stroke in women. Female-specific factors such as sex hormone- and pregnancy-related disorders were included in our studies and we explored microvascular health through heart- and brain imaging and functional measurements of the vasculature.

SUMMARY

Chapter 1 introduces the clinical features and epidemiology of migraine and the association with cardiovascular disease including stroke. The possible mechanisms behind this association are explored, with a focus on the role of female-specific factors.

Chapter 2 presents a review of knowledge on migraine and the associated risk of cardiovascular disease at the time of the start of this thesis. It emphasizes how the increased risk for cardiovascular disease and in particularly stroke is most consistently described in women, especially those with migraine with aura. The role of cortical spreading depolarizations, genetics, sex hormones and vascular pathology including endothelial dysfunction are reviewed as underlying mechanisms for the assocation between migraine and cardiovascular disease. For clinical practice, it is advised that migraine with aura should be incorporated in cardiovascular risk assessments in women, even though the absolute risk remains low. Clinical advice for women with migraine remains limited to revising possible oral contraceptives use and lifestyle advise mainly aimed at quitting smoking.

Further exploration of the vascular component that may be altered in migraine patients is described in **chapter 3**, which presents a case-control study exploring cold extremities and sleep in men and women with migraine and healthy controls. This study focused on body temperature as a measure of dysfunctional autonomous vascular regulation that is a possible common pathophysiological mechanism linking migraine and cardiovascular disease. Women with migraine reported cold extremities more than twice as often as women without migraine, whereas such a difference was not found in men. This possibly indicates a vascular vulnerability in migraine patients that is sex-specific. No difference was found comparing the migraine subtypes with or without aura. Among the women with migraine, cold extremities were associated with higher attack frequencies, which was partly mediated by difficulty falling asleep. Further studies are needed to investigate whether cold extremities are associated with increased cardiovascular risk in migraine patients and could serve as a non-invasive marker for risk assessment in these patients.

Gonadal hormone levels likely play a role in the sex differences found in the increased cardiovascular risk in migraine patients. Previously, an altered pattern of estradiol levels and trigeminal nerve-mediated microvascular reactivity throughout the menstrual cycle in women with menstrually related migraine (MRM) was described by the Erasmus MC vascular pharmacology study group. These findings coincide with the results of our case-control study described in **chapter 4** that investigated the differences in pain perception in women with MRM at two different stages of the menstrual cycle, compared to women without migraine and postmenopausal women. Women without migraine reported higher pain sensation in the trigeminal dermatome at mid-luteal phase compared to the early follicular phase. Similar to the findings on estradiol levels and vascular reactivity, these cyclical changes were not found in women with MRM. Interestingly, differences in cyclicity of perceived pain were only detected in the trigeminal dermatome and not outside of this region. Our findings suggest that the fluctuation of trigeminal pain throughout the cycle is compromised in women with MRM; which may point to an altered trigeminovascular sensitivity that may aid in understanding pathophysiology and treatment response in these patients.

In **chapter 5** a cross-sectional study in women with PCOS is described, aimed at comparing microvascular function in women with and without migraine using novel non-invasive techniques. Measurements of dermal blood perfusion during local heating (local thermal hyperaemia; LTH) were used to assess regulation of the microvasculature. Measurements were performed with and without blockade of axon reflex induced neuropeptide release and nitric oxide availability, two important components of the vasoregulatory response to heating. The application of EMLA anesthetic cream resulted in less inhibition of axon reflex mediated vasodilation in women with migraine compared to women without migraine (86.7%, SD = 26.5 versus 67.9 %, SD = 24.2; p = 0.014). This finding suggests that vasoregulation in women with migraine is altered, however this difference was not found in the control conditions without blockade. The observed affected peripheral neuropeptide activity was not reflected by a conventional cardiovascular risk score (Framingham Risk Score; FRS) in these women, which may indicate that the altered vasoregulation in women with migraine is not associated with the conventional cardiovascular risk factor profile.

In **chapter 6** ischemic stroke characteristics and cardiovascular risk profile were assessed in women with a history of pregnancy-related complications associated with vascular disease (preeclampsia, HELLP syndrome and placental abruption). While no differences were found in ischemic stroke etiology, age at onset was 10 years younger and the risk for young stroke (onset <50 years) was increased almost five-fold in these women compared to women without a history of pregnancy complications. No differences in stroke characteristics were found when women with and without migraine were compared, however age at onset was 9 years younger in these patients. Our results suggest that both pregnancy-related disorders and migraine are associated with a risk for CVD at a younger age. This could imply that both disorders should be incorporated in cardiovascular risk assessment of women. Whether the combination of these factors leads to an even further increased cardiovascular disease risk needs further study.

Chapter 7 describes a nested case-control study with data from electronic health records and a subsequent systematic review and meta-analysis on the ischemic stroke risk in women with migraine using combined oral contraceptives (COC). In the case-control study, risk of stroke was substantially increased in women with migraine using COC compared to women with neither of these risk factors (aOR: 6.83; 95% CI: 3.95–11.68) The risk even further increased when the factor smoking was added (aOR: 30.24; 95% CI: 4.22–610). The systematic review including the results from our nested case control study showed a substantially increased ischemic stroke risk for women who both had migraine and used COC compared to women without migraine and COC use (pooled aOR: 4.95; 95%CI: 2.13–11.48). In a subanalysis in women with migraine using lower estrogen dose COC (<50 µg) ischemic stroke risk was still increased (pooled aOR: 3.14; 95%CI: 1.75–5.62). Evidence on the risk of ischemic stroke in women with migraine using COC remains limited and heterogenous. Further studies are needed to improve personalized decision making regarding COC prescription in women with migraine.

The increased risk for stroke in patients with migraine seems to be sex-dependent, however the underlying mechanisms remain unclear. **Chapter 8** presents a large prospective study based on the multicenter Dutch Parelsnoer Institute CVA database, in which we investigated sex differences and the role of migraine in cardiovascular risk profile and stroke characteristics. We concluded that migraine was not associated with sex differences in cardiovascular risk profile. It is important to note that only conventional, and not female-specific risk factors were taken into account in this analysis. In women as opposed to men, migraine was associated with an almost twofold increased risk for young stroke. In men, but not in women, migraine was associated with stroke from 'other determined etiology' according to the trial of ORG 10172 in acute stroke treatment (TOAST) classification and with a poorer outcome according to the mRS. This study suggests that there are sex differences in the pathophysiology underlying the association between migraine and ischemic stroke that are not related to conventional cardiovascular risk factors. This stresses the importance of sex-specific cardiovascular risk assessment and the need to study what factors give rise to sex differences in future research.

DISCUSSION

Although there is extensive literature describing the association between migraine and cardiovascular risk there are still many unknowns. Current knowledge gaps are: 1) which mechanisms may drive this association, 2) why the increased cardiovascular risk appears to be more prominent in women with migraine, 3) how migraine as a risk factor relates to other female-specific conditions associated with increased cardiovascular disease risk, such a pregnancy-related complications and sex hormone disorders and 4) which clinical recommendations might be given to women with migraine to reduce the risk of cardiovascular events. The studies described in this thesis aim to investigate these topics and provide a basis for further research.

1. POSSIBLE MECHANISMS FOR THE ASSOCIATION OF MIGRAINE WITH CARDIOVASCULAR DISEASE

1.1 VASCULAR VULNERABILITY

There is increasing evidence that the association between migraine and cardiovascular disease is not based on conventional risk factors resulting in atherosclerosis.[1,2] Our results in **chapter 8** support this view, as the prevalence of conventional cardiovascular risk factors was not increased in men or women with migraine in a population of ischemic stroke patients. In the study population of women with PCOS described in **chapter 5** we also found no increased cardiovascular risk scores according to the conventional Framingham Risk Score (FRS) in women with migraine. It is suggested that instead of these conventional cardiovascular factors, there are other mechanisms responsible for the migraine – cardiovascular disease association. Besides the factors summarized in **chapter 2**, including vascular comorbidities and a shared genetic predisposition, there is an increased interest in the role of the microvasculature. Therefore, we focused in several studies of this thesis on microvascular function and vulnerability. A possible cardiovascular pathophysiological mechanism that lies outside of the conventional scope is described **chapter 3**, in which we show that women with migraine more often suffer from cold extremities than women without migraine. Cold extremities indicate peripheral vascular autonomous dysregulation, which may indicate the presence of a systemic vascular vulnerability. Interestingly, the higher prevalence of cold extremities was only found in women, which may suggest that the altered vascular reactivity in migraine is (at least partly) sex-specific. This finding strengthens the idea that sex differences in cardiovascular risk for migraine patients may be based on a more 'female type' cardiovascular etiology; presenting as dysregulation of the microvasculature rather than large artery atherosclerosis.[3,4] In the field of cardiology, this female-specific pathophysiology is observed in the large percentage of women with angina pectoris and cardiac ischemia with no obstructive coronary artery disease (ANOCA and INOCA).[5] In these patients, coronary vasomotor dysfunction is the common etiology, which interestingly is associated with systemic dysfunction beyond the cardiac vasculature.[6,7]

The microvascular vulnerability possibly underlying the increased CVD risk in patients with migraine may be caused by endothelial dysfunction. Where the endothelium has vasoprotective properties when in healthy homeostasis, dysfunction leads to inflammation, increased vessel wall permeability, altered vascular tone, platelet activation and thrombogenicity.[8,9] Serum levels of several biomarkers for endothelial dysfunction are altered in patients with migraine, including endothelial precursor cells (EPCs), endothelial microparticles and secondary factors involved in inflammation and coagulation.[10,11] Levels of the vasoactive protein endothelin-1 (ET-1) rise in the early ictal phase in patients with migraine.[12] The ET-A and ET-B receptors have contrasting effects on the vasculature and the ratio of their expression varies greatly among vascular locations. The ET-A receptor has vasoconstrictive effects and is the predominant receptor in the trigeminovascular system. Besides its vasoactive role, ET-1 is associated with cortical spreading depolarization, induction of pain and sensitization, and the release of substances involved in migraine initiation such as nitric oxide (NO) and calcitonin gene-

related peptide (CGRP).[12,13] Increased ET-1 levels are also observed after ischemic stroke, both in patients with and without migraine.[13,14] Levels of NO synthase inhibitor ADMA are also increased in migraine patients, especially in the interictal period.[15] Interestingly, higher ADMA levels were also associated with white matter hyperintensities (WMH) in these patients, confirming the idea that the observations of subclinical damage on MRI are related to endothelial dysfunction.[16]

Assessment of endothelial vasoregulation can be performed by a wide variety of functional tests, including (brachial artery) flow-mediated dilation (FMD), venous occlusion plethysmography and pulse wave velocity. In several systematic reviews, vasoregulation measured with these modalities was not clearly impaired in women with migraine. However, the heterogeneity and small sizes of the studies complicate interpretation of the results.[17,18] Many of the techniques assessing microvascular function are invasive or prone to inter- and intra-observer variability. Moreover, any of these functional measurements is challenged by having to control a variety of artefact-inducing circumstances, such as temperature, stress and breathing patterns to which the microvasculature inherently responds. The novel technique LTH (laser Doppler assessment of blood flow changes induced by local thermal hyperemia) that we used to assess microvascular reactivity in **chapter 5** is non-invasive, has good reproducibility and allows for controlling of interfering circumstances. Moreover, using blockades of axon reflex induced neuropeptide release and NO-dependent responses allows for further assessment of the role of the involved vasoactive components and endothelium. Our findings in a study population with PCOS show that neuropeptide release is inhibited less after blockade with anesthetic EMLA cream in women with migraine than in women without migraine. This suggests that an altered microvascular response, occurs in these women. No differences were found in overall responses without blockade or in after blockade of NO was established by application of LNMMA, which suggests the involved mechanisms are likely complicated and masked by compensatory effects. Further investigation of the mechanisms of microvascular function in both women and men with migraine is needed. Although the vasodilatory component of microvascular function may certainly play an important role in the migraine – CVD association, more attention is also needed for the pathogenic roles of inflammation, platelet activation and thrombosis and their interplay with the endothelium.

1.2 MIGRAINE SEVERITY, SUBTYPE AND CVD RISK

An important question to address to improve individual risk assessment for patients with migraine, is whether more frequent, more severe migraine or particular migraine subtypes are associated with a further increased underlying microvascular vulnerability and thus increased CVD risk.

There is conflicting evidence on whether patients with a higher migraine attack frequency have a higher CVD risk compared to those with a lower attack frequency.[19,20] Literature on the subject is limited to the assessment of migraine severity in terms of attack frequency and is mostly focused on women and the risk of ischemic stroke.[19] In patients with migraine with aura, a frequency of more than one migraine attack per month was associated with a further

increased risk of ischemic stroke compared to lower frequencies.[20,21] Interestingly, risk for angina and myocardial infarction was decreased in women with an attack frequency of more than once a month, while risk for ischemic stroke was increased in women with more than one attack per week.[20] In men, no evidence was found that migraine attack frequency was related to further increase of CVD risk.[22] In MRI studies, infarct like lesions in the posterior circulation were found more extensively in patients with higher migraine attack frequencies (≥1 per month).[23,24] However, progression of white matter hyperintensities was not associated with number or frequency of migraine attacks nor with activity (attacks in the past year) or use of migraine therapy.[25] Moreover, it is unknown whether white matter hyperintensities or infarct like lesions are associated with a higher CVD risk in migraine patients.

If increased CVD risk in migraine patients would be the consequence of repeated attacks, reducing the attack frequency would be a major therapeutic goal to minimize this risk. However, even if a robust association between migraine severity and CVD risk would be confirmed, it should not directly be concluded that it is a causal relation. It is possible and maybe even more likely that such an association represents a shared underlying pathology responsible for both (more severe) migraine and developing CVD. For example, in **chapter 7** of this thesis, we found that women with migraine who report cold extremities have a higher attack frequency than those who do not have cold extremities. This could indicate that a higher level of vascular vulnerability in these women is responsible for both cold extremities and more severe migraine. In this light, cold extremities may provide a useful clinical marker for vascular vulnerability and risk in migraine patients. However, migraine frequency was also indirectly affected by difficulty falling asleep as a result of experiencing cold extremities. Thus, it might not be purely the vascular vulnerability itself leading to migraine severity. To aid migraine patients to alleviate sleep difficulties caused by cold feet, the LUMC has recently launched a campaign promoting the use of warm socks.

It is evident that migraine with aura is associated with an increased cardiovascular risk, however for migraine without aura the evidence is less conclusive. There is evidence that CVD risk for migraine patients without aura may also be increased, although the risk seems to be significantly lower than the for migraine with aura.[26] The difference in stroke risk between these two subtypes could be caused by the underlying physiological substrate of a migraine aura; cortical spreading depolarizations (CSDs). These propagating waves of neurovascular uncoupling in the cortex are in turn associated with ischemia and thus would be a logical link to explain increased stroke in patients with migraine auras. However, this theory does not explain the increased cardiovascular risk for events occurring outside the vasculature of the brain. Moreover, there is evidence that CSDs also occur asymptomatically in migraine patients without aura.[27] In this thesis, we found no differences in reported cold extremities in patients with migraine with versus without aura.

1.3 MIGRAINE AND CVD RISK IN RELATION TO HORMONES

Sex hormones play an extensive role in both migraine and cardiovascular disease and it is likely that the association between the two is likewise affected by gonadal hormone levels. The role of sex hormones are also likely a part of the explanation behind the observed sex differences in CVD risk in patients with migraine.

Both female and male sex hormones are under scrutiny for their influence on migraine and CVD. However, it is unlikely that the susceptibility for migraine and the related cardiovascular risk are dependent on the absolute level of a single hormone. Rather, fluctuations of hormone levels and possibly ratios of hormones are involved. A clear example of abrupt hormonal fluctuations effecting both migraine and the vasculature is pregnancy and the postpartum period, during which migraine activity often surges and the female vasculature is undergoing enormous changes, sometimes leading to pregnancy-related vascular disorders. Women with active migraine during pregnancy are more at risk of vascular complications including ischemic stroke and myocardial infarction.[28,29] Within two weeks after delivery, post-partum angiopathy may occur. This is a rare condition of multifocal vasoconstriction of cerebral arteries of unknown cause, that presents with severe headache and visual disturbances and can lead to ischemic stroke.[30] There is still much to uncover in the field of neuro-obstetrics about the intersection of such cerebrovascular complications of pregnancy with migraine and ischemic stroke, and the possible involvement of sex hormones.

Estradiol is an especially interesting hormone in relation to migraine and CVD because of its wide array of genomic and non-genomic influences and its different receptors in many tissues of the body. Estradiol receptors present in vascular endothelial cells and smooth muscle cells and are involved in the regulation of lipid metabolism, inflammation, fibrinolysis and thrombosis.[31] Likewise, estradiol stimulates vasodilation through nitric oxide synthesis in the endothelium, increases nerve excitability by stimulating norepinephrine (NE) release and influences pain sensitization through serotonergic and GABA-ergic pathways.[32,33] Moreover, increased estradiol levels are associated with enhanced cortical excitability and spreading depolarizations, which might increase susceptibility to cerebral ischemia.[34] In the trigeminovascular system, estradiol interacts in a not yet understood way with CGRP, possibly further enhancing hypersensitivity and vasodilation.[35] The complexity of the role of estrogens increases further, as the effects vary dependent on the interplay with healthy or dysfunctioning endothelium and whether it is exogenous or endogenous of origin.[36] It is through any of these roles that estrogen may influence the vascular status of patients with migraine. Although it is unlikely that only the level of this single hormone is responsible for the association between migraine and CVD by itself, it may influence the proposed vascular vulnerability underlying this association as well as its observed sex specificity.

The effect of gonadal hormones on migraine severity and risk of CVD makes it important to carefully consider the use of hormonal therapy in patients with migraine. This is especially important for post-menopausal women on hormone replacement therapy (HRT), for

transgender patients on gender affirming hormonal therapy and for women using oral contraceptives. Whereas endogenous female hormones seem to have a vasoprotective role, this does not seem to be the case for exogenous hormones.[31,36] The influence of exogenous hormones is investigated in **chapter 7**, where we summarize the evidence for an increased stroke risk in women with migraine using combined oral contraceptives. Risk of ischemic stroke in these women was increased substantially compared to women without these two risk factors (pooled aOR: 4.95; 95%CI: 2.13–11.48), even in a sub-analysis that focused on low dose estrogen COC (pooled aOR: 3.14; 95%CI: 1.75–5.62). The use of exogenous estrogen is associated with increased hypercoagulability due to platelet hyperactivity. Possibly, this increased hypercoagulability interacts with the endothelial dysfunction that is already present in patients with migraine and results in a supra-additive increase of cardiovascular risk.

In this thesis we also took a closer look at the influence of sex hormones on migraine pathophysiology. In **chapter 4**, we found that women with menstrually-related migraine (MRM) have an altered cyclical pattern of trigeminal pain perception compared to women without migraine and postmenopausal women. Pain outside of the trigeminal dermatome was not different in these women. These findings are in line with the absence of cyclicity in estradiol levels and vascular reactivity which was described in a previous study from the Erasmus migraine study group in the same population.[38] Further studies should further explore the vascular status and CVD endpoints in relation to sex hormone level cyclicity in migraine patients.

Women with polycystic ovary syndrome (PCOS) are interesting to study in the perspective of both cardiovascular disease and migraine because of their sex hormone level imbalance. This hormone imbalance may present as increased levels of androgens including testosterone. To dissect sex differences in the association between migraine and cardiovascular disease, it is of interest to discover whether women with a more androgenous hormone profile suffer less from migraine and are less at risk for 'female type' microvascular dysfunction. In **chapter 5** of this thesis we evaluated the microvascular response to local thermal hyperemia in women with PCOS with and without migraine. Our findings indicated an altered vasoreactivity after axon reflex blockade in women with PCOS and migraine as opposed to women with PCOS without migraine. Interestingly, another study found a slightly increased axon reflex peak phase after administration of estradiol, progesteron or a combination in women with PCOS.[39] Moreover, when testosterone levels are suppressed in women with PCOS, microvascular dilation is improved and this effect is mediated through the endothelin-1 ET-B receptor.[40] These findings support the idea that neuropeptide release is influenced by gonadal hormones and might be involved in vascular dysfunction in women with migraine.

2. SEX DIFFERENCES IN MIGRAINE AS A RISK FACTOR

Whether the increased cardiovascular risk is exclusive to women with migraine or whether men share this risk partly or even equally is uncertain. Even though the main body of research concerning migraine as a cardiovascular risk factor addresses women, there is some evidence

that men with migraine are also at risk.[22,41,42] However, most of the existing data on the migraine – CVD association come from the studies including only women such as the Women's Healthy Study, and only very few studies investigated sex differences in migraine as a CVD risk factor.[42] In cohorts including both men and women, stratification by sex is often lacking and statistical power is often still poorer for men as they have a lower migraine prevalence. It might be that sex differences that are currently observed in the risk for cardiovascular disease in patients with migraine are based on underpowered evidence. In order to provide clarity, the focus of future research on the risks of migraine should shift more towards men.

In **chapter 8** we assessed sex differences in stroke characteristics in patients with migraine. Slight sex differences in age at onset, etiology and stroke outcome were found, however no differences were observed in cardiovascular risk factors. It is important to emphasize that only information on conventional cardiovascular risk factors was available in this study, whereas we believe that the association between migraine and CVD is likely influenced by other factors. Men with migraine had a higher risk of poor stroke outcome compared to men without migraine, whereas no differences were found in women. Interestingly, previous studies on stroke outcome in patients with migraine only included women, in whom a poorer stroke outcome was mostly found. More studies including patients from both sexes are needed to confirm our findings of a poorer stroke outcome in men with migraine.

Interestingly, there were differences in stroke etiology in men with migraine compared to men without migraine, whereas no such differences were found in women. The TOAST classification stroke subtype 'other determined etiology' was more common in men with migraine compared to men without migraine; which includes non-atherosclerotic vasculopathies, hypercoagulable states, vasculitis, microangiopathies and hematological disorders. This suggests that men with migraine have an underlying vascular pathology that is usually more often associated as 'female type', than men without migraine. Possibly, this could be related to the evidence that men with migraine have a more feminine sex hormone profile with higher estradiol levels, lower testosterone levels and more often present with clinical androgen deficiency symptoms compared to men without migraine.[43–45] Moreover, it has been shown that gender affirming hormonal therapy in male to female transsexuals (MFTs) increase circulating NO levels and NO dependent vasodilation and leads to a similar rate of migraine as in genetic females.[46,47]

Future research should focus on the non-conventional and female-specific risk factors and their possible interaction with sex hormone profiles as well as further investigate the stroke causes that fall under the 'other' and 'undetermined' categories of the TOAST classification in more detail.

In this same population of ischemic stroke patients, only in women, migraine was associated with a younger age at stroke onset. This was confirmed in the group of women with a history of pregnancy-related complications described in **chapter 6**, where not only the hypertensive disorders of pregnancy, but also migraine was associated with a younger age at stroke onset.

Another sex disparity in migraine as a risk factor for CVD was described in this thesis in **chapter 3**. The increased prevalence of cold extremities in migraine patients was exclusively found in women. If cold extremities are considered as a marker for vascular vulnerability, this seems to indicate a higher cardiovascular risk for women.

In this thesis, several findings point towards sex differences in the cardiovascular profile of migraine patients. However, the same limitation occurred in the studies of this thesis as in much of the other migraine literature; the under-inclusion of men. It is important to investigate possible sex differences further, to be able to understand the pathophysiological mechanisms. For instance, there could be common underlying mechanisms shared by men and women with migraine, which are more apparent in women due to their sex hormone profile. The importance of including more men in migraine-oriented research to be able to provide them with substantiated clinical advice regarding their risk of CVD should not be underestimated.

3. MIGRAINE IN COMBINATION WITH FEMALE-SPECIFIC RISK FACTORS

Allthough more research is needed to investigate whether migraine is a female-specific cardiovascular risk factor, current evidence clearly indicates that women with migraine have an increased risk of CVD. Therefore, it is interesting to investigate how migraine acts as part of the female-specific cardiovascular profile in terms of pathophysiology and interaction with other female-specific co-morbidities. With a combination of female risk factors, an additive or even a supra-additive risk of CVD could arise. Reproductive disorders, such as preeclampsia and polycystic ovary syndrome are examples of female specific risk factors for cardiovascular disease with interesting associations with migraine that we have investigated in this thesis.

Women with pregnancy-related complications associated with vascular disease (preeclampsia, HELLP syndrome and placental abruption) have a higher risk of young stroke (onset <50 years) and have an on average 10 years earlier stroke onset compared to women without these complications (**chapter 6**). This new finding is in line with the results of a systematic review that found that hypertensive disorders of pregnancy increase stroke risk and are associated with an earlier onset of type 2 diabetes mellitus and hypertension.[48,49] Although it is possible that these common conventional risk factors play a role, the pathophysiology of preeclampsia itself is under scrutiny for its possible long term effect on the vasculature after pregnancy, for example on coronary artery calcification and endothelial dysfunction.[50–52] We found no differences in ischemic stroke characteristics in women with and without migraine within this group, although this may have been a result of underpowered analyses. A lack of data on female-specific risk factors and the need to include very large groups is a common problem in studies aiming to investigate multiple risk factors simultaneously. The association of preeclampsia and migraine is interesting because of possible overlap in vascular pathophysiology. A history of migraine is associated with an increased risk of hypertensive disorders of pregnancy.[53] Not only do these disorders share similar, though slightly differing clinical characteristics

of headache with visual disturbances, both are associated with pathological changes in the vasculature including altered vasoreactivity and platelet activity and a resulting increased risk of CVD.[53] Similar to migraine, MRI studies show progressive white matter lesions in women with a history of hypertensive pregnancy disorders.[54,55] A higher rate of endothelial dysfunction has been demonstrated by several modalities.[50] Endothelin-1 (ET-1) is shown to be an important player in the clinical manifestations of hypertension, proteinuria and vasospasm observed in preeclampsia and thus could be part of the vascular vulnerability seen in both disorders.[56] Similarly, CGRP levels are altered in pregnancy and are lower in women with preeclampsia.[57] However, in contrast to migraine, preeclampsia is associated with atherosclerotic type vascular risk and, depending on subtype (early or late onset) its pathogenesis may involve defective placentation and genetic maternal factors varying per individual.[58] These differences show that understanding how the pathophysiology of migraine and preeclampsia interacts is complicated. It is hypothesized that hypertensive disorders of pregnancy do not play a causative role in vascular disease but rather act as a stress test for underlying vascular dysfunction which is brought to light by pregnancy. It could be argued that the similar vascular vulnerability that is observed in migraine patients occurs in women prone to hypertensive disorders of pregnancy as well. Further exploration of the possible supra-additive risk for CVD of migraine and preeclampsia could prove valuable for clinical management in these women.

In **chapter 5** we focused on cohort of women with the common hormone disorder PCOS. PCOS is associated with an increased CVD risk factor profile and a hyperandrogenous sex hormone profile. There is of yet only limited and contradicting evidence of a positive association between PCOS and migraine.[59,60] Although our findings describe a different vasodilatory reaction in women with PCOS with migraine, we did not include a control group to appraise differences in women without PCOS. Future research should investigate the epidemiology of migraine in women with PCOS and their associated CVD risk.

A recurrent theme with important clinical implications for female-specific vascular risk management is the use of exogenous hormones; in the form of hormone replacement therapy, gender affirming hormone therapy or combined oral contraceptives (COC). In the case-control study and systematic review presented in **chapter 7** we find confirmation that the use of COC is an additional risk factor for stroke in women with migraine, even in case of low dose estrogen COC. The risk in these women was further increased by smoking, which reinforces the importance to address smoking in women with migraine using COC as a serious modifiable risk factor.

Other female-specific CVD risk factors that have a possible association with migraine but that have remained outside the scope of this thesis are early onset menopause, depression, auto-immune disease and social factors such as domestic violence.[61–63] Whether certain combinations of these factors increase the CVD risk of women with migraine even further, remains to be investigated. For future research it is important to include pregnancy- and hormone related disorders and gender related psychosocial factors in our analysis of sex specific differences in cardiovascular risk for patients with migraine.

4. CLINICAL RECOMMENDATIONS

As we described in **chapter 2**, current clinical recommendations concerning cardiovascular risk reduction for patients with migraine are limited.

Our case control study and meta-analysis in **chapter 7** on the effect of combined oral contraceptives (COC) use in combination with migraine found a substantially increased risk for ischemic stroke in women even for low dose estrogen COC. However, the studies included in the meta-analysis were heterogeneous, few in number and did not distinguish between migraine with and without aura. Despite the limited extent, the literature concerning increased CVD risk for women with migraine has led to some clinical recommendations. The World Health Organization (WHO) and American Congress of Obstetricians and Gynecologists (ACOG) strongly recommends against the use of COCs by women with migraine with aura, classifying the use as an unacceptable health risk.[64,65] For migraine without aura, the WHO also advises against COC use, but only for women with migraine older than 35 years. For younger women, it is advised to make a careful assessment about starting or continuing COC use, weighing benefits against possible harms. The consensus statement by the European Headache Federation (EHF) and the European Society of Contraception and Reproductive Health (ESC) also provides separate recommendations for migraine with and without aura.[66] For migraine with aura COC use is contraindicated, whereas for migraine without aura, substitution by progesterone-only alternatives is advised. For women with migraine with or without aura it is advised to take additional cardiovascular risk factors into consideration. Our case control study showed a supra-additive effect of smoking on ischemic stroke risk in women with migraine using COC. Clinical advice for now should at least focus on the importance of quitting smoking in young women with migraine (with aura) and COC use. Personalized decision making is essential in these cases, as for many women with migraine, the benefits of using COCs (including decreased migraine severity) may outweigh the relatively small absolute risk of cardiovascular complications.

Currently, there is not enough evidence to support preventative pharmacotherapeutic measures for young women with migraine, such as starting antihypertensive or antiplatelet therapy. Although no such recommendations exist, it would be sensible to screen women with migraine, especially those with aura, for cardiovascular risk factors, including hypertension and pregnancy-related complications. Likewise, screening for migraine as cardiovascular risk factor should be part of follow up assessments in women with a history of pregnancy-related complications such as preeclampsia and cardiovascular events. In the Erasmus Medical Center in Rotterdam, this screening is now implemented as part of the follow up care for patients with hypertensive disorders of pregnancy. Moreover, ischemic stroke and migraine should be incorporated in primary care cardiovascular risk management. Because of the detrimental effect on sleep and concurrently increased migraine attack frequency, it might be advisable for women with migraine to combat cold feet by wearing warm socks when going to bed. For that reason, the LUMC headache research group has set up a warm sock campaign for patients with migraine.

There is also limited evidence for recommendations about the use of triptans or the new generation CGRP (receptor) inhibitors in migraine patients with prior CVD. Based on their pharmacological vasoconstrictive effects, the American Academy of Neurology and the Dutch National Health Care Institute (ZIN) consider triptans to be contraindicated in patients with a history of ischemic vascular disease.[67] The Nederlandse Vereniging voor Neurologie states that there is no solid evidence to support contraindication of triptan use after a cardiovascular event. Theoretically, CGRP (receptor) inhibitors could increase risk of vasoconstriction and ischemia, however, no confirmation from long term clinical trials yet exists.[68,69] Erenumab, one of the large CGRP monoclonal antibodies, was associated with adverse events of hypertension, which would be a highly undesirable side effect for women with migraine.[70] In contrast, the small molecule CGRP receptor antagonists called gepants do not seem to cause vasoconstriction and are therefore considered safe for patients with a history of vascular events.[71] It is speculated that women in particular are more prone to be at risk for the cardiovascular complications of CGRP (receptor) blockade. In women, levels of CGRP are higher and female sex hormones further amplify the vasodilatory effects.[57] Moreover, CGRP induced vasodilation of coronary arteries is much more prominent in the most distal portions, which in women is more often the part involved in ischemic pathophysiology.[72] Further research is needed to assess the safety of triptans and CGRP (receptor) inhibitors and their interaction with other female-specific risk factors in migraine patients. There is currently no evidence that suggests that decreasing migraine attack frequency, for example through the use of prophylactic treatment, reduces future CVD risk.[73]

The absolute risk for cardiovascular disease for young women remains low. However, migraine with aura and possibly also without aura should be included as an important risk factor in clinical risk assessment. It is important for health care providers to be aware of migraine as a risk factor for cardiovascular disease and to properly inform their patients.

FUTURE PERSPECTIVES

This thesis was written during a time of growing awareness of the differences in medical science and clinical practice for women. Sex differences in cardiovascular health are being increasingly acknowledged over the past few decades. The field of cardiology has been at the forefront of this movement, with more recognition for heart disease in women as a result. However, women remain significantly underrepresented in clinical trials on cardiovascular disease.[74] Even in fields such as experimental migraine science, the majority of preclinical studies investigating migraine involves male animals.[75] For both research concerning cardiovascular disease and migraine, there remains a vast lack of comparisons between sexes. The first and most important future perspective is to continue to bring the standards of medical science and care for women to the same level as it is for men, which can only be attained by continued prioritization of appraisal of sex differences in future research and education. Whereas for cardiovascular science, this means shifting the focus towards women, for migraine science, it means including more men.

It is necessary to increase awareness among medical professionals about sex differences in cardiovascular disease. Stroke may present itself very differently in women. For example, headache as a symptom of stroke occurs much more often in women than in men.[76] It is important to educate health professionals about the risk of misdiagnosing stroke in patients with a history migraine as another attack, thereby withholding proper clinical care for these patients. Similarly, it is important to educate women themselves about cardiovascular disease, whether they have migraine or not, so that they are able to recognise symptoms, seek proper care and optimize their lifestyles to minimise risk. In this new view of assessing cardiovascular disease, it is important to include migraine as a cardiovascular risk factor, especially in the presence of other (female-specific) risk factors.

An important topic for further research on sex differences is better recognition of cardiovascular risk for women by improving prediction-models used in primary healthcare. Although there is a large amount of cardiovascular risk prediction models for women in the general population, these rarely include female specific predictors.[77] Further exploration of the interaction between migraine and other (female-specific) risk factors, including pregnancy-related complications and sex hormone disorders could provide vital information for cardiovascular risk assessment. Big data and machine learning are emerging methods that can help to interpret vast amounts of data. Our nested case control study on COC use in patients with migraine is an example of routine data collected from different sources and analysed to improve our understanding of risks and additive risks. In the future, the foundation of (personalized) clinical recommendations could advance significantly when collecting, sharing and combining routine data from clinical settings is improved.

Research on the unconventional (female type) vascular disease calls for different techniques to assess the microvasculature and endothelial function, especially in relation to migraine status. Although some of them are used in this thesis, there is still much to learn. Further use of the local thermal hyperaemia (LTH) technique as a non-invasive tool to assess vascular health in migraine patients is promising. A first step could be to compare microvascular function measured with this new technique in women and men with migraine to controls and to investigate possible sex differences. Moreover, MRI studies exploring the heart-brain axis could enable identification of markers for microvascular damage in patients with migraine, focussing on sex differences and differences in migraine subtype.

The foundations of these aims and perspectives have been addressed by the CardiovasculaR hEalthy ageing in Women (CREW) project, which was recently finished and further investigated several new modalities of cardiovascular health, possible sex-specific risk factors and pathophysiology. This research project was a great example of the potential in bringing together experts of different fields, including neurology, cardiology, gynaecology, radiology and epidemiology. Increased interdisciplinary collaboration, such as seen in the novel fields of neuro-obstetrics and gynecardiology, could greatly advance medical care and research. Continued funding of projects similar to CREW, appraising sex differences in cardiovascular

health and the role of migraine is essential. In the best case, elucidating the association between migraine and cardiovascular disease could improve understanding of the complex pathophysiology of migraine and sex disparities in cardiovascular disease and deliver therapeutic targets to minimise the risk for both men and women.

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CHAPTER 10

| Nederlandse samenvatting

In dit proefschrift wordt de rol van migraine als cardiovasculaire risicofactor onderzocht in populaties met een verschillende voorgeschiedenis, zoals een herseninfarct of polycysteus ovariumsyndroom (PCOS). Hierbij ligt de nadruk op vrouwspecifieke factoren en sekseverschillen, en werden zowel conventionele als nieuwe maten voor vasculaire gezondheid onderzocht. Dit proefschrift is geschreven in het kader van het CREW consortium. Het CREW-consortium was een onderzoeksverband van grote medische centra in Nederland in samenwerking met de Nederlandse Hartstichting en het Nederlands Hartinstituut, met als doel om vrouwspecifieke cardiovasculaire pathofysiologie te onderzoeken. Het CREW-MIST project (Cardiovascular risk profile in Women – microvascular status) dat deel uitmaakte van dit onderzoek, richtte zich op migraine in relatie tot herseninfarcten bij jonge vrouwen, met aandacht voor vrouwspecifieke aandoeningen zoals zwangerschaps- en geslachtshormoongerelateerde aandoeningen en het beoordelen van de (micro)vasculaire gezondheid door middel van beeldvorming van het hart en brein en functionele vaatmetingen.

SAMENVATTING

Hoofdstuk 1 bevat een introductie over migraine, de klinische kenmerken, epidemiologie en de associatie met hart- en vaatziekten, waaronder herseninfarcten. De mogelijke mechanismen verantwoordelijk voor deze associatie worden beschreven, met een focus op de rol van vrouwspecifieke componenten.

Hoofdstuk 2 geeft een overzicht van de kennis over migraine en het bijbehorende risico op hart- en vaatziekten ten tijde van de start van dit proefschrift. Het beschrijft hoe het verhoogde risico op hart- en vaatziekten, en herseninfarcten in het bijzonder, het meest consistent wordt beschreven bij vrouwen, vooral met het subtype migraine met aura. De rol van *cortical spreading depolarization* (CSD), genetica, geslachtshormonen en microvasculaire pathologie, waaronder endotheeldysfunctie, worden onderzocht als onderliggende mechanismen voor de associatie tussen migraine en hart- en vaatziekten. De conclusie van dit hoofdstuk is dat migraine met aura moet worden meegewogen in cardiovasculaire risicobeoordeling van vrouwen, ook al blijft het absolute risico op eindpunten zoals een herseninfarct laag. Klinisch advies voor vrouwen met migraine is momenteel beperkt tot leefstijladviezen gericht op stoppen met roken en het herzien van het gebruik van oral anticonceptiva.

Verdere verkenning van de vasculaire component die mogelijk anders is bij migraine patiënten wordt beschreven in **hoofdstuk 3**, waarin een case-control studie gepresenteerd wordt waarin koude ledematen en het effect op slaap worden onderzocht bij mannen en vrouwen met migraine en gezonde controles. Deze studie focust zich op lichaamstemperatuur als maat voor autonome vasculaire regulatie die als mogelijk onderliggend pathofysiologisch mechanisme migraine en hart- en vaatziekten met elkaar verbindt. Vrouwen met migraine rapporteerden meer dan twee keer zo vaak koude ledematen dan vrouwen zonder migraine, terwijl een dergelijk verschil niet werd gevonden bij mannen. Dit duidt mogelijk op een vasculaire kwetsbaarheid bij migraine patiënten die seksespecifiek is. Er werd geen verschil gevonden

tussen migraine met of zonder aura. Bij de vrouwen met migraine waren koude ledematen geassocieerd met slechter in slaap vallen, wat weer kan leiden tot het uitlokken van migraine aanvallen. Verdere studies zijn nodig om te onderzoeken of koude ledematen geassocieerd zijn met een verhoogd cardiovasculair risico voor patiënten met migraine en of dit een marker kan zijn voor cardiovasculaire risico inschatting.

Geslachtshormoonspiegels spelen waarschijnlijk een rol in de geslachtsverschillen die worden gevonden in het verhoogde cardiovasculaire risico bij migraine patiënten. Eerder werd een veranderd patroon van oestradiol spiegels en trigeminale zenuw-gemedieerde microvasculaire reactiviteit gedurende de menstruatiecyclus beschreven bij vrouwen met menstrueel gerelateerde migraine (MRM). Deze resultaten vielen samen met onze bevindingen in **hoofdstuk 4**, waarin een case-control studie wordt gepresenteerd die de verschillen in pijnperceptie bij vrouwen met MRM in twee verschillende stadia van de menstruatiecyclus onderzocht, vergeleken met vrouwen zonder migraine en postmenopauzale vrouwen. Vrouwen zonder migraine rapporteerden een hogere pijnsensatie in het trigeminale dermatoom in de mid-luteale fase in vergelijking met de vroege folliculaire fase. Vergelijkbaar met onze bevindingen betreffende oestradiolspiegels en vasculaire reactiviteit, werden deze cyclische veranderingen niet waargenomen bij vrouwen met MRM. Interessant is dat verschillen in cycliciteit van pijn alleen werden gedetecteerd in het trigeminale dermatoom en niet buiten deze regio. Onze bevindingen suggereren dat de fluctuatie van trigeminale pijn gedurende de cyclus is aangetast bij vrouwen met MRM; wat een aanwijzing kan zijn voor een veranderde trigeminovasculaire gevoeligheid die van belang kan zijn voor ons begrip van de pathofysiologie en de behandelingsrespons bij deze patiënten.

In **hoofdstuk 5** wordt een cross-sectionele studie beschreven in een subgroep van vrouwen met PCOS, waarin de microvasculaire functie bij vrouwen met en zonder migraine wordt vergeleken met behulp van nieuwe niet invasieve technieken. Metingen van de bloedperfusie in de oppervlakkige lagen van de huid tijdens lokale verwarming (lokale thermische hyperemie; LTH) werden gebruikt om de reactiviteit van de microvasculatuur te beoordelen. Metingen werden uitgevoerd met en zonder blokkade van de door de axonreflex geïnduceerde afgifte van neuropeptiden en van het vrijkomen van stikstofmonoxide, twee belangrijke componenten van de vasoregulatoire respons op verwarming. Het aanbrengen van anesthetische EMLA crème resulteerde in minder remming van axonreflex-gemedieerde vasodilatatie bij vrouwen met migraine in vergelijking met vrouwen zonder migraine (86,7%, SD = 26,5 versus 67,9%, SD = 24,2; p = 0,014). Deze bevinding suggereert dat vasoregulatie bij vrouwen met migraine anders reageert op remming van de afgifte van neuropeptide. Echter werden er geen verschillen gevonden in de reactie zonder blokkade. De waargenomen veranderde activiteit van perifere neuropeptiden was bij deze vrouwen niet geassocieerd met de conventionele cardiovasculaire risicoscore (Framingham Risk Score; FRS). Dit suggereert dat de veranderde vasoregulatie bij vrouwen met migraine niet geassocieerd is met het conventionele cardiovasculaire risicofactorprofiel.

Hoofdstuk 6 beschrijft de kenmerken van herseninfarcten en het cardiovasculaire risicoprofiel bij vrouwen met een voorgeschiedenis van zwangerschapsgerelateerde complicaties (pre-eclampsie, HELLP-syndroom en placenta-abruptie). Hoewel er geen verschillen werden gevonden in de etiologie, was de leeftijd ten tijde van het herseninfarct 10 jaar lager en was het risico op een *young stroke* (aanvang <50 jaar) bij deze vrouwen bijna vervijfvoudigd in vergelijking met vrouwen zonder een voorgeschiedenis van zwangerschapscomplicaties. Er werden geen verschillen in de kenmerken van het herseninfarct gevonden tussen vrouwen met en zonder migraine, hoewel de leeftijd bij aanvang 9 jaar lager was bij vrouwen met migraine. Onze resultaten suggereren dat zowel zwangerschapsgerelateerde aandoeningen als migraine moeten worden opgenomen in de vrouwspecifieke cardiovasculaire risicobeoordeling. Of de combinatie van deze factoren leidt tot een nog verder verhoogd risico op hart- en vaatziekten moet nader worden onderzocht.

In **hoofdstuk 7** voerden we een nested case-control studie uit en verwerkten we de resultaten daarvan in een systematische review en meta-analyse over het risico op een herseninfarct bij vrouwen met migraine die gecombineerde orale anticonceptiva (OAC) gebruiken. In de case-control studie was het risico op een herseninfarct aanzienlijk verhoogd bij vrouwen met migraine die OAC gebruikten in vergelijking met vrouwen zonder deze risicofactoren (aOR: 6,83; 95% BI: 3,95-11,68). Het risico werd zelfs nog verder verhoogd door de factor roken (aOR: 30,24; 95% BI: 4,22-610). De systematische review en meta-analyse inclusief de resultaten van onze case-control studie toonden een verhoogd risico op een herseninfarct voor vrouwen die zowel migraine hadden als OAC gebruikten in vergelijking met vrouwen zonder migraine en OAC-gebruik (gepoolde aOR: 4,95; 95%CI: 2,13–11,48). In een subanalyse van vrouwen met migraine die een OAC met een lagere dosis oestrogeen (<50 µg) gebruikten was het risico op een herseninfarct nog steeds verhoogd (gepoolde aOR: 3,14; 95%CI: 1,75–5,62). De risicofactoren migraine en OAC gebruik gezamenlijk resulteerde in een aanzienlijk verhoogd risico op een herseninfarct, zelfs bij gebruik van OAC met een lage dosis oestrogeen. De geïncludeerde studies waren echter beperkt in omvang en heterogeen. Aanvullend onderzoek is daarom nodig om de gepersonaliseerde besluitvorming over het voorschrijven van OAC's bij vrouwen met migraine te verbeteren.

Het verhoogde risico op een een herseninfarct bij patiënten met migraine lijkt sekse-afhankelijk te zijn, maar de onderliggende mechanismen hiervoor blijven onduidelijk. In **hoofdstuk 8** presenteren we een grote prospectieve studie gebaseerd op de multicenter Nederlandse Parelsnoer Instituut CVA database, met als doel om sekseverschillen en de rol van migraine in het cardiovasculair risicoprofiel en kenmerken van herseninfarcten te onderzoeken. We concludeerden dat migraine niet geassocieerd was met sekseverschillen in het cardiovasculair risicoprofiel. Het is belangrijk om op te merken dat in deze analyse alleen rekening werd gehouden met conventionele risicofactoren, en niet met vrouwspecifieke risicofactoren. Bij vrouwen was migraine geassocieerd met een bijna tweevoudig verhoogd risico op *young stroke*. Bij mannen was dit niet het geval, maar was migraine geassocieerd met herseninfarcten van 'andere vastgestelde etiologie' volgens de TOAST-classificatie en met een

slechtere uitkomst volgens de mRS. Onze studie suggereert dat er sekseverschillen zijn in de pathofysiologie achter de associatie tussen migraine en herseninfarcten, die niet gerelateerd zijn aan conventionele cardiovasculaire risicofactoren. Dit benadrukt het belang voor een seksespecifieke cardiovasculaire risico inschatting en de noodzaak om in toekomstige studies te onderzoeken welke factoren verantwoordelijk zijn voor sekseverschillen.

APPENDICES

SUPPLEMENTARY MATERIAL: CHAPTER 3

1. LUMINA BACKGROUND INFORMATION

Dutch migraine patients aged 18-80 years were recruited via nationwide public announcement, advertising in lay press and our research website (www.lumc.nl/hoofdpijn). They were considered eligible after a two-step inclusion process using validated questionnaires via the dedicated Leiden University MIgraine Neuro-Analysis (LUMINA) website. Additionally, patients attending our outpatient headache clinic were invited to participate by a letter. Patients were first asked to fill out a validated web-based screening questionnaire with a sensitivity of 0.93 and specificity of 0.36.[1] Patients who fulfilled the screening criteria, were sent a validated web-based extended migraine questionnaire[2], based on the International Classification of Headache Disorders criteria (previously ICHD-2, now ICHD-3 version) criteria.[3] The specificity of the second questionnaire was 0.95 and sensitivity was 0.45.[2] This questionnaire is accessible for patients via our research website and is described in English in detail by van Oosterhout et al. 2011.[2] We consider the cohort a well-defined web-based cohort. Four percent of subjects were included from our headache outpatient clinic and 87% of the participants were previously diagnosed with migraine by a physician. In addition to questions that were necessary to diagnose migraine accurately, the extended questionnaire also included items on demographic factors, aura and headache characteristics, acute and prophylactic headache medication use, and allodynia. Healthy controls were free of any known neurological or psychiatric disorders and did not have any primary or secondary headaches apart from an occasional episodic tension type headache. Participants unable to use the web-based questionnaires due to lack of the needed internet skills were allowed to fill out the questionnaires on paper.

2. QUESTIONNAIRES AND RATING ON THERMAL DISCOMFORT AND COLD EXTREMITIES (TDCE) AND DIFFICULTIES INITIATING SLEEP (DIS)

To accommodate the participants of this research, the following questionnaires were translated to Dutch. Questionnaire and rating are adapted from Kräuchi et al.[4]

Thermal Discomfort and Cold Extremities (TDCE)

In order to calculate a score, the following two questions were asked:
(A) During the past month, how intensely did you suffer from cold hands?
Answer categories were:1='not at all', 2='a little', 3='quite', 4='extraordinary'.
(B) During the past month, how intensely did you suffer from cold feet?
Answer categories were:1='not at all', 2='a little', 3='quite', 4='extraordinary'.

For categorical analyses TDCE was rated as relevant when the answer to question A or the answer to question B was option 3 or 4.

Difficulties Initiating Sleep (DIS)

(A) During the past month, how often was your sleep onset latency (SOL) longer than 30 min?
Answer categories were:1='never', 2='seldom', 3='1–2 times per week', 4='≥3 times per week'.

(B) During the past month, how long (in minutes) has it usually taken to fall asleep?
Answer: … minutes
(C) During the past month, was it a problem for you to fall asleep?
Answer categories were:1='not at all', 2='a little', 3='quite', 4='extraordinary'.

For categorical analyses, DIS was categorized as relevant when the answer to question A was option 3 or 4 and the answer of question C was option 3 or 4.

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SUPPLEMENTARY MATERIAL: CHAPTER 4

See Supplementary material: Chapter 3, 1. LUMINA background information.

SUPPLEMENTARY MATERIAL: CHAPTER 5

Supplemental Table 1. Additional general, cardiometabolic, gynaecological/obstetric and endocrine characteristics of the total study population, that includes women with and without migraine who were diagnosed with PCOS.

	All subjects (N = 49)	**Women with migraine (N = 23)**	**Women without migraine (N = 26)**	***P-value***
General parameters and intoxications[1]				
Education				0.128
Pre-vocational secondary	9 (18.4%)	5 (21.7%)	4 (15.4%)	
Secondary vocational	10 (20.4%)	2 (8.7%)	8 (30.8%)	
Senior general secondary/ Pre-university	5 (10.2%)	3 (13.0%)	2 (7.7%)	
Higher professional	18 (36.7%)	7 (30.4%)	11 (42.3%)	
University/ PhD/ Postdoc	6 (12.2%)	5 (21.7%)	1 (3.8%)	
Unknown	1 (2.0%)	1 (4.3%)	0 (0%)	
Alcohol use of consumers (units/week)	1.0 (0.3–3.8)	1.3 (0.3–5.0)	0.8 (0.1–1.5)	0.415
Practitioner of a sport[2]	21 (43.8%)	6 (27.3%)	15 (57.7%)	0.045*
Anthropometrics				
Waist (cm)	98.0 (87.3–108.3)	95.0 (86.0–109.0)	100.0 (92.3–109.0)	0.312
Hip (cm)	112.0 (104.0–121.0)	109.0 (101.5–116.3)	116.5 (107.8–123.0)	0.055
Cardiometabolic parameters				
Non-fasting glucose (mmol/L)[3]	5.0 (4.0–5.7)	5.0 (4.0–5.5)	4.9 (4.0–6.6)	0.923
Total cholesterol (mmol/L)[3]	5.2 (4.4–6.1)	5.3 (4.5–6.0)	5.1 (4.4–6.4)	0.886
HDL cholesterol (mmol/L)[3]	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.942
Total cholesterol to HDL ratio[3]	3.6 (2.9–4.6)	3.7 (2.7–4.5)	3.5 (3.1–4.7)	0.728
LDL cholesterol (mmol/L)[3]	3.1 (1.0)	3.0 (1.0)	3.2 (1.0)	0.575
Triglycerides (mmol/L)[3]	1.2 (0.8–1.8)	1.0 (0.7–1.8)	1.3 (1.0–1.9)	0.356
ApoA1 (g/L)[3]	1.8 (0.4)	1.7 (0.3)	1.8 (0.5)	0.393
ApoB (g/L)[3]	0.8 (0.7–1.0)	0.8 (0.7–1.0)	0.8 (0.7–1.0)	0.854
Lp(a) (g/L)[3]	0.09 (0.05–0.2)	0.07 (0.04–0.2)	0.10 (0.06–0.2)	0.623
Gynaecological/obstetric parameters				
Age at menarche (years)[4]	13.0 (12.0–14.0)	13.0 (12.0–14.0)	13.5 (12.3–15.0)	0.093
Number of pregnancies in total	2.0 (1.0–3.0)	2.0 (1.0–4.0)	2.0 (1.0–3.0)	0.310
Number of pregnancies that lasted at least 6 months	2.0 (1.0–2.0)	2.0 (1.0–2.0)	2.0 (1.0–2.0)	0.763

Supplemental Table 1. Continued

	All subjects (N = 49)	Women with migraine (N = 23)	Women without migraine (N = 26)	*P-value*
Endocrine parameters[2]				
LH (U/L)	6.4 (1.8–25.4)	3.1 (1.3–17.9)	11.1 (2.0–25.9)	0.230
FSH (U/L)	8.7 (3.4–38.6)	6.7 (2.7–23.6)	11.6 (3.8–42.6)	0.243
AMH (µg/L)[5]	0.1 (0.01–0.7)	0.08 (0.01–0.7)	0.1 (0.01–0.8)	0.913
Androstenedione (nmol/L)	2.2 (1.6–3.7)	2.2 (1.6–4.1)	2.1 (1.6–2.9)	0.515
DHEA (nmol/L)[6]	8.5 (5.4–11.2)	9.1 (5.1–11.6)	8.3 (5.4–11.0)	0.723
DHEA sulfate (µmol/L)	2.7 (1.3)	2.7 (1.2)	2.6 (1.4)	0.682

Values of all subjects are displayed as means (standard deviation) or medians (interquartile range, $Q_3 - Q_1$), or as numbers (percentage). Differences between participants with and without migraine were tested with Student's T-test for variables with a normal distribution, while the Mann-Whitney U test was used for variables with a skewed distribution and for variables with a non-normal distribution in one group. The Fisher's exact test was used for categorical data.
Abbreviations: AMH, Anti-Müllerian hormone; DHEA, dehydroepiandrosterone; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LH, luteinizing hormone
[1] Self-reported; [2] Data are missing in one migraine patient, that has been excluded from the analyses; [3] Data are missing in one patient without migraine, that has been excluded from the analyses; [4] Data are missing in two patients without migraine, that have been excluded from the analyses; [5] Hormone levels below the detection limit have been considered to be at the detection limit, which is 0.01. This was the case in 6 migraine patients and 8 patients without migraine; [6] Hormone levels below the detection limit have been considered to be at the detection limit, which is 3.6. This was the case in 1 migraine patient and 3 patients without migraine.

SUPPLEMENTAL ANALYSES

CARDIOVASCULAR RISK SCORE IN RELATION TO LTH MEASUREMENTS AND ENDOPAT MEASUREMENTS

Furthermore, we examined the correlation between the FRS and outcome measures of the EndoPAT in 42 participants, including 21 migraine patients. We observed no relation between the natural log-transformed FRS (lnFRS) and the lnRHI scores (Pearson's r = 0.17 (95% CI [-0.14–0.45]; $p = 0.279$ and $R^2 = 0.03$). Similarly, no correlation was found between the lnFRS and the AI@75 (Pearson's r = 0.14 (95% CI [-0.17–0.43]; $p = 0.375$ and $R^2 = 0.02$). Further stratification according to the migraine diagnosis did not result in any significant correlations between the lnFRS and the (transformed) lnRHI or AI@75 scores either (data not shown).

Lastly, we examined associations between the FRS and LTH measurements in 47 participants. To capture the total effect of the LTH response, without blockade of any underlying mechanism, we studied the AUC of the entire LTH response, corrected for its baseline, under control conditions.

No association was found between the lnFRS and the natural log-transformed AUC of the total LTH response under control conditions in all participants (Pearson's r = -0.12 (95% CI [-0.40–0.17]; $p = 0.415$ and $R^2 = 0.01$). Further stratification according to the migraine diagnosis did not result in any significant correlations in patients with or without migraine either (data not shown).

SUPPLEMENTARY MATERIAL: CHAPTER 6

ANALYSES OF BASELINE CHARACTERISTICS AMONG MIGRAINEURS VS NON-MIGRAINEURS

Migraine diagnosis was established using the Migraine for Stroke Screener (MISS). See supplementary material: chapter 7.

Table 1. migraine vs no migraine

	Migraine (n=46)	No migraine (n=107)	P-value
Age at event; median (IQR)	59.0 (18.3)	68.0 (19.5)	0.00
BMI; median (IQR)	26.0 (7.4)	26.1 (6.6)	0.82
Penumbra surface; median (IQR)	12184 (12440)	10021 (14726)	0.65
Infarct core; median (IQR)	1405 (3276)	2207 (6487)	0.39
Penumbra/infarct core index; median (IQR)	0.9 (0.2)	0.8 (0.4)	0.56
Infarctvolume; median (IQR)	10.9 (44.6)	16.6 (36.0)	0.84
Toast classification			0.93
Large vessel disease	14 (30)	26 (24)	
Small vessel / lacunar infarct	7 (15)	19 (18)	
Cardial embolic	8 (17)	20 (19)	
Dissection	3 (7)	1 (1)	
Other/unknown	16 (35)	39 (36)	
mRS, pre-admission ≥ 2	2 (4)	9 (10)	0.51
mRS, 3 months FU ≥ 2	20 (43)	50 (47)	0.64
NIHSS ≥ 5	22 (48)	63 (59)	0.21
EQ5D ≥ 6	28 (61)	71 (66)	0.86
BI ≤ 17	2 (4)	11 (10)	0.35

Data are expressed as n (%) unless otherwise stated

SUPPLEMENTARY MATERIAL: CHAPTER 7

MIGRAINE SCREENER FOR STROKE (MISS) QUESTIONNAIRE

Migraine Screener for Stroke (MISS) is a 5 question reliable screening tool for migraine diagnosis in patients with transient ischemic attack or stroke. The screener was validated through semi-structured telephone interviews with the International Classification of Headache Disorders, second-edition criteria as gold standard. The sensitivity of all questions combined was 0.47 (95% CI 0.31-0.62), the specificity was 0.97 (95% CI 0.93-0.99), the positive predictive value (PPV) was 0.80 (95% CI 0.59-0.93) and the negative predictive value (NPV) was 0.87 (95% CI 0.82-0.92). For assessing migraine with aura, the question about visual disturbances had a good NPV (0.99, 95% CI 0.96-1.00), but a low PPV (0.38, 95% CI 0.24-0.53). To prevent misclassification, especially for the aura symptoms, patients with a positive screener should be interviewed more extensively to confirm the migraine diagnosis.[1]

Question 1. Have you ever had a migraine attack or do you still have migraine attacks?
Never
1–4 times
≥5 times
Unknown

Question 2. Have you ever been diagnosed with migraine by a physician?
Yes
No
Unknown

Question 3. Have you ever had severe headache attacks accompanied by nausea or vomiting?
Never
1–4 times
≥5 times
Unknown

Question 4. Have you ever had severe headache attacks accompanied by hypersensitivity to lights and sounds?
Never
1–4 times
≥5 times
Unknown

Question 5. Have you ever had visual disturbances lasting 5–60 min followed by headache?
Never
1–2 times
≥3 times
Unknown

Table 3. a Screener versus final ICHD-II diagnosis of migraine as gold standard

Screener question	Answer to screener question	Final diagnosis		Test characteristics (95% CI)
		migraine	no migraine	
(1) Self-reported migraine	Yes, ever (n) No, never (n)	43 5 Sens 0.90 (0.77–0.97)	19 149 Spec 0.89 (0.84–0.94)	PPV 0.69 (0.57–0.82) NPV 0.97 (0.93–0.99)
(2) Diagnosed with migraine by a physician	Yes, ever (n) No, never (n)	26 20 Sens 0.57 (0.41–0.71)	8 160 Spec 0.95 (0.91–0.98)	PPV 0.76 (0.59–0.89) NPV 0.89 (0.84–0.94)
(3) Severe headache with nausea	Yes, ever (n) No, never (n)	42 5 Sens 0.89 (0.77–0.97)	17 148 Spec 0.90 (0.85–0.94)	PPV 0.71 (0.58–0.82) NPV 0.97 (0.93–0.99)
(4) Severe headache accompanied by hypersensitivity to light and sound	Yes, ever (n) No, never (n)	44 2 Sens 0.96 (0.85–1.00)	18 149 Spec 0.89 (0.85–0.94)	PPV 0.71 (0.58–0.82) NPV 0.99 (0.95–1.00)
(5) Visual disturbances followed by headache	Yes, ever (n) No, never (n)	28 15 Sens 0.65 (0.49–0.79)	20 144 Spec 0.88 (0.83–0.93)	PPV 0.58 (0.43–0.72) NPV 0.91 (0.85–0.95)
All questions combined	Yes, ever (n) No, never (n)	20 23 Sens 0.47 (0.31–0.62)	5 156 Spec 0.97 (0.93–0.99)	PPV 0.80 (0.59–0.93) NPV 0.87 (0.82–0.92)

Sens = Sensitivity; Spec = specificity; missing data <5%.

b Visual disturbances followed by headache versus final ICHD-II diagnosis of migraine with aura as gold standard

Screener question	Answer to screener question	Final diagnosis		Test characteristics (95% CI)
		migraine with aura	no migraine with aura	
(5) Visual disturbances followed by headache	Yes, ever (n) No, never (n)	18 2 Sens 0.90 (0.68–0.99)	30 157 Spec 0.90 (0.68–0.99)	PPV 0.38 (0.24–0.53) NPV 0.99 (0.96–1.00)

Sens = Sensitivity; Spec = specificity; missing data <7%.

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SUPPLEMENTARY MATERIAL: CHAPTER 8

SEE SUPPLEMENTARY MATERIAL: CHAPTER 7: MISS QUESTIONNAIRE.

In the present study, migraine diagnosis was considered positive when participants answered confirmatively to a combination of the three questions selected to obtain the highest possible predictive value (ever migraine attack, ever severe headache with nausea/vomiting or ever severe headache with photo- and/or photophobia, positive predictive value: 0.80 (0.66-0.90) negative predictive value: 0.96 (0.91-0.98))[5] or in case of migraine diagnosis by physician's report.

ABBREVIATIONS

ACOG	american congress of obstetricians and gynecologists
ADMA	asymmetric dimethylarginine
ASCO	atherosclerosis small-vessel disease cardiac pathology other causes
ATC	anatomical therapeutic chemical
AUC	area under curve
BMI	body mass index
BP	blood pressure
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CI	confidence interval
CGRP	calcitonin gene related peptide
CSD	cortical spreading depolarization
CVD	cardiovascular disease
COC	combined oral contraceptives
COX-1	cyclooxygenase-1
CREW	cardiovascular healthy aging in women
CREW-MIST	cardiovascular healthy aging in women microvascular status
CT	computed tomography
DBF	dermal blood flow
DM	diabetes mellitus
DIS	difficulty initiating sleep
DUST	dutch acute stroke study
EDHF	endothelium derived hyperpolarizing factor
EMLA	eutectic mixture of local anesthetics
EPC	endothelial precursor cells
ET-1	endothelin-1
FAI	free androgen index
FHM	familial hemiplegic migraine
FMD	flow mediated dilation
FRS	framingham risk score
GABA	gamma-aminobutyric acid
GWAS	genome wide association study
HDL	high density lipoprotein
lHR	hazard ratio
HRT	hormone replacement therapy
ICD	internation classification of diseases
ICHD	international classification of headache disorders
ICPC	international classification of primary care
IHS	international headache society
LAA	large artery atherosclerosis
LACI	lacunar infarct

LH	luteinizing hormone
LTH	local thermal hyperaemia
LNMMA	ng-monomethyl-l-arginine
LUMINA	leiden university migraine neuro-analysis
MA	migraine with aura
MI	myocardial infarction
MISS	migraine screener for stroke
MO	migraine without aura
MRI	magnetic resonance imaging
MRM	menstrually related migraine
mRS	modified rankin scale
NIHSS	national institutes of health stroke scale
NO	nitric oxide
NPRS	numeric pain rating scale
OCP	oral contraceptives
OR	odds ratio
PACAP	pituitary adenylate cyclase activating peptide
PACI	partial anterior circulation infarct
PE	preeclampsia
PCOS	polycystic ovary syndrome
PFO	patent foramen ovale
POCI	posterior circulation infarct
PSI-CVA	parelsnoer institute cerebrovascular accident initiative
PVD	peripheral vessel disease
RR	relative risk
RHI	reactive hyperaemia index
RVCL-S	retinal vasculopathy with cerebral leukodystrophy and systemic manifestations
SHBG	sex hormone-binding globulin
STIZON	stichting informatievoorziening voor zorg en onderzoek
SVD	small vessel disease
TACI	total anterior circulation infarct
TIA	transient ischemic attack
TDCE	thermal discomfort and cold extremities
TOAST	trial of org 10172 in acute stroke treatment
TRPV-1	transient receptor potential vanilloid 1
WHO	world health organization
WML	white matter lesions

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CURRICULUM VITAE

Katie Linstra was born on the 13th of February, 1987 in Gouda, the Netherlands. She attended secondary school at the bilingual department of the “Wolfert van Borselen” in Rotterdam. In 2005 she was accepted at University College Utrecht, where she graduated cum laude with a premedical science major. During her bachelor, she participated in an exchange semester at the National University of Singapore in 2007, following courses in Pharmacology, Nanotechnology and Health psychology. Her bachelor thesis research concerned painful diabetic neuropathy. In 2008 she started a master in medicine at the Selective Utrecht Medical Masters (SUMMA) programme at the University Medical Centre in Utrecht. As part of her research traineeship, she investigated a new method of spinal cord stimulation for chronic pain at the department of Neurosurgery, Anaesthesiology and Medical Technology. After obtaining her medical degree in 2012, she worked as a resident (ANIOS) at the Department of Neurology of the Tergooi ziekenhuis in Blaricum. In 2014, she started her PhD research at the Neurology department of the Leiden University Medical Center and at the department of Internal Medicine, Division of Vascular Medicine and Pharmacology at the Erasmus Medical Centre in Rotterdam, under the guidance of prof.dr. M.J.H. Wermer, prof. dr. A. MaassenvandenBrink and prof. dr. G. M. Terwindt. Katie is currently working at a.s.r. as medical advisor.

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