The Metabolic Face of Migraine

Abnormalities in Energy Metabolism, Mitochondrial Functioning, Oxidative Stress and the Therapeutic Potential of Ketone Bodies in Migraine

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Summary

Migraine – a common, complex, and debilitating headache disorder - can be regarded as a conserved (mal)adaptive response pattern that occurs in genetically predisposed individuals with a mismatch between the brain's energy reserve and workload. Given the high prevalence of migraine it seems unlikely that migraine genotypes have not conferred some evolutionary advantage. Technological advances, such as those in neuroimaging and genetics, have enabled the examination of different aspects of (cerebral-) metabolism in migraine patients, while recent complementary animal research has highlighted possible mechanisms in migraine pathophysiology. An increasing amount of evidence – much of it clinical - points towards migraine being a response to cerebral energy deficiency, or oxidative stress levels that exceeding antioxidant capacity. The attack itself might then help to restore brain energy homeostasis and reduce potentially harmful oxidative stress levels.

The current PhD thesis is divided into two major topics: 1.) Metabolic abnormalities in migraine 2.) The potential migraine-protective mechanisms and the efficacy and safety of a potentially novel migraine prophylaxis: ketone bodies (KBs). These two topics are divided into 5 major sections, as presented in chapters 2 and 4.

In the first major section of the thesis, the *Nature Neurology* review discusses the evidence for abnormalities in energy metabolism and mitochondrial functioning in migraine with a particular focus on clinical data, including neuroimaging, biochemical, genetic, and therapeutic studies, and their relation with the abnormal sensory processing and cerebral hyperresponsivity found in migraine patients between attacks. Experimental data is examined to elaborate on potential mechanisms of such metabolic abnormalities with regards to migraine attack generation. Finally, potential treatments targeting cerebral metabolism, such as nutraceuticals, ketone bodies, and dietary interventions are highlighted.

Despite increasing evidence pointing towards the role of mitochondrial functioning, energy metabolism and oxidative stress in migraine pathophysiology, not all previous research has been conclusive and some mitochondrial function / oxidative stress markers have not yet been examined in migraine. To address this insufficiency, alpha-lipoic acid (ALA), total thiols, total plasma antioxidant capacity (TAC), lipid peroxide (PerOx), oxidised LDL (oxLDL), HbA1c and lactate were determined in the serum of 32 higher frequency episodic migraineurs (5-14 migraine days/ months, 19 with aura, 28 females), as described in the second major section of the thesis. It was determined that the majority of patients had abnormally low ALA and lactate levels (87.5% and 72%, respectively). About half (46.9%) of the patients had abnormally high PerOx values, while for thiols and TAC over one third of patients had abnormally low values (31.2% and 37.5%, respectively). 21.9% of patients had abnormally low HbA1c and no one's HbA1c was above 5.6%. The oxLDL levels were normal in all but one patient. This original research study provides further evidence for the role of oxidative stress and altered metabolism in migraine pathophysiology, which might represent a suitable therapeutic target. ALA, being too low in almost 90% of patients, might represent a potential biomarker for migraine. Further research is required to replicate these results, in particular a comparison with a control group.

The increased understanding of migraine metabolism offers exciting novel and likely well-tolerated therapeutic opportunities. The ketogenic diet (KD), a diet that mimics fasting and leads to the elevation of KBs, is a therapeutic intervention targeting cerebral metabolism that has recently shown great promise in the prevention of migraine. KBs are an alternative fuel source for the brain and are hence likely able to circumvent some of the abnormalities in glucose metabolism and transport found in migraine. In addition, recent research has shown that KBs – D-β-hydroxybutyrate (D-BHB) in particular – are more than metabolites: As signalling molecules, they have the potential to positively influence other pathways commonly believed to be part of migraine pathophysiology, including mitochondrial functioning, oxidative stress, cerebral excitability, inflammation, and the gut microbiome. In the third major section of the thesis,

the *Nutrients* review describes the mechanisms by which the presence of KBs, D-BHB in particular, could influence such migraine pathophysiological mechanisms. Common abnormalities in migraine are summarised with a particular focus on clinical data, including phenotypic, biochemical, genetic, and therapeutic studies. Experimental animal studies will be discussed to elaborate on the potential therapeutic mechanisms of elevated KBs in migraine pathophysiology with a particular focus on the actions of D-BHB. In complex diseases such as migraine a therapy that can target multiple possible pathogenic pathways seems advantageous. Further research is needed to determine whether the absence / restriction of dietary carbohydrates, the presence of KBs or both are of primary importance for the migraine protective effects of the KD.

A proof-of-concept-open-label pilot study on ten treatment refractory patients (age range: 25-61 years, 1 male, attack frequency range: 6-24 migraine days/months) was conducted to 1) assess the pharmacokinetics of a one-time dose of various ketogenic substances (L-Lysine, L-Leucine, racemic and D- beta-hydroxybutyrate (βHB) mineral salts) and 2) examine the effect of a one month supplementation with daily 20g racemic βHB, 40g racemic βHB, 10g racemic or 10g D- βHB on migraine days compared to a one month baseline period. As described in the fourth major section of the thesis, it was observed that 10g racemic βHB (n=5) lead to a quick elevation in blood βHB levels (peak 0.62mmol/l after 1 hour, SEM=0.08). The one month of intervention with 20g of racemic βHB per day led to an average reduction of 51% in migraine days compared to baseline could be observed (mean baseline = 16.25 days, SEM= 3.71; mean after βHB= 8 days, SEM= 2.92). This perceived benefit from βHB seemed to coincide with a drop in average peak βHB blood levels from 0.62 mmol/l to 0.3 mmol/l after 1-2 weeks of ingestion. While this heterogeneous patient data from a small sample may not lead to conclusive deductions, they warrant the conduction of a controlled clinical trial to assess the potential efficacy and safety of exogenous ketogenic substances in migraine prevention.

To address the above shortcoming, a randomised, placebo-controlled, double-blind, crossover, single-centre trial was planned and undertaken at the University Hospital of Basel, Switzerland. As discussed in the fifth major section of the thesis, 45 episodic migraineurs (5-14 migraine days/months), with or without aura, aged between 18 and 65 years, were recruited at various headache clinics in Switzerland, Germany, and Austria and via internet announces. After a 4-week baseline period, patients were randomly allocated to one of the two trial arms and received either the βHB mineral salt or placebo for 12 weeks. This was followed by a 4-week washout period, a subsequent second baseline period and finally another 12-week intervention with the alternative treatment. Co-medication with triptans (10 days per months) or analgesics (14 days per months) was permitted. The primary outcome was the mean change from baseline in number of migraine days (meeting ICHD-3 criteria) during the last 4 weeks of intervention compared to placebo. Secondary endpoints included mean changes in headache days of any severity, acute migraine medication use, migraine intensity as well as migraine and headache related disability. In addition to routine laboratory analysis, exploratory outcomes were: genetic profiling and expression analysis, oxidative and nitrosative stress, serum cytokine analysis, as well as blood βHB and glucose analysis (pharmacokinetics). A crossover design was chosen, as it greatly improves statistical power and participation rates, without increasing costs. To our knowledge this is the first controlled trial using βHB salts world-wide. If proven effective and safe, βHB might not only offer a new prophylactic treatment option for migraine patients, but might additionally pave the way for clinical trials assessing its use in other neurological diseases with a metabolic component, such as Alzheimer's Disease.

Abbreviations

AcAc = acetoacetate

AD = Alzheimer's Disease

ADP = adenosine diphosphate

ALA = alpha-lipoic acid

ASIC = acid sending ion channel

ATP = adenosine triphosphate

BBB = blood–brain barrier

BD = 1,3-butanediol

BDH1 = βHB dehydrogenase

βHB = Beta-hydroxybutyrate

CAT = catalase

CGRP = Calcitonin gene-related peptide

CM = chronic migraine

CNS = central nervous system

CoQ10 = coenzyme Q10

COX = cyclooxygenase

CSD = cortical spreading depression

CSF = cerebrospinal fluid

CRF = clinical report form

EM = episodic migraine

EMA = European Medical Association

^{18}FDG-PET = 18-fluorodeoxyglucose-PET

FFA = free fatty acids

FGF21 = fibroblast growth factor 21

FOXA2 = forkhead box transcription factor A2

Foxo3 = forkhead box O3

GABA = gamma-aminobutyric acid

GLUT = glucose transporter

GMP = good medical practice

GST = glutathione-S transferase

GTT = glucose tolerance test

GWAS = genome wide association studies

HbA1c = glycated haemoglobin A1c

HDAC = histone deacetylaes

HMGCL = 3-hydroxy-3-methylglutaryl -CoA lyase

HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA

HMGCS2 = 3-hydroxy-3-methylglutaryl-CoA synthase 2

IHS = International Headache Society

IMP = investigational medicinal product

KB = ketone body

KD = ketogenic diet

KE ketone ester

IHS = international headache society

LL = L-Leucine

LY = L-Lysine

MA = migraine with aura

mAbs = monoclononal antibody

MCT = medium-chain triglyceride

MCT1/2/4 = monocarboxylic acid transporter 1/2/4

MPP+ = 1-methyl-4-phenylpyridinium

Mt DNA= mitochondrial DNA

MO = migraine without aura

MRI = magnetic resonance imaging

mTOR = mechanistic Target of Rapamycin

^{1}H- MRS = proton magnetic resonance spectroscopy

^{31}P- MRS = phosphorus magnetic resonance spectroscopy

Mt = mitochondrial

NAD = nicotinamide adenine dinucleotide

NADH = reduced nicotinamide adenine dinucleotide

NAT2 = N-acetyltransferase 2

NO = nitric oxide

Nrf2 = nuclear factor erythroid 2-related factor 2

OXCT1 (SCOT) = succinyl-CoA:3-ketoacid coenzyme A transferase

oxLDL = oxidised LDL

OXPHOS = oxidative phosphorylation

PACAP = pituitary adenylate cyclase-activating peptide

PerOx = lipid peroxide

PD = Parkinson's disease

PDH = pyruvate dehydrogenase

PET = positron-emission tomography

PPARα = peroxisome proliferator-activated receptor alpha

RCT = randomised controlled trial

RNS = reactive nitrogen species

ROS = reactive oxygen species

SIRT1/3 = member of the sirtuin family 1/3

SLC16A6 = Solute Carrier Family 16 Member 6

SNP = single nucleotide polymorphism

SOD = superoxide dismutase

TAC = total plasma antioxidant capacity

TBI = traumatic brain injury

TCA cycle = tricarboxylic acid (=Krebs) cycle

TRP = transient receptor potential channels

1. Introduction

1.1 Migraine

1.1.1 Overview

Migraine is a complex, debilitating and common headache disorder that affects approximately 17% of women and 8% of men in Europe [1]. In addition to gender, the number of individuals affected by migraine also differs across countries and ethnic groups [2]. According to the WHO Migraine is the eighth most burdensome disease and when considering "years of life lost to the disability" migraine is the first cause of disability in individuals below 50 years of age [3]. With a peak incidence during the most productive years of life, migraine not only causes a huge amount of suffering, but also inflicts a substantial amount of costs on society: approximately €111 billion per year in Europe alone [4], when both estimations of costs of health care-related expenditures, as well as losses due to reduced productivity are included.

Migraine is characterised by recurrent moderate to severe, typically throbbing and unilateral headache attacks that last between 4-72h, which are aggravated by any kind of physical activity and accompanied by either photo-, phono-, or osmophobia, nausea or a combination of these. It is a multifactorial / multigenic disease that develops from the interaction of a genetically predisposed individual in an enabling environment. Migraine can be seen as a spectrum disorder, in which clinical and pathophysiological features may progress over time, with episodic migraine (EM) on one end and chronic migraine (CM) on the other end of the continuum [5,6]. CM, which affects between 1.4 – 2.2 % of the population worldwide [7,8], is defined by 15 or more headache days per month out of which 8 have to meet the criteria for migraines [9,10]. Each year approximately 3 % of EM become chronic [11].

Migraines are more than the headache (ictal) phase, as they are typically accompanied by neurological symptoms during a premonitory phase, which precedes the headache by up to 12 hours and a postdrome phase, which follows the migraine headache and can last hours or days. Most commonly reported symptoms preceding and/or following the attack are fatigue, irritability, cognitive difficulties, mood change, yawning, stiff neck, phonophobia, nausea, change in appetite, food cravings, bloating etc. [12–14]. In one third of migraineurs the headache phase is preceded by a specific clinical syndrome known as aura [15], a phase of visual or motor disturbances that typically occurs up to one hour before the attack itself.

While migraine is a very heterogeneous disorder, it is divided into only two major subgroups, based on the presence (migraine with aura (MA)) or absence (migraine without aura (MO)) of this aura, MA occurs in approximately one third of migraineurs [15].

While the physiological correlate of the aura is likely to be an event called cortical spreading depression (CSD), a slowly propagating wave of neuronal and glial depolarisation, followed by suppressed activation [16,17], the primary migraine pathogenic mechanisms are still largely unknown. Current anti-migraine therapies are far from satisfactory and, apart from the recent addition of Calcitonin gene-related peptide (CGRP) monoclonal antibodies (mAbs), their mechanisms of action are also not completely understood. Three migraine-specific prophylactic agents of the CGRP mAbs class have recently become available: Aimovig, Emgality and Ajovy. Several others of the same class are in development. Anti-CGRP mAbs are injection-based drugs that chronically inhibit pain transmission by blocking the action of the peptide CGRP, which is expressed in the whole body [18]. While phase 3 results are encouraging concerning side-effect wise, the long-term consequences of blocking CGRP's action in humans are not yet known [18] and increasing evidence accumulates to show that there might be an adaptation to it or a

loss of efficacy (International Headache Conference abstracts, 2019). In addition, these drugs are far from a cure. Old, non-migraine-specific prophylactic agents, such as topiramate and Botox produce a 1–2 day reduction compared to placebo in monthly migraine days in patients with CM [19,20]. In comparison, in phase 3 data, the anti-CGRP mAbs resulted in an approximately 1.5–2 day reduction in monthly migraine days in patients with EM and a 2–2.5 day reduction in patients with CM patients [21]. This means on average patients are left with 8-15 days with migraine per months (or more). These results are similar to those for topiramate and Botox, although a subset of patients treated with anti-CGRP mAbs experience a 75–100% reduction in monthly migraine days, some patients administer fewer doses of acute drugs, and some patients report that pain severity is decreased [21]. All in all, even if anti-CGRP mAbs were safe to be taken over years, a large proportion of patients are still left without an efficacious treatment option.

1.1.2 Current understanding of migraine pathophysiology

Originally it was believed that migraine is a vascular disorder, with vasodilation as the major contributor to migraine headache [22]. This pure vascular theory has been 'defeated by facts' [23], as there is substantial evidence today that vasodilation of both extra- and intracerebral blood vessels might only be an epiphenomenon of migraine [23–27]. The current understanding of the origin of the migraine headache is that it results from the activation and sensitization of the trigeminal pain pathway, whose afferents densely innervate the meninges and its associated blood vessels [28–30]. How and where exactly this process is initiated is still a matter of debate. While the CSD underlying the migraine aura might be able to explain the activation of the trigeminal ganglion in MA [31–33], migraine premonitory symptoms which occur up to 12 hours before the headache onset, suggest that the initial trigger happens long before. Furthermore, the existence of an asymptomatic 'silent aura' in MO is controversial and has yet to be demonstrated [34]. In addition, the mechanisms that elicit the CSD itself are still unclear.

Since susceptibility to migraine is determined by genetic factors, it is subject to the forces of natural selection. It seems fairly unlikely that the common gene polymorphisms underlying a condition that affects more than 15% of the population worldwide do or at least did not confer any evolutionary advantage. A migraine-prone nervous system may be, or at least might have been, associated with reproductive or survival advantages [35,36].

Has our environment become inadequate or suboptimal for the conserved adaptive genetic response patterns associated with migraine? One factor that has drastically changed within the last 10'000 years is nutrition. The agricultural revolution ensured that one macro nutrient was constantly available: carbohydrates. In drosophila, depending on genotype, in particular mitochondrial (mt) DNA-haplotype, a suboptimal diet, e.g. containing too many carbohydrates, can lead to reduced mitochondrial function and increased oxidative stress [37]. Individuals with specific mtDNA variations may metabolise carbohydrates differentially, which would have implications for a variety of diseases such as migraine. Already in 1935 migraine has been referred to as a "hypoglycaemic headache" [38]. Despite this early connection between migraine and energy metabolism, for several decades the focus of clinical and basic research has shifted towards (neuro-)vasculature, cerebral excitability and neurotransmission. Within recent years metabolism and mitochondrial (dys-)function have regained interest in the pathophysiology of various neurological diseases, including migraine. An increasing amount of evidence – much of it clinical - points towards migraine being - at least partially - an energy deficit syndrome.

1.1.3 Migraine trigger factors and oxidative stress

Aggravating & trigger factors

When trying to decipher to the pathogenesis of migraines, it makes sense to look at the perceived start of migraine attacks: trigger factors. Two recent systematic reviews [39,40] and a study on 1207 patients [41] have identified the following most common migraine trigger factors with only slight differences in absolute frequency: stress / relaxation thereafter, fasting / skipping a meal, sleep changes (too much or too little), hormonal changes (including menses or oral contraceptives), weather changes (including hypoxia and high altitude), physical exercise (including sexual activity), alcohol, strong odours (especially perfume or cigarette smoke), intense light (especially bright or blue light) and loud noises. The distinction between trigger factors and premonitory symptoms of the migraine attack is not always easy. Fatigue and craving for calorie dense foods such as chocolate (and hence its consumption) are more likely to represent premonitory symptoms [42,43]. The same is probably true for neck pain [44]. Individual triggers seem to have an additive effect [45], with a subsequent attack resulting only once an individual attack generation threshold has been reached. This suggests that trigger factors are acting on common pathways. While for some of the more "metabolic" triggers a direct link to energy homeostasis seems apparent, most of the seemingly unrelated triggers also have a potential common denominator: oxidative stress [46].

Fasting / skipping a meal

Fasting / skipping a meal is not only amongst the most commonly cited migraine triggers [39,40,47], but it can also be used experimentally to elicit migraine attacks in susceptible patients. Blau and Cumings (1966) fasted 12 migraine patients for 19 hours. 9 patients developed an attack subsequently, with blood sugar dropping to 44-65mg/dl in all of them [48]. Increased migraine frequency was observed during Ramadan [49]. Migraine prevalence in type 2 diabetics was found to be proportionally increased with the number of hypoglycaemic episodes [50], however, both type 1 and 2 diabetes appear protective against migraine [51]. In the rat, repeated cerebral hypoglycaemia leads in the rat to impaired oxidative phosphorylation characterized by a decreased mitochondrial membrane potential and ATP levels [52].

Stress (mental or physical)

While low levels of aerobic exercise can be beneficial in migraine prevention [53], more intensive (aerobic) exercise / physical effort is frequently reported as migraine trigger [41,54] and can even be used to experimentally trigger migraine [55]. Many studies have confirmed that prolonged or short-duration high intensity exercise results in increased reactive oxygen species (ROS) production [56,57], which remains elevated during recovery from exercise [58].

Mental stress is a prospectively validated migraine trigger [59]. Similar to physical stress, severe mental or psychological stress was also shown to increase oxidative stress in the central nervous system [60,61]. In mice chronic but mild stress is able to damage the structure of brain mitochondria due to excessive oxidative stress [62] and is associated with changes in energy metabolism [63].

Sleep changes

Sleeping patterns and hence circadian disruptions are known to disturb gene expression of all genes regulated by Clock genes, thereby impairing brain function [64] and other body systems, such as metabolism [65]. In shift-working nurses,–insomnia or shift work disorder is associated with higher prevalence of migraine, chronic headache and medication overuse headache [66]. It is well known that sleep deprivation uses up metabolic reserves. In animal models of chronic sleep deprivation glycogen

stores become depleted, while oxidative stress and inflammation are increased [67,68]. In healthy humans, one night of sleep deprivation was enough to significantly reduce glutathione, ATP, cysteine, and homocysteine levels [69]. Morning cortisol levels were also blunted, which has a negative impact on gluconeogenesis. Irregular or insufficient sleep can thus favour a migraine attack via various metabolic disturbances. The reason why excessive sleep can be migraine generating is less clear, but delaying breakfast and calorie intake could compromise metabolic homeostasis in the brain.

Ovarian hormone changes

During reproductive years, migraine is much more prevalent in women than men [70]. Hormones are also amongst the most frequently reported migraine triggers in women [41], which might be due to falling oestrogen levels before the menses, as seen in menstrual migraine [71]. In women's health, oestrogen plays an important role not only in the oestrous cycle, but also in the brain via neuroprotective and antioxidant modes of action [72]. Physiological levels of 17β-oestradiol are able to preserve mitochondrial function in the face of inhibition of oxidative phosphorylation (OXPHOS), protecting against ATP depletion, mitochondrial membrane potential decline and the generation of ROS in human neuroblastoma cells [73]. 17β-oestradiol is also involved in insulin sensitivity, the regulation of insulin secretion and nutrient homeostasis [74]. Both progesterone and 17β-oestradiol have been shown to regulate cerebral oxidative metabolism in the rat [75] and 17β-oestradiol was shown to modulate mitochondrial calcium flux in the brain stem of the rat [76]. Both sex hormones also increase susceptibility to CSD in rodents [77] and modulate the CSD inhibiting effect of 5-hydroxytryptamine, the serotonin precursor [78].

Oral combined hormonal contraceptives typically tend to increase migraine frequency [79], possibly by increasing oxidative stress levels [80–83]. By contrast, an oestrogen-free contraceptive pill containing only desogestrel improves MA [84].

Alcohol

When it comes to food-related triggers, alcohol is among the most frequently and consistently mentioned substances [41]. Alcohol consumption causes numerous biochemical changes in (and outside) the central nervous system, in which mitochondria are the primary organelles affected [85,86]. The metabolism of ethanol by alcohol dehydrogenase or cytochrome P450-2E1 generates ROS and reactive nitrosative species (RNS) and decreases antioxidant activity, especially superoxide dismutase 2 (SOD2) and glutathione [85,87,88]. Alcohol administration to rodents decreased mitochondrial complex I, III and IV activities, Na(+)/K(+)-ATPase activity; in addition, SOD2 mRNA and protein expression was decreased [86,88]. The latter two effects have specifically been linked to migraine. Furthermore, hypoglycaemia typically develops 6-24 h after a moderate or heavy intake of ethanol in a person who has had an insufficient intake of food [89] and this decreased glucose metabolism is particularly affecting the human brain [90]. Alcohol-induced hypoxia and increases of free iron in the cell promote further ROS generation [91]. Increased free iron was reported in the periaqueductal grey of migraine patients [92], a brain area that is associated with migraine attack generation [93].

Sensory triggers

The way the migraine brain responds to stimuli is so peculiar that for some researchers migraine constitutes primarily a disorder of sensory processing [94]. The senses do not only play a role in premonitory symptoms [39,43], but olfactory, visual and auditory triggers are also among frequently reported migraine triggers [39–41]. While it is not certain whether sensory trigger factors are attack

generators or resulting from stimulus hypersensitivity due to the premonitory phase [95], intense sensory stimulation can be linked to increased oxidative stress.

Odorant inhalation modulates physiological pathways and odour compounds can influence stress biomarkers and oxidative stress levels [96]. Perfumes contain a number of chemicals among which phthalates and degraded limonene are susceptible to induce oxidative stress, mitochondrial dysfunction, and eventually apoptosis [97–100] Cigarette smoke was shown to increase markers of oxidative stress in healthy adults after a single exposure [101].

Bright light, especially blue light, another commonly reported sensory trigger, is known to increase oxidative stress in the retina [102], but also in other tissues, where it can damage mitochondria [103] and leads to significant reduction of intracellular glutathione and in anti-oxidant status [104]. Experimentally, however, light failed to trigger an attack in susceptible patients with MA [105], and comparable studies are lacking in MO.

Similar to other known sensory triggers, loud noises were shown to increase oxidative stress [106,107].

Atmospheric conditions

The exact connection between weather and migraine attack generation is unclear. However, there are several potential mechanisms by which weather changes could be involved in migraine pathophysiology, such as extreme temperatures, hypoxia and increased air pollutants.

Extreme hot or cold conditions stress the entire body and extra energy is needed to maintain a constant body temperature. In addition, sunlight irradiance influences sensory processing in migraine [108].

Low atmospheric pressure per se is not thought to trigger migraine [109], unless accompanied by other factors. Such other factors could be particles with microorganisms and minerals like iron contained for instance in Saharan dust, which activates the trigeminovascular system but also increases blood nitrate and nitrite in experimental animals [110]. Another potential link between weather and migraine is air oxygen content and consequently hypoxia. Migraine prevalence is increased in high-altitude populations [111]. Experimental hypoxia is able to trigger migraine headache [43,112], but much less so migraine aura [112]. It can even trigger migraine-like headaches in most healthy subjects [113]. Hypoxia was shown to increase oxidative stress and impair mitochondrial functioning in the mouse [114].

In summary, most reported migraine trigger factors have some link to oxidative stress. The publications in the main chapters of this thesis will discuss further metabolic abnormalities in migraine, including detailed tables summarising the research studies (see *Nature Neurology* review chapter 2.1).

1.2 Ketosis and (endogenous) ketone bodies

1.2.1 Overview

Ketone bodies (KBs) are produced by the liver and used peripherally as an energy source when glucose is not readily available in times of fasting or a low-carbohydrate, medium-protein diet (known as ketogenic diet; KD). The state of increased KBs (>0.5 mmol/l) in the blood is called ketosis. Beta-hydroxybutyrate (βHB), also known as beta-hydroxybutyric acid, 3-hydroxybutyric acid or 3-hydroxybutyrate, is an endogenous metabolite with the formula $CH_3CH(OH)CH_2CO_2H$. It is a beta hydroxy acid and a keto acid. It is a chiral compound having two enantiomers, D-3-hydroxybutyric acid and L-3-hydroxybutyric acid. Together with acetoacetate (AcAc) βHB is one of the main KBs, while acetone is the third, least abundant and metabolically least important KB. βHB is water soluble, absorbed in the gastrointestinal tract, and able to cross the blood–brain barrier (BBB) [115]. The D- form

of βHB is believed to be the physiologically more important isomer [116–118], but isoenzymes for the utilization of both isomers (D- and L-βHB) are expressed in various tissues from early gestational age [118].

The normal blood concentration of KBs in healthy adults eating a standard Western diet is very low, typically less or equal to 0.2 mmol/l, compared to glucose (≅ 4-5 mmol/l) [119]. KB levels increase up to over 20-fold during fasting, a KD or very prolonged exercise (4-8 mmol/l). For example, after an overnight fast, KBs provide for merely 2-6% of the body's energy requirements, while they supply 30-40% of the energy needs after a 3-day fast. This is when KBs become essential for the survival of the organism because unlike most other tissues, the brain cannot utilize fatty acids for energy when blood glucose levels become compromised [116,120]. In this case, KBs supply the brain with an alternative source of energy, amounting to nearly 2/3 of the brain's energy needs during periods of prolonged fasting and starvation [120,121]. Higher KB levels are also found in neonates and pregnant women [122]. In the neonate, normal βHB functioning is essential for survival [123]. The KB ratio, defined as the ratio of circulating βHB to AcAc, is approximately 1 following a meal, but this rises to nearly 6 after prolonged fasting [116,124].

The KD was developed about 100 years ago after the observation that prolonged fasting has anticonvulsive effects [125]. With its high fat, low carbohydrate and moderate protein content it simulates the metabolic effects of starvation. Within recent years the KD has received new interest, in particular since KBs might be beneficial for a variety of neurological disorders via various different mechanisms [126–128], including improved energy metabolism. Elevated KB levels have been shown to be well tolerated for extended periods of time (up to several years [129–142]). Recently, some case studies [132,143–145] and a first short proof of concept [146] study have demonstrated a reduction in migraine attack frequency, severity and use of acute anti-migraine medication during ketosis- with effects sizes ranging from total absence of attacks [143] to a reduction to 1/5th of the run-in period [146]. In addition, preliminary evidence suggests that the protective effect may outlast the duration of ketosis [143,144,146]. This might be a result of longer-lasting gene-expression changes [126,147].

1.2.2 Sources

In humans, βHB is synthesized in the liver from AcAc, the first KB produced in the fasting state. The biosynthesis is catalysed by the enzyme βHB dehydrogenase (see figure 1). AcAc is a product of fat oxidation.

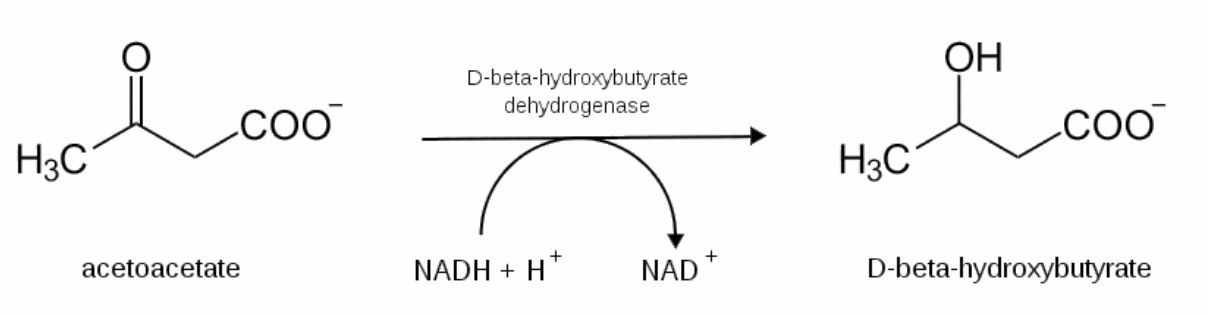

Figure 1. Synthesis of beta-hydroxybutyrate from acetoacetate.

There are no direct natural sources of βHB as KBs are not typically present in foods, unless one was to eat an animal in ketosis (such as a starving cow). Medium-chain triglycerides (MCTs) can be considered an indirect source for βHB. MCTs are triglycerides whose fatty acids have an aliphatic tail of 6–12 carbon

atoms. Rich natural sources of MCTs include palm kernel oil and coconut oil and approximately 10-20% of the fatty acids in milk from horses, cows, sheep, and goats are medium chain fatty acids. MCTs passively diffuse from the gastrointestinal tract and are metabolised in the liver to βHB irrespective of blood glucose levels. The 8 carbon MCTs are particularly ketogenic.

Another indirect natural source of βHB are the two ketogenic amino acids L-Leucine and L-Lysine. Via a number of steps, unused ketogenic amino acids are metabolised into KBs and are hence also able to raise βHB levels if the given dose exceeds metabolic demand [148,149]. High lysine foods include beef, cheese, turkey, chicken, pork, soy, fish, shrimp, shellfish, nuts, seeds, eggs, beans, and lentils. Similarly, foods high in leucine include cheese, soybeans, beef, chicken, pork, nuts, seeds, fish, seafood, and beans.

1.2.3 Biosynthesis

As mentioned above, KBs are small lipid-derived molecules that serve as a circulating energy source for tissues in times of fasting or prolonged exercise. Fatty acids in adipose tissue contain over 80% of the human body's stored energy [117]. In brief, during fasting, muscle and liver stores of glycogen are depleted first. Then, fatty acids are mobilized from adipocytes and transported to the liver for conversion to KBs. Most KB synthesis occurs in the liver [117], nevertheless smaller amounts can be produced in other tissues, such as the brain [149,150] through expression of ketogenic enzymes [151,152] or reversal of the ketolysis pathway [153,154].

In healthy adults, the liver is capable of producing up to 185 g of KBs per day [155]. In hepatic ketogenesis (Figure 2), fatty acids are first metabolized to acetyl-CoA via mitochondrial β-oxidation. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) condenses acetyl-CoA with acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). 3-hydroxy-3-methylglutaryl -CoA lyase (HMGCL) then liberates AcAc from HMG-CoA (Figure 2). HMGCS2 is expressed mostly in the liver, but is also highly expressed in neonatal rat intestine [156] and to a lesser extent in muscle, kidney and brain of neonates and adults [157]. HMGCL is expressed in most tissues [158]. AcAc is the common precursor of the other two circulating KBs, acetone and βHB. Most AcAc is metabolized by βHB dehydrogenase (BDH1) to βHB. βHB is the most abundant circulating KB and is less likely to spontaneously degrade into acetone than AcAc. The D- form of βHB is believed to be the physiologically more important isomer [116–118], but isoenzymes for the utilization of both the D- and L- isomers of βHB are expressed from early gestational age in various tissues [118]. The L-form of βHB is a product of β-oxidation, while the D-form is the product of KB synthesis through HMG CoA [119].

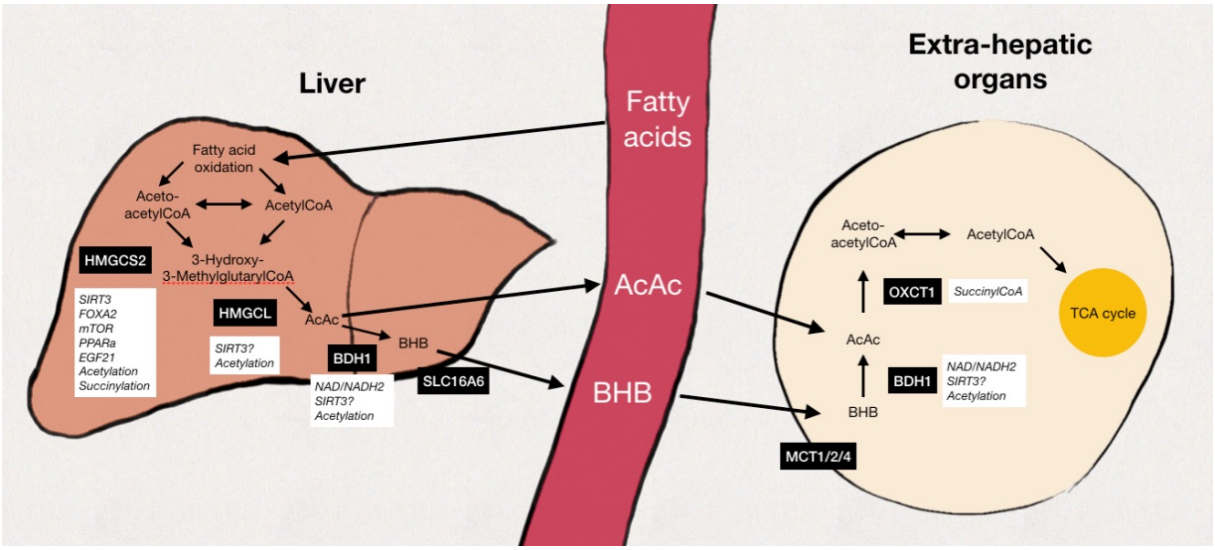

Figure 2: Beta-hydroxybutyrate (βHB) synthesis includes the following steps: β-oxidation of fatty acids to acetyl CoA, formation of acetoacetyl CoA, conversion of acetoacetyl CoA to 3-hydroxy-3-methylglutaryl CoA (HMG CoA) and then to acetoacetate (AcAc) and finally the reduction of AcAc to βHB. βHB is distributed via the circulation to metabolically active tissues, such as muscle or brain,where it is converted back into AcAc by the same enzyme as in the synthetic pathway, but from there, the pathway of KB utilization diverges. Succinyl-CoA donates its CoA to AcAc to form acetoacetyl-CoA, a reaction catalyzed by succinyl-CoA:3-ketoacid coenzyme A transferase (OXCT1, also known as SCOT) in most tissues. Acetoacetyl-CoA is then converted to two acetyl-CoA and fed into the TCA cycle for oxidation and ATP production.

AcAc = acetoacetate; BDH1 = βHB dehydrogenase; FOXA2 = forkhead box transcription factor A2; HMGCL = 3-hydroxy-3-methylglutaryl -CoA lyase; HMGCS2 = 3-hydroxy-3-methylglutaryl-CoA synthase 2; MCT1/2/4 = monocarboxylic acid transporter 1/2/4; mTOR = mechanistic Target of Rapamycin; NAD = nicotinamide adenine dinucleotide; NADH = reduced nicotinamide adenine dinucleotide; OXCT1 (SCOT) = succinyl-CoA:3-ketoacid coenzyme A transferase; PPARα = peroxisome proliferator-activated receptor alpha; SLC16A6 = Solute Carrier Family 16 Member 6; SIRT3 = member of the sirtuin family 3; TCA cycle = tricarboxylic acid (=Krebs) cycle;

Adapted from Newman & Verdin 2014 [159].

In humans, basal serum levels of βHB are in the low micromolar range (typically below 0.2mmol/l), but begin to rise after 12–16 hours of fasting, reaching 1–2 mmol/l after 2-3 days of fasting [159], and 6–8 mmol/l with prolonged starvation [160]. KB levels above 2 mmol/l are also reached with a KD that is almost devoid of carbohydrates and moderate in protein [161]. Children produce and utilize βHB more efficiently than adults, a capability crucial in the days immediately after birth when the brain depends on KBs as an energy source, and serum levels can reach 2–3 mmol/l [160]. At the other end of life, the elderly generate KBs after a ketogenic meal or a fast to the same extent as younger adults [162,163].

1.2.4 Metabolism

Once synthesized from AcAc or absorbed from the gastrointestinal tract, βHB is distributed via the circulation to metabolically active tissues, such as muscle or brain, where it is used as a glucose-sparing energy source [164]. Circulating KBs are mostly taken up by extrahepatic tissues, but 10–20% of KBs may be lost in the urine or lung [164,165]. The rate of KB utilization is proportional to their circulating levels [166–168].

Once taken up by the target tissue, βHB is converted back into AcAc by the same enzyme as in the synthetic pathway, but from there, the pathway of KB utilization diverges (see Figure 2). Succinyl-CoA donates its CoA to AcAc to form acetoacetyl-CoA, a reaction catalysed by succinyl-CoA:3-ketoacid coenzyme A transferase (OXCT1, also known as SCOT) in most tissues. This reaction bypasses the essentially irreversible reaction catalysed by HMG-CoA synthase. The differing enzymatic routes of

synthesis and utilization prevent a futile cycle of βHB synthesis and utilization in the liver since OXCT1 is not expressed in the liver [169]. Acetoacetyl-CoA can then be converted to two acetyl-CoA and fed into the TCA cycle for oxidation and ATP production [170].

KBs are able to produce more energy in comparison to glucose because of the metabolic effects of ketosis—the high chemical potential of βHB leads to an increase in the ΔG0 of ATP hydrolysis [119]. A further point to underline is that glucose levels, even though reduced, remain within physiological levels during either fasting or the KD (Table 1). This is because glucose can be produced endogenously from lactate (Cori cycle), glucogenic amino acids and from glycerol liberated via lysis from triglycerides [171]. Insulin levels also remain within the physiological range.

Table 1. Blood levels during a standard Western (higher carb) diet, a ketogenic diet and diabetic ketoacidosis.

Blood levels	*Standard (higher carb) diet)*	*Ketogenic diet*	*Diabetic ketoacidosis*
Fasting glucose (mg/dl)	80-120	65-95	>300
Insulin (uU/l)	6-23	< 10	0
BHB (mmol/l)	0.1	2-8	>25
pH	7.4	7.4	>7.3

1.2.5 Regulation

The rate-limiting step of KB synthesis is the condensation of acetyl-CoA and acetoacetyl-CoA into HMG-CoA by mitochondrial HMGCS2 [172]. HMGCS2, and therefore production of KBs, is transcriptionally regulated at least two nutrient-sensitive pathways. The first involves the forkhead box transcription factor A2 (FOXA2), which binds to the HMGCS2 promoter and activates transcription [173]. FOXA2 itself is regulated by two hormonal signals: insulin signalling leads to inactivation of FOXA2 via phosphorylation and nuclear export [174], while glucagon activates FOXA2 via p300 acetylation [175]. FOXA2 deacetylation is controlled by yet another nutrient-responsive enzyme, member of the sirtuin family 1 (SIRT1), which works in cooperation with class I or II histone deacetylaes (HDACs) [175]. The second pathway of HMGCS2 transcriptional regulation involves the mechanistic Target of Rapamycin C1 (mTORC1), peroxisome proliferator-activated receptor alpha (PPARα), and lastly fibroblast growth factor 21 (FGF21) [170,176,177]. Both PPARα and its target gene FGF21 are strongly up-regulated in the liver during or KD, and mice lacking either one have reduced ketogenesis [176]. The mTORC1 complex suppresses PPARα, hence inhibition of mTORC1 is required for the induction of PPARα [177], and in turn PPARα is required to induce FGF21 [176].

The activity of HMGCS2 is also post-translationally regulated by succinylation and acetylation. HMGCS2 is deacetylated and activated by the primary mitochondrial deacetylase SIRT3 [178]. SIRT3 regulates many pathways involved in fasting metabolism, and mice lacking SIRT3 have reduced levels of βHB during fasting [178]. Of note, all of the enzymes involved in the generation of KBs from lipids are acetylated, many of them heavily, and contain at least one site for SIRT3 deacetylation [179,180]. Similar to acetylation, succinylation of HMGCS2 reduces its activity [181]. The mechanisms that drive succinylation are still unknown.

In contrast, the interconversion of AcAc and βHB by BDH1 appears to be readily reversible and is regulated primarily by the ratios of substrates and cofactors (NAD/NADH) [170]. BDH1 contains several

SIRT3-regulated acetylation sites, though their functional significance is not yet known [179,180]. Little is known about OXCT1 regulation, but its activity may be inhibited by tyrosine nitration [182].

1.2.6 Transport

βHB transport is relatively less well understood than its synthesis and utilization. A small, polar molecule, βHB is readily soluble in water and blood [117]. Several monocarboxylic acid transporters (MCT1, MCT2 and MCT4) carry it into cells, such as neurons, mitochondria and across the BBB (see figure 4) [183].

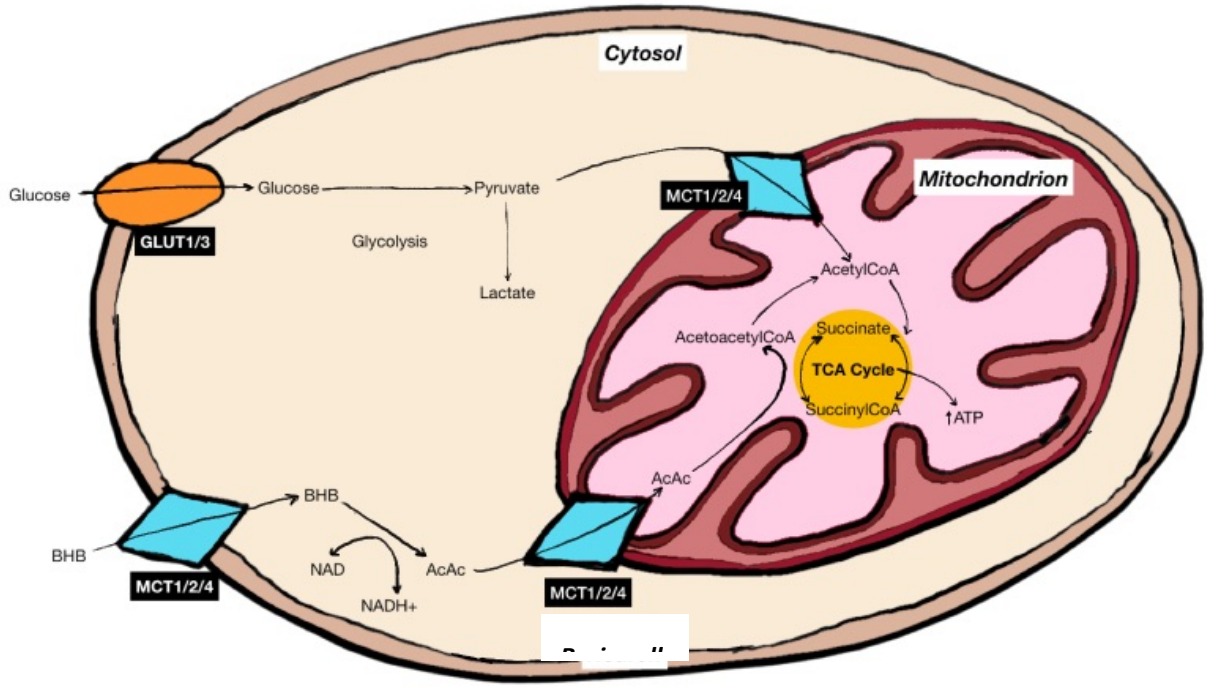

Figure 3: Glucose and the ketone bodies, beta- hydroxybutyrate (βHB) and acetoacetate (AcAc), enter brain cells via different plasma membrane transporters, namely, glucose transporter 3 (GLUT1/3) and monocarboxylate transporter 1/2/4 (MCT1/2/4), respectively.

AcAc = acetoacetate; ATP = adenosine triphosphate; βHB = Beta-hydroxybutyrate, GLUT1/3 = glucose transporter1/3; NAD = nicotinamide adenine dinucleotide; NADH = reduced nicotinamide adenine dinucleotide; MCT1/2/4 = monocarboxylic acid transporter 1/2/4; TCA cycle = tricarboxylic acid (=Krebs) cycle

Adapted from [141].

Up-regulation of MCT1 in particular is associated with high utilization of KB in the neonatal period and on a KD [184]. Recently, the monocarboxylate transporter of the Solute Carrier Family 16 Member 6 (SLC16A6) was identified as the key transporter for exporting βHB from the liver [185].

1.2.7 Evolution

The use of βHB as a fasting energy source is evolutionarily ancient. Many species of bacteria synthesize polymers of βHB (poly βHB) to store energy [160]. A complete set of ancestral βHB biosynthetic enzymes, from HMG-CoA synthase through βHB dehydrogenase, emerged early in eukarya and can even be found in plants. As these cytoplasmic enzymes are not known to participate in ketogenesis, this conservation likely reflects important roles in cholesterol biosynthesis. Specialization for KB metabolism, along with

mitochondria- and tissue-localisation, emerged more recently and gradually. Mitochondrial HMGCS2 was the latest enzyme involved in KB metabolism to diverge from its cytoplasmic counterpart, and is conserved throughout amniota (including birds and humans) [186].

1.2.8 Pathology

It is important to emphasize that ketosis is a physiological mechanism and must be differentiated from the pathological ketoacidosis seen in type 1 diabetes [187]. In physiological ketosis (which occurs during KDs or calorie restriction), ketonemia reaches maximum levels of 7-8 mmol/l (it does not go higher precisely because the central nervous system (CNS) and other tissues efficiently use these molecules for energy in place of glucose) and with no change in pH, whereas in uncontrolled diabetic ketoacidosis it can exceed 20 mmol/l [188] (Table 1). Diabetic ketoacidosis arises because of a lack of insulin in the body. The lack of insulin and corresponding elevation of glucagon leads to increased release of glucose by the liver from glycogen via glycogenolysis and also through gluconeogenesis. This process is normally suppressed by insulin. The absence of insulin also leads to the release of free fatty acids (FFA) from adipose tissue (lipolysis), which are converted through the aforementioned process of beta oxidation into KBs (AcAc, βHB and acetone). The body is initially able to buffer the change with the bicarbonate buffering system, but this system is quickly overwhelmed [189]. Since both AcAc and βHB are acidic the pH of the blood drops, when levels of these KBs are too high (i.e. over 8 mmol/l), resulting in ketoacidosis. Ketoacidosis is known to occur in untreated type I diabetes, under certain circumstances in some cases of type II diabetes and also in alcoholics after prolonged binge-drinking without intake of sufficient carbohydrates. Ketoacidosis does not happen in healthy individuals on a fast or KD, or when using currently existing exogenous βHB supplements, where the highest achieved KB elevation is still within low physiological ranges.

There are also patients with hereditary deficiencies of the enzymes of KB synthesis or degradation. These patients tend to be asymptomatic between episodes, but when fasting severe disturbances of energy metabolism can arise. Patients with deficiencies of ketolytic enzymes have episodes of ketoacidosis, whereas patients with hereditary deficiencies of ketogenesis have episodes of hypoketotic hypoglycaemia [190]. Fortunately, these deficiencies are rare.

1.2.9 Mechanisms of endogenous ketosis (via KD, fasting or calorie restriction)

With regards to mechanisms of elevated βHB, most evidence still comes from KD animal models or cellular studies. While the unique effects of βHB may help explain the therapeutic benefit of fasting, calorie restriction, low-carbohydrate and ketogenic diets, teasing apart the specific role of βHB and the effects of a KD is a challenging task. KDs inextricably combine reduced carbohydrate consumption, reduced glucose utilization, reduced insulin signalling, dependence on beta-oxidation of lipids for energy, and increased glucagon signalling, along with increased KB levels. Exogenous supplementation of βHB with unchanged carbohydrate intake has recently gained increased research interest and is necessary for a specific manipulation of KB levels only, outside the confines of a KD.

In the following, the mechanisms of endogenously raised βHB levels due to the aforementioned dietary changes will be summarized briefly. Afterwards, a more detailed summary of existing studies on exogenous elevation of βHB with unchanged carbohydrate intake will be given.

Animal and cellular studies suggest that ketosis is likely to have many complicated effects. The following potentially disease- modifying mechanisms of elevated βHB have been shown:

- KBs provide an alternative and more efficient energy substrate for the tricarboxylic acid cycle (Kerbs cycle), which in turn leads to increased ATP production per O_2 consumed [191–194]. This could hence mitigate any cerebral energy deficit.
- In the rat, a KD leads to increased mitochondrial biogenesis [195].
- Compared to glucose, KBs generate lower levels of oxidative stress in combination with a bigger cellular energy output and antioxidant capacity [194]. In turn there is decreased oxidative damage otherwise caused by various kinds of metabolic stressors.
- KD has been shown to lead to a marked upregulation of both glucose transporters (GLUT-1) and MCT [196], enhancing available energy to the brain.
- Neural excitability depends, at least in part, also on energy metabolism [197,198]. A KD has been shown to activate inward rectifying potassium channels (metabolically sensitive K(ATP) channels) and in turn to stabilize central neural excitability [147,198,199].
- Higher synthesis of inhibitory neurotransmitter GABA and reduction of neuronal firing in GABAergic neurons have also been demonstrated in response to KBs, with the extent of slowing being greater in faster-firing neurons [198].
- Another effect of KBs on neural excitability seems to be mediated by an inhibition of glutamate transport and hence excitatory synaptic transmission [200].
- Expression of markers of mTOR pathway activation (pS6 and pAkt) was reduced in the hippocampus and the liver of rats fed a KD, suggesting an inhibition of mTOR [201].
- Both short- and long-term treatment with a MCT-enriched KD resulted in significant reduction in the velocity of cortical CSD in young rats [131].
- Furthermore, a reduction in pain and inflammation in rats fed the KD has been demonstrated [202–204].

1.3 Exogenous ketone bodies

1.3.1 Overview

Direct exogenous sources of βHB of chemical origin have recently become available, mostly in mineral salt (sodium, calcium, potassium and magnesium βHB) or ester form. They are produced and sold in several production sites around the world. The biggest sales market exists in the United States where they are sold as a sport supplement. Production sites, available products and formulation, as well as application patents are steadily increasing and are the topic of excessive research in distinguished laboratories all around the world (e.g. at the University of Oxford, California and Florida). KB supplements have originally been developed for the US army and navy, where available products have been used for over 10 years [119]. For example, D- βHB has been used as a replacement for the potentially toxic D-L-lactate in Ringer's solution, a fluid resuscitation [205–207].

In the MigraKet trial – a vital part of this PhD thesis – mineral βHB salts were used as the investigational medicinal product (IMP). βHB-sodium-salt (Na-βHB) has the formula C4H7NaO3, βHB-calcium-salt (Ca-βHB) has the formula C8H14CaO6 (see Figure 4), βHB-potassium-salt (K-βHB) has the formula C4H7NaO3 and βHB-magnesium-salt-trihydrate (Mg-βHB) with the formula C8H14MgO6·*3H2O (see Figure 4). In the body, these molecules are dissolved into the cations Na+, Ca2+, K+ or Mg2+ respectively and βHB.

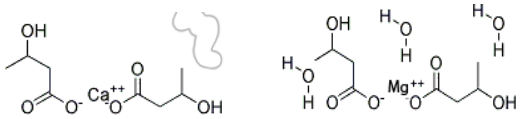

Figure 4 . Structure of beta-hydroxybutyrate -calcium-salt and beta-hydroxybutyrate -magnesium-salt-trihydrate.

A strict KD that leads to KB levels high enough to combat some of the neurological diseases might not provide a feasible long-term solution for many patients, because it is difficult to implement in an ambulatory setting and patient adherence may be limited. In addition, there might be negative long-term consequences with a concomitant drop in vitamins, minerals and other plant based nutrient uptake in a less well formulated KD.

Dietary supplementation with exogenous KBs, such as βHB salts[138,208,209] could not only be much more feasible than KD in larger patient populations, but also avoid the potentially negative consequences of a none-well formulated KD, with drastically reduced vegetable intake as well as increased highly processed protein, dairy and (trans-/ polyunsaturated-) fats, all of which could lead to mineral and vitamin deficiency as well as increased inflammation.

1.3.2 Uptake and transport

Mineral- βHB is dissolved into the mineral cation and βHB once ingested. As a small polar molecule, βHB is readily soluble in water and blood, absorbed in the gastrointestinal tract, and able to cross the BBB [115]. HMG-CoA synthase and other enzymes necessary for ketogenesis are present in the intestine and are highly expressed during the suckling period and are also present in the adult in smaller amounts [156]. Hence, uptake of exogenous βHB via the intestinal tract into the blood stream is a natural phenomenon. Indeed, various studies have demonstrated that orally administered βHB is absorbed and able to significantly elevate blood βHB [115,138,208,210]. For example, βHB was orally administered in a sodium DL-βHB -salt form at 1g/kg per day in two toddlers [115]. This led to increased blood levels comparable to a 16-24 hour fast (1.5- 2.5 mmol/l).

Various MCTs, including MCT1 and MCT2, carry βHB across the BBB [37]. The aforementioned study by Plecko et al. showed that exogenous βHB in mineral salt form also crosses the BBB and can be found in the cerebrospinal fluid (CSF) in a substantial amount, leading to levels in the comparable to a 24-40h fast after a single dose of 1g/kg (0.2 - 0.7 mmol/l).

During hyperketonemia, the rate-limiting step for KB utilization is their transport into brain, with the utilization rate increasing nearly proportionally with plasma KB concentration [211]. A small peak level of only 0.48 mmol/l with a consistent average elevation of 0.29 mmol/l of plasma KB levels over the

duration of 1 months in 8 healthy volunteers was estimated to contribute up to 9% of brain energy metabolism [212].

1.3.3 Metabolism

The metabolism of exogenous mineral-βHB is identical to the endogenous pathways described above. In solution (i.e. the stomach) mineral-βHB dissolves into βHB and the mineral cation(s) and is subsequently absorbed from the gastrointestinal tract. As described above, once taken up by a target tissue, βHB is converted back into AcAc by the same enzyme responsible for its synthesis. From there the pathway of KB utilization is identical to the endogenous metabolism described in 1.2.4.

1.3.4 Potential Mechanisms of Exogenously Elevated Beta-Hydroxybutyrate

Glucose metabolism

Insulin levels have been found to be unaltered by 1g/kg βHB during 7 months of supplementation [115], suggesting no negative effect of βHB supplementation in patients where insulin signalling is intact.

Where insulin signalling is suboptimal though, βHB seems to somewhat mimic the acute effects of insulin: The administration of 4 mmol/l D-βHB and 1 mmol/l AcAc to the glucose perfused, increased cardiac hydraulic work in a working rat heart, while decreasing net glycolytic flux and O2 consumption, improving net cardiac efficiency by 28%, analogous to the addition of insulin [213]. Addition of both insulin and KBs to the glucose perfusate increased the efficiency of cardiac hydraulic work by 35%. The ability of a physiologic ratio of KBs to correct most of the metabolic defects of acute insulin deficiency suggests therapeutic roles for these natural substrates during periods of impaired cardiac performance and in insulin-resistant states. Both insulin and KBs have the same effects on the metabolites of the first third of the TCA cycle, on mitochondrial redox states and both increase the hydraulic efficiency of the working perfused heart. Viewed in this light, mild ketosis provides similar metabolic effects as insulin, but at the metabolic or primitive control level which by-passes the complex signalling pathway of insulin. During prolonged fasting, when insulin levels approach zero, ketosis might compensate metabolically for the absence of insulin effects. It follows that the induction of mild ketosis would be therapeutic in insulin resistant states.

With regards to glucose levels, βHB supplementation was shown to increase the intracellular glucose concentration, potentially by providing an alternative metabolic substrate by increasing mitochondrial acetyl CoA concentration, thereby by-passing the pyruvate dehydrogenase (PDH) complex and instead providing acetylCoA from AcAcCoA [213]. Another study on the effects of exogenous elevation of βHB showed that after a 4 hour D-βHB infusion in 12 septic patients, glucose levels in the plasma were reduced as compared to the treatment with a control solution [214]. These results could also be interpreted as an insulin-like effect of βHB or alternatively a decreased gluconeogenesis due to the alternative metabolic substrate.

Gene Expression Changes

βHB was found to be an endogenous inhibitor of HDACs [172]. HDACs are a family of proteins that broadly regulate gene expression and may have specific roles in glucose metabolism and diabetes (see [159]). Treating cultured cells with βHB induced dose-dependent histone hyperacetylation, as does infusing βHB into mice [215]. The histone hyperacetylation seen in mouse tissues with βHB treatment is similar to

that seen after KD, fasting or calorie restriction, suggesting that at least some of the mechanisms of endogenous versus exogenous βHB elevation might be similar. Consistent with HDAC inhibition, βHB induces specific changes in gene expression, such as induction of forkhead box O3 (Foxo3), the mammalian ortholog of the stress-responsive transcriptional factor that regulates lifespan in worms [215].

HDAC inhibition by βHB might have life-extending properties. Studies in knockout mice have shown that class I HDACs have a key role in regulating metabolic disease. HDAC3 regulates expression of gluconeogenic genes [177] and HDAC3 knockout mice have reduced fasting glucose and insulin levels [178–180]. In fact, chronic treatment with butyrate, a broad HDAC inhibitor, keeps mice essentially metabolically normal on a high-fat-high-carb diet [181] and this treatment is also associated with lower insulin and glucose levels, better glucose tolerance and prevention of weight gain amongst others. Butyrate also provides some of these benefits even to mice already obese [181].

Mitochondrial Functioning and Neuroprotection

Similarly, the class I HDAC inhibitor SAHA improves insulin sensitivity, increases mitochondrial biogenesis and increases metabolic rate and oxidative metabolism in a mouse diabetes model [216]. The mechanism for these metabolic benefits of class I HDAC inhibition may be up-regulation of PGC1α in in a variety of tissues by relief of HDAC3-mediated transcriptional repression [181,216]. Transcription of FGF21 is similarly up-regulated via butyrate inhibition of HDAC3, activating ketogenesis in obese mice [217]. Several single nucleotide polymorphisms in HDAC3 have been associated with an elevated risk of type 2 diabetes in a Chinese population [182]. βHB may have similar effects on glucose homeostasis, mitochondrial function, and obesity through endogenous inhibition of HDAC3.

Traumatic brain injury (TBI), which results in immediate changes in energy metabolism, has been shown to increase adult brain uptake and oxidation of the supplemented βHB (3h of βHB infusion) and the TBI-induced 20% decrease in ipsilateral cortical ATP concentration was alleviated by βHB infusion beginning immediately after injury [218].

The heroin analogue 1-methyl-4-phenylpyridinium (MPP+) both in vitro and in vivo, produces death of dopaminergic substantia nigral cells by inhibiting the mitochondrial NADH dehydrogenase multienzyme complex, producing a Parkinson's Disease (PD) like syndrome. Similarly, a fragment of amyloid protein (Aβ1–42) is lethal to hippocampal cells, producing recent memory deficits characteristic of Alzheimer's Disease (AD). In cell culture, it was shown that 4 mmol/l D-βHB protected cultured mesencephalic neurons from MPP+ toxicity and hippocampal neurons from Aβ1–42 toxicity [191]. KBs are able to correct defects in mitochondrial energy generation. The ability of KBs to protect neurons in culture suggests that defects in mitochondrial energy generation contribute to the pathophysiology of both brain diseases. These findings further suggest that KBs may play a therapeutic role in these most common forms of human neurodegeneration and in turn that exogenous βHB could ameliorate both AD and PD.

Similarly, in neural cell lines and an animal model of AD induced by injecting Amyloid beta into the hippocampus neuroprotective effects of exogenous βHB could be demonstrated [219]. The administration of exogenous βHB effectively prevented Amyloid beta deposition and neuron apoptosis in this rat model. βHB pre-treatment also relieved the oxidative stress in Amyloid beta -induced PC-12 cells, as shown by decreased intracellular ROS and Ca2+ levels, activated nuclear factor erythroid 2-related factor 2 (Nrf2) and recovered superoxide dismutase (SOD) and catalase (CAT) activities.

Tumor Cell Progression

Cancer cells express an abnormal metabolism characterized by increased glucose consumption owing to genetic mutations and mitochondrial dysfunction [220]. In a recent study, proliferation and viability were measured in the highly metastatic VM-M3 cells cultured in the presence and absence of βHB [210]. Adult male inbred VM mice were implanted subcutaneously with firefly luciferase-tagged syngeneic VM-M3 cells. Mice were fed a standard diet supplemented with either 1,3-butanediol (BD) or a ketone ester (KE), which are metabolized to the ketone bodies βHB and AcAc. Survival time, tumour growth rate, blood glucose, blood βHB and body weight were measured throughout the survival study. KB supplementation decreased proliferation and viability of the VM-M3 cells grown in vitro, even in the presence of high glucose. Dietary KB supplementation prolonged survival in VM-M3 mice with systemic metastatic cancer by 51 and 69%, respectively. KB administration elicited anticancer effects in vitro and in vivo independent of glucose levels or calorie restriction.

Oxidative Stress

The protective effect of KBs have been mainly attributed to the improvement of mitochondrial function – as KB levels increase, their oxidation in the brain rises. For this reason, they have been used as protective molecules against refractory epilepsy and in experimental models of ischemia and excitotoxicity. The mechanisms underlying the protective effect of these compounds are not completely understood. All KBs, AcAc and both the physiological and non-physiological isomers of βHB (D- and L-βHB, respectively) were shown to be protective for diverse ROS, as well as hypoglycaemia in vitro and in vivo [221]. Hydroxyl radicals were effectively scavenged by D- and L-βHB. In addition, the three KBs were able to reduce cell death and ROS production, while only D- βHB and AcAc prevented neuronal ATP decline. Finally, in an in vivo model of insulin-induced hypoglycaemia, the administration of D- or L- βHB, but not of AcAc, was able to prevent the hypoglycaemia-induced increase in lipid peroxidation in the rat hippocampus. The data suggest that the antioxidant capacity contributes to protection by KBs against oxidative damage in both in vitro and in vivo models associated with free radical production and energy impairment.

In a similar study, the protective effect of βHB against neuronal death induced by severe non-coma hypoglycaemia in the rat in vivo and by glucose deprivation in cortical cultures was examined [222]. Results showed that systemic administration of D-βHB reduces ROS production in distinct cortical areas and subregions of the hippocampus and efficiently prevented neuronal death in the cortex of hypoglycaemic animals. In vitro results supported the finding of reduced ROS levels and stimulates ATP production with D-βHB, while the non-physiologic isomer of βHB, L- βHB, had no effect on energy production, it also reduced ROS levels. Data therefore suggest that protection by βHB, not only results from its metabolic action but is also related to its capability to reduce ROS, rendering this KB as a suitable candidate for the treatment of traumatic and ischemic injury.

Another study also showed that βHB is able to protect cultured neurons from hypoxic or hypoglycaemic insults, as well as from oxidative stress from hydrogen peroxide [223]. In neural cell lines and an animal model of AD induced by injecting Amyloid beta (Aβ) into the hippocampus, βHB pre-treatment relieved the oxidative stress in Aβ-induced PC-12 cells, as shown by decreased intracellular ROS and Ca2+ levels [219]. This suggests the potential benefit of βHB supplementation also for neurodegenerative disorders.

Brain Excitability

The mechanisms responsible for the therapeutic response of KBs in epilepsy remain controversial. Some authors have attributed the effects to increases in the inhibitory neurotransmitter GABA [224,225]. For example, when ketotic mice were administered both acetate and a nitrogen donor, like alanine or leucine, they manifested an increased forebrain concentration of glutamine and GABA. These findings supported the hypothesis that in ketosis there is greater production of acetyl-CoA and a consequent alteration in the equilibrium of the aspartate aminotransferase reaction that results in diminished aspartate production and potentially enhanced synthesis of glutamine and GABA [224]. The metabolism of ketones in brain is likely to raise the ΔG′ of ATP hydrolysis, and with it the extent of the Na+ and Ca2+ gradients which depend upon ΔG′ATP [226], thus raising the so-called resting membrane potential and inhibiting the synchronous neuronal discharge characteristic of epilepsy.

Inflammation

Prolonged fasting or calorie restriction is known to reduce inflammation [227,228]; however, the specific role of KBs in that process was previously undetermined. A very recent combined cellular and animal study using exogenous βHB suggests that the anti-inflammatory effects of caloric restriction or KDs may be linked to βHB-mediated inhibition of the NLRP3 inflammasome [229]. It was found that only βHB, but neither AcAc nor butyrate and acetate supplementation, suppresses activation of the NLRP3 inflammasome in response to urate crystals, ATP and lipotoxic fatty acids.

Fat Metabolism

Oral administration of exogenous D,L-βHB lead to drastically reduced blood levels of FFA from 0.6 mmol/l to less than 0.1 mmol/l in 3 patients [230]. This was achieved with as little as 5g Na-βHB daily, corresponding to 0.4 mmol/l βHB levels at 1 h only. While this is a significant increase, it is a very low level relative to the Km for brain endothelial transport of 5 mmol/l, but similar to the Km for neuronal or mitochondrial transport of 0.5 mmol/l. How exactly KB levels affect FFA release from adipocytes is still largely unknown, nevertheless, a larger study administering D-βHB versus a control solution for 4 hours in 12 septic patients also showed inhibited lipolysis/ decreased FFA [214], supporting the assumption of a positive effect of βHB on FFA.

1.3.5 Therapeutic Roles of BHB Supplementation

In comparison to the KD itself, the therapeutic efficacy of βHB supplementation in neurological diseases is less established to date and remains to be examined in migraine. The aforementioned studies on mechanisms of the KD and βHB supplementation suggest that similar mechanisms characterise both therapies. The implication from this is that many diseases that profit form a KD would also benefit from βHB supplementation.

Case studies suggest that βHB salts are well tolerated and, albeit to a lesser extent than the KD, starvation or fasting, they can also lead to a substantially increase in blood KB levels [138,207–209,230,231] . An advantage is that this can be achieved in a more feasible manner than consuming a KD. In addition, βHB salts seem to be able to elevate KB blood concentrations much higher (up to 0.9 mmol/l) than other available exogenous ketogenic substances, such as medium chain triglycerides (MCTs; < 0.4mmol/l) [133] or leucine (< 0.4mmol/l) [231]. Even though fasting blood KB levels tend to be higher (between 4-8mmol/l) than what can be achieved with most exogenous ketogenic substances, existing studies, e.g. on AD [133]

or diseases with abnormal glucose metabolism [138,208,232], strongly suggest that modest externally induced ketosis can already be of benefit for certain diseases. In the following, interventions using βHB supplementation will be reviewed.

1.3.6 Relevance for the PhD

While the evidence for a benefit of a KD on several neurological diseases is increasing steadily, the mechanisms driving the effect remain largely unknown. Of particular importance is the question whether it is the absence of glucose or the presence of KBs that is of major importance. There are several observations which suggest that at least some of the benefits observed during endogenously induced nutritional ketosis could be achieved by exogenously increasing blood KBs levels. Of migraine relevance is, for instance, that βHB has been shown to influence several mechanisms that are believed to be part of migraine pathophysiology, such as inflammation, oxidative stress, brain excitability and brain energy metabolism (reviewed below). Moreover, during pregnancy migraines are known to be significantly reduced in around 60% of cases, out of which around half experience a complete resolution of the disorder [233]. While carbohydrate content in the diet of pregnant women is unlikely to have changed, a defining feature of pregnancy is an elevation in KBs [122,234]. It seems plausible that it might be this presence of KBs that is responsible for some of the migraine protective effect seen during pregnancy.

1.4 Main objectives of this PhD

1.) Aim 1: Highlight the metabolic abnormalities in migraine.

2.) Aim 2: Examine some potential peripheral biomarkers of metabolism and oxidative stress in migraine that have produced mixed results or have not yet been examined.

3.) Aim 3: Review the potential therapeutic mechanisms of ketosis in migraine.

4.) Aim 4: Examine the pharmacokinetics of various ketogenic supplements and the potential efficacy of exogenous ketone body substances in migraine.

5.) Aim 5: Plan and conduct an efficacy and safety phase 2 trial on exogenous ketones bodies (beta-hydroxybutyrate mineral salts) in migraine.

1.5 Contributions of the PhD student

During the first year of my PhD I was mainly involved in MIGSPO, a migraine neuroimaging sport intervention study that had already been running before I came to Basel. I was responsible for recruitment, screening, multi-modal magnetic resonance imaging (MRI) scanning and preliminary neuroimaging data management and analysis. Additionally, I started planning a side-project with an intranasal cooling company, where we wanted to assess the acute migraine aborting effect of intranasal cooling and TRPM8 channel activation, respectively. Since the company lost its research funding, this project was later aborted. During this first year, I additionally had the chance to start planning MigraKet (a phase 2 trial on "Exogenous ketone bodies in migraine prevention", see *Trials* paper, chapter 2.5), the project idea I came to Basel with. I started writing and submitting the ethics approval, started looking

for possible study drugs etc. The idea behind MigraKet was fairly simply: instead of providing the migraine brain with an alternative energy substrate to glucose by forcing the liver to produce KBs (endogenous KBs), the goal was to use exogenous KBs in the form of ketogenic supplements instead.

When my first primary supervisor (Dr. med. Till Sprenger) left for a leading clinical position in Germany after a few months into my first year at the University of Basel, I had the chance to make my dream PhD topic MigraKet the primary focus of my PhD under the supervision of Prof. Dr. med. Dirk Fischer (as long as I raised enough funding for the phase 2 trial and my PhD). I therefore had the great opportunity to be part of the conceptualization of MigraKet and its sub-projects from the very onset. I was encouraged to come up with own ideas and had the freedom to develop new study plans/ ideas / study assessments and biomarkers. in collaboration with my supervisors and external experts. I consequently had a substantial role in all aspects of the studies mentioned in this dissertation, i.e. from financing, design, planning and conduct trough analysis and dissemination of study results.

Financing:

During the course of my PhD, I raised or received the following grants, stipends and prizes, adding up to a total of approximately 585'000 CHF:

- Best Poster Prize at International Headache Conference, Vancouver, Canada (2017)
- Stipend to attend the iHEAD / IHA academy in Vancouver, Canada, by the International Headache Society (IHS) (2017) (2'000 CHF)
- Swiss National Science Foundation (SNSF) project grant for PhD project: "Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A randomised, placebo-controlled, double-blind study" (525'000 CHF) (2017)
- PhD project stipend for excellent young scientists from the Free Academic Society (FAG) Basel (12'000 CHF) (2016)
- Project award by the Clinical Trial Unit, Department of Medicine, Basel University Hospital (150 hours data - and on-site management) (2016)
- Second place at BioBusiness 2016 start-up pitch (2016)
- iHEAD 2016 winner of the young headache researcher debate (2016)
- Stipend to attend the iHEAD academy 2016 in London, UK, by the International Headache Society (IHS) (2016) (1'000 CHF)
- UKBB Research Fellowship (Brian Fowler Fund) to stay at the laboratory of Prof. Rami Burstein, Professor of Anaesthesia and Neuroscience at Harvard Medical School (3'000 CHF) (2016)
- Scholarship to attend BioBusiness, a 5 day program on BioEntrepreneurship at the USI, an exclusive learning platform and network where academia, industry and venture capitalists interact fruitfully (2016) (2'000 CHF)
- antelope@university stipend aimed at highly qualified female doctoral students to systematically plan and promote their careers and prepare them for future leadership and management assignments in academia (2016)
- Hans Ruedi Isler Prize of the Schweizer Kopfschmerzgesellschaft (SKG) for the best migraine research project (5'000 CHF) (2015)
- PPHS PhD Top-Up stipend for highly qualified graduates to fund PhD student-initiated research projects (19'250 CHF) (2015)
- Novartis Biotechnology Leadership Camp 2015 individual and group winner (2015)

Planning:

After conceptualization and identification of the most suitable study design with my supervisors, I drafted the first version of the study protocol. This included literature reviews, endpoint definitions according to international migraine guidelines, analysis plans for main and secondary objectives,

genetic, epigenetic, safety, metabolism and oxidative stress markers, contacting chemical laboratories for finding the study drug, preliminary pharmacokinetic studies on available ketogenic supplements, trial execution and patient visit plans, laboratory analysis plans, data management plans, monitoring plans. I coordinated the different teams involved, including pharmacy, study drug manufacturers, Abbott (glucose and ketone body monitoring), statisticians, geneticists, laboratory staff, data managers, monitors, study nurses and study physicians. I helped set up an electronic data entry system with the Clinical Trial Unit, which can now be used for all subsequent clinical trials, in order to reduce manual data entry time and error rates; this included the SecuTrial design. I drafted and submitted all the study relevant documents, such as the ethics proposal (and 5 amendments), the Swissmedic application (and amendments), the Risk and Safety Information on the study drug (100 pages), clinical report forms (CRFs) and the patient information for MigraKet. I also came up with the idea for MigraGlu, the use of permanent glucometers in migraine during baseline and the ketogenic intervention and MigraMit, the analysis mitochondrial function markers in migraine. MigraGlu and MigraMit were integrated as a sub-analyses into MigraKet and ethics and patient information documents were adapted accordingly.

In order to find the best ketogenic supplement, prove our concept and have some preliminary data for the grant applications and the subsequent patent application, we collected some pilot pharmacokinetic and efficacy on several substances: the ketogenic amino acids L-Lysine and L-Leucine, Sodium- , Calcium- and magnesium-D/L-beta-hyroxybutyrate (βHB) salts and mixed mineral D- βHB salts (the details on study set-up and results and can be found in the published patent in section 2.4). I was responsible for the data analysis and writing the patent application with the help of the University of Basel tech transfer office (unitectra). With regards to our IMP, I found a Dutch good medical practice (GMP) supplier of Ca-Mg- βHB, which we sourced and packaged for MigraKet. In order to enhance recruitment, I made a 5min information video for MigraKet, with was later used for social media (Facebook etc.) advertisement, uploaded on self-help and other migraine organisations. I also designed flyers and handouts for hospitals, private clinics and pharmacies.

Conduct:

In the conduct phase, I was responsible for recruitment, telephone pre-screening of patients, data collection, i.e. the study visits, study assessments, education of patients, study drug administration, CRFs, patient folders etc. (everything apart from adverse events and neurological assessments), the data entry, project management and coordination of the teams contributing to MigraKet. I also educated the study nurses and other parties involved on how to run MigraKet.

In addition, I was responsible for amendments and any communication with regulatory or funding agencies; for example, when the placebo designed by the University Pharmacy (mannitol) turned out to be a laxative in our quantities used or the recruitment was too slow and we had to change the trial design to cross-over (see *Trials* paper). Moreover, I helped with data entry / analysing data of other projects running in the lab, patent search and gave critical feedback on manuscript drafts.

Analysis / Writing:

Finally, I wrote the drafts for all the manuscripts below, including the design and drawing of the display items, I coordinated the critical revision by co-authors, submitted and revised manuscripts as first author and presented and discussed our work at several international and national conferences, more recently also as invited speaker. Since the clinical trial MigraKet is still running, unfortunately, I came up with MigraMit, for which I selected mitochondrial functioning markers to be analyzed from the baseline blood samples, found a laboratory that was able to determine these novel markers, and interpreted the data together with my supervisors and the clinical trial unit statisticians.

2. First author publications

2.1 Manuscript 1: The Metabolic Face of Migraine

In press: **Gross, E.C.,** Lisicki, M., Fischer, D., Sandor, P.S. & Schoenen, J. (2019). The Metabolic Face of Migraine. *Nature Reviews Neurology.* (Impact factor= 20.3)

The Metabolic Face of Migraine

Clinical, biochemical, genetic, therapeutic and experimental evidence supporting the crucial role of energy metabolism in migraine pathophysiology.

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E.C.G. was responsible for literature search and the main composition of the manuscript, including the majority of display items. M.L. edited the manuscript and provided additional text and display items. D.F. and P.S.S. edited the manuscript. J.S. was responsible for the design, edited in depth the manuscript, display items and provided additional text and citations. All authors proofread the final manuscript prior to submission.

nature REVIEWS

NEUROLOGY

The Metabolic Face of Migraine

Clinical, biochemical, genetic, therapeutic and experimental evidence supporting the crucial role of energy metabolism in migraine pathophysiology.

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Contributions

E.C.G. was responsible for literature search and the main composition of the manuscript, including the majority of display items. M.L. edited the manuscript and provided additional text and display items. D.F. and P.S.S. edited the manuscript. J.S. was responsible for the design, edited in depth the manuscript, display items and provided additional text and citations. All authors proofread the final manuscript prior to submission.

Competing interests

E.C.G. is the founder of KetoSwiss AG and E.C.G. and D.F. are the inventors of the patent WO 2018/115158 Al) held by the UKBB and University of Basel on the use of beta-hydroxybutyrate in migraine prevention. M.L., P.S. and J.S. have no conflicts of interest for this publication.

Key points

- Prevalent migraine attack triggers have unbalanced cerebral energy metabolism and/or oxidative stress as common denominators.
- MRS studies show decreased mitochondrial phosphorylation potential and ATP in the brain of migraineurs between attacks. Glucose (and lipid) metabolism and mitochondrial functions are abnormal in the peripheral blood.
- Migraine patients have increased prevalence of various single nucleotide polymorphisms in non-coding and in nuclear-encoded mitochondrial DNA. Common migraine GWAS variants are functionally involved in mitochondrial metabolism.

- Metabolic enhancers like riboflavin or co-enzyme Q10, and dietary or pharmacological ketogenesis improve migraine, but novel more efficient metabolic strategies are warranted.
- Experimental studies provide a possible link between cerebral energy disequilibrium and migraine attack generation including cortical spreading depression and trigemino-vascular system activation via pannexin-1 and/or TRP/ASIC channels.
- CGRP, a major actor in the migraine headache, could also be part of an antioxidant response and metabolic changes, which might help restore energy homeostasis.
- Migraine can be regarded as a conserved (mal)adaptive response pattern that occurs in genetically predisposed individuals with a mismatch between the brain's energy reserve and workload.

Abbreviations

ADP = adenosine diphosphate
ALA = alpha-lipoic acid
ASIC = acid sending ion channel
ATP = adenosine triphosphate
BMI = body mass index
CGRP = Calcitonin gene-related peptide
CoQ10 = coenzyme Q10
COX = cyclooxygenase
CSD = cortical spreading depression
^{18}FDG-PET = 18-fluorodeoxyglucose-PET ()
FFA = free fatty acids
GABA = gamma-aminobutyric acid
GLUT = glucose transporter
GTT = glucose tolerance test
GWAS = genome wide association studies
KB = ketone bodies
KD = ketogenic diet
MA = migraine with aura
MO = migraine without aura
^{1}H- MRS = proton magnetic resonance spectroscopy
^{31}P- MRS = phosphorus magnetic resonance spectroscopy
Mt = mitochondrial
NADH = reduced nicotinamide adenine dinucleotide
NO = nitric oxide
OXPHOS = oxidative phosphorylation
PACAP = pituitary adenylate cyclase-activating peptide
PET = positron-emission tomography
RCT = randomised controlled trial
RNS = reactive nitrogen species
ROS = reactive oxygen species
SOD2 = superoxide dismutase 2
TRP = transient receptor potential channels

Abstract

Migraine can be regarded as a conserved (mal)adaptive response pattern that occurs in genetically predisposed individuals with a mismatch between the brain's energy reserve and workload. Given the high prevalence of migraine it seems likely that migraine genotypes have conferred some evolutionary advantage. Technological advances (e.g. in neuroimaging and genetics) have enabled the examination of different aspects of (cerebral-) metabolism in migraine patients, with recent complimentary animal research highlighting possible mechanisms in migraine pathophysiology. An increasing amount of evidence – much of it clinical - points towards migraine being a response to cerebral energy deficiency or oxidative stress levels that exceed antioxidant capacity, the attack itself helping to restore brain energy homeostasis and reducing potentially harmful oxidative stress levels. The increased understanding of migraine metabolism offers exciting novel and likely well-tolerated therapeutic opportunities. In this review, we describe the evidence for abnormalities in energy metabolism and mitochondrial functioning in migraine with a particular focus on clinical data (including neuroimaging, biochemical, genetic and therapeutic studies) and their relation with the abnormal sensory processing and cerebral hyperresponsivity found in migraine patients between attacks. Experimental data will be discussed to elaborate on potential mechanisms of such metabolic abnormalities with regards to attack generation. Finally, potential treatments targeting cerebral metabolism, such as nutraceuticals, ketone bodies and dietary interventions, are highlighted.

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Introduction

Susceptibility to migraine is determined by genetic factors and hence subject to the forces of natural selection. It seems likely that the common gene polymorphisms underlying migraine, which affects more than 15% of the population worldwide, does confer some evolutionary advantage. A migraine-prone nervous system may be, or at least might have been associated with reproductive or survival advantages [1,2]. Has our environment become inadequate or suboptimal for the conserved adaptive genetic response patterns associated with migraine, possibly because of the change in nutrition over time or is migraine the price the human species has to pay for having a highly developed and performing brain?

Already in 1935 migraine was referred to as a "hypoglycemic headache" [3]. Despite this early connection between migraine and energy metabolism, clinical and basic research has focused on (neuro-)vasculature and neurotransmission until Willem Amery revived the metabolic pathogenesis of migraine in his hypothesis-generating review in Cephalalgia entitled "Brain hypoxia: the turning-point in the genesis of the migraine attack?" (1982). Since then there is accruing evidence – much of it clinical - that migraine is - at least partially - an energy deficit syndrome with mitochondrial dysfunction. Technological advances (e.g. in neuroimaging and genetics) have allowed to examine different aspects of (cerebral-) metabolism in migraine patients, with complimentary animal research deciphering possible links between metabolic factors and trigeminovascular activation in migraine pathophysiology.

Since it was shown in parallel that cortical responsivity and sensory processing are abnormal in migraine patients between attacks (review by [4]), a combination of sensory overload and lowered energy reserve was postulated to ignite the major pain-signalling system of the brain, the trigeminovascular system, leading to the migraine attack [5].

This review will describe the abnormalities in energy metabolism in migraine with a particular focus on clinical data including phenotypic, biochemical, genetic and therapeutic studies. Experimental data will be discussed to elaborate on the potential role of such metabolic abnormalities in attack generation. Finally, therapeutic approaches targeting cerebral metabolism (antioxidants, nutraceuticals, pharmacological or dietary ketogenesis) will be highlighted.

1. Aggravating & trigger factors suggesting metabolic dysfunction

When trying to decipher the pathogenesis of migraines, it makes sense to look at the perceived triggers of migraine attacks and at factors that on a longer time scale may aggravate migraine, the two being sometimes related. Two recent systematic reviews [6,7] and a study on 1207 patients [8] have identified the following most common migraine trigger factors: stress or relaxation thereafter, fasting or skipping a meal, sleep changes (too much or too little), ovarian hormone changes (including menses and oral contraceptives), weather changes (including certain winds, hypoxia and high altitude), physical exercise (including sexual activity), alcohol, strong odours (especially perfume or cigarette smoke), intense light (especially bright or blue light) and loud noises. The distinction between trigger factors and premonitory symptoms of the migraine attack is not always easy. For example, pre-attack photophobia (see [25]), fatigue and craving for calorie dense foods such as chocolate (and hence its consumption) are more likely to represent premonitory symptoms, rather than trigger factors, which is probably also true for neck pain [9]. By contrast, the glare of sunlight or the consumption of alcohol can trigger an attack if the migraine threshold is low. Individual triggers seem to have an additive effect [10], with a subsequent attack resulting only once an individual attack generation threshold has been reached. This suggests that trigger factors are acting on common pathways. While for some of the more "metabolic" triggers (such as skipping a meal/fasting, exercise, dehydration and hypoxia, lack of sleep) a direct link to energy homeostasis seems obvious, most of the seemingly unrelated triggers, including hormonal changes, also have a potential common denominator: changes in mitochondrial metabolism and/or oxidative stress [11].

For instance, intense physical [12,13], and severe mental or psychological stress are able to increase oxidative stress in the central nervous system [14]. In healthy humans, one night of sleep deprivation is enough to significantly reduce glutathione, ATP, cysteine, and homocysteine levels [15]. Intense sensory stimuli can increase oxidative stress. This has been shown for odours [16], perfumes containing phthalates [17], blue light [18] and loud noises [19]. Migraine prevalence is increased in high-altitude populations [20]. Experimental hypoxia is able to trigger migraine headache [21,22], but much less so migraine aura [21], and it can even induce migraine-like headaches in most healthy subjects [23].

In animals, alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties [24]. While in rodents estrogen, and to a lesser extent progesterone, increase susceptibility to cortical spreading depression (CSD) [25], the culprit for the migraine aura, and modulate the CSD inhibiting effect of 5-hydroxytryptamine, the serotonin precursor [26], both hormones also influence oxidative metabolism in the rat brain [27] and 17β-oestradiol is involved in insulin sensitivity, the regulation of insulin secretion and nutrient homeostasis [28].

In summary, most migraine trigger or aggravating factors have some link to energy metabolism and oxidative stress.

2. Biochemical studies

2.1. Brain OXPHOS, ATP and lactate

Magnetic resonance spectroscopy (MRS) allows determining non-invasively the concentration of numerous substances in various tissues. Some of these substances, like lactate, magnesium, and ATP provide pivotal information about energy metabolism. The results obtained in migraine are summarized in table 1.

Using ^{31}P-MRS, an impairment of mitochondrial oxidative phosphorylation (OXPHOS) was detected in the brain of migraine patients both during[29] and in-between migraine attacks [30–37]: increased ADP, decreased organic phosphate and phosphorylation potential. Similar patterns were observed in skeletal muscles[30,38,39], suggesting a generalized rather than a brain-specific alteration (see reviews by Reyngoudt et al[36] & Cevoli et al. [40] for details). More recently, brain ATP was directly quantified with a modified ^{31}P-MRS methodology and concordantly found to be decreased by 16%in migraine without aura patients between attacks (n=19) compared to healthy controls (n=26)[41],. The lowest ATP concentrations were detected in the more severely affected patients, which concords in part with other studies showing modest correlations between brain hypometabolism and attack frequency.[33,38,41] Because it is as a crucial co-factor for ATP-production, magnesium is often included in ^{31}P-MRS studies of neural metabolism. In line with OXPHOS alterations, cytosolic free magnesium was significantly reduced in the occipital lobes of migraineurs [32,33,42].

With the more accessible ^{1}H-MRS technique, concentrations of lactate, a key cellular metabolite, can be determined. Because of variability in methodology and patients' selection criteria data on brain lactate levels in migraine does not permit to draw strong conclusions (see review by Reyngoudt et al (2012) [36] & Cevoli et al [40] for details). Elevated brain lactate was found in migraine with aura [43,44], but not in migraine without aura [45–48]. Occipital baseline lactate levels were increased in patients having strictly visual auras but not in those having complex neurological auras, while lactate increased significantly during photic stimulation in the latter, but not in the former [43]. An important issue that must be considered is that stimulus-induced increases of cortical lactate are physiological [49]. They are explained by the astrocyte-to-neuron lactate shuttle [50], the mechanism by which astrocytes provide energetic supplies for neurons when they become activated. Hence, the absence of a stimulus-induced lactate increase in migraine patients, whose neuronal activation is likely more energy-demanding [51], could be considered as pathological, as it may render them vulnerable to an energetic crisis. A study combining lactate quantification in the cortex and electrophysiological testing of brain-evoked responses is clearly worthwhile.

Another useful method in assessing energy dynamics in the brain is ^{18}-FDG-PET. The procedure consists of measuring the positron emission by radiotracer-labelled glucose absorbed by metabolically active tissues after systemic injection. It does not allow distinguishing the glucose uptake by neurons or non-neuronal cells like astrocytes. In a recent study comparing glucose uptake at rest with 18-FDG PET and visual evoked potentials, neuronal activation exceeded glucose uptake in visual areas in 90% of interictal migraine without aura patients, but in only 15% of healthy controls [52]. Given that at least 50% of glucose uptake in the brain goes to the astrocytes, the CNS cells where energy is stored, this suggests that energy reserves are reduced in migraine patients and supports the concept that a mismatch

between brain activation and glucose metabolism may be a cornerstone in migraine pathophysiology. Further studies along this line of research are clearly needed to confirm this hypothesis.

2.2. Peripheral metabolic abnormalities

Metabolic abnormalities in peripheral tissues will be described in this section and are synoptically presented in table 2.

Abnormal mitochondrial enzyme functions

Support for a generalized metabolic dysfunction in migraine comes from reduced activity of mitochondrial enzymes, such as monoamine-oxidase, succinate-dehydrogenase, NADH-dehydrogenase, cyclooxygenase (COX) and citrate-synthetase in the platelets of migraine with (MA) or without aura (MO) patients [53 54]. Interestingly, these biochemical changes are restricted to enzymes of the respiratory chain that are encoded by mtDNA (see section 3), which are much more vulnerable to oxidative stress, as they do not have the same repair mechanisms as nuclear DNA. Some further evidence for mitochondrial abnormalities in migraine comes from elevated lactate and pyruvate plasma levels, but those are mostly found in patients with migrainous stroke [55,56].

Oxidative / nitrosative stress and antioxidant capacity

As aforementioned, all common migraine triggers are likely to negatively impact oxidative stress levels. This link can be supported by studies that directly examine markers of increased oxidative or nitrosative stress and / or decreased anti-oxidant capacity[57–70] (table 2). All studies on oxidative stress / antioxidant capacity in migraine demonstrate at least one marker being abnormal [57–70]. Of all biomarkers examined, superoxide dismutase (SOD) activity seems to be the only one consistently reduced in migraine patients, also intericatlly [71]. The inconsistent results for the other markers could be due to differences in methodology, patients' selection (low vs high frequency migraine, MA or MO etc.) and variations depending on the migraine cycle. Regarding the latter, nitrosative and oxidative stress [70] and NO [71] are significantly elevated during migraine attacks, but not interictally.

Free iron is highly pro-oxidant and accumulates in the brain stem of migraine patients in proportion to disease duration [72]. Other heavy metals with pro-oxidant properties may also be increased in migraine [73].

Glucose metabolism

Due to limited glycogen stores, high energy needs and the unavailability of large energy dense molecules due to the blood brain barrier, the human brain is highly dependent on energy sources from the circulation and hence particularly vulnerable to their potential shortcomings. Hypoglycaemia has been associated with migraine for almost a century [3,74,75]. A simple comparison between migraine associated symptoms, premonitory symptoms in particular, and symptoms of hypoglycaemia [76] show several similarities: for example, dizziness, pale skin, cold hands and feet, binge eating/sugar cravings, yawning, nausea, low blood pressure, shaking, cognitive difficulties, tiredness, fatigue, visual dysfunction and slurred speech. These symptoms are caused by an insufficient supply of glucose to the brain and/or release of catecholamines due to sympathetic activation [76].

The hypothalamus controls homeostasis and it is activated early during the premonitory phase of both triggered and spontaneous migraine attacks [77–79]. This could represent the underlying physiological correlate of premonitory symptoms themselves or be part of an adaptive behavioural response [2] to a

hypoglycaemic or energy-compromised brain, initiating increased yawning (-> elevation of brain oxygen), craving (-> restoration of energy balance), fatigue, sickness and hypersensitivity (-> "energy conserving behaviours") etc.

There is circumstantial evidence from early experimental studies that metabolic changes induced by fasting, glucose or insulin administration can trigger migraine attacks. Although insulin-induced hypoglycaemia elicited an attack in only 2 out of 20 patients during an observation time of 2 hours [80], a 50g Glucose Tolerance Test (GTT) after a 10-hour fast did so within the test period of 8 hours in 6 out of 10 migraine patients reporting attacks associated with fasting or craving [81]. Interestingly, the metabolic responses in patients who developed an attack differed substantially from those that did not: free fatty acid (FFA) and ketone body (KB) levels increased significantly in the former, already before headache onset, and amplified despite similar food intake [81]. There was no apparent difference in glucose and glycerol levels. Like for nitroglycerin [81], attacks triggered by metabolic stress seem to develop after a latency of several hours, probably needed to activate the trigeminovascular system.

Abnormal metabolic responses were reported in a number of GTT studies (table 2). Comparing iv GTT in the same migraine with aura patients during and outside of an attack, Shaw et al. [82] found ictally an impaired glucose tolerance, and an increase in FFA, KB, beta-hydroxybutyrate: acetoacetate ratio, glycerol and cortisol, but a decrease in insulin levels, which was considered as an ictal stress response with increased lipolysis and ketogenesis. The latter can also be interpreted as counter-regulatory responses to a cerebral energy deficit. Since KBs are an efficient alternative fuel for the brain when glucose availability is low, their elevation would be expected to restore brain energy homeostasis. However, given our western carbohydrate laden diets, most brains are not keto adapted and hence do not necessarily have the enzymatic composition and transporters in place to actually utilize the KB produced during the energy crisis.

Interictal impaired glucose tolerance and insulin resistance were found in various other studies [83] [84] [85] [86] [87] (see table2). Insulin, the anabolic hormone of the body and the key regulator of glucose homeostasis, promotes the absorption of glucose from the blood into predominantly fat and muscle cells with the help of insulin-sensitive glucose transporters (GLUT), in particular GLUT4. Insulin also blocks carnitine transporters and thus also penetration of FFA into the cells. Of note, in endothelial cells of the blood brain barrier, astrocytes and oligodendrocytes, an insulin-independent GLUT1 is responsible for glucose transport under basal conditions. The evidence for an association between migraine and insulin resistance was reviewed by Rainero et al. (2018) [88] and seems robust although it was not found in one study [89] or only in female migraineurs in another [90]. The degree of insulin resistance was shown to correlate with disease severity, while β-cell function remained normal [91].

Rather than being directly involved in migraine pathogenesis, reduced insulin sensitivity, could be part of a temporary adaptive response to ensure that the brain's energy needs are met. Such a "glucose-sparing effect" is typically observed when glucose availability is low (e.g. during fasting or carbohydrate restriction) [92–94]. The finding that diabetes seems to be migraine protective [95] [84] supports the assumption that insulin resistance might be an adaptive response, rather than a causal factor. In the long-term, however, chronic insulin resistance might contribute to metabolic diseases, such as the metabolic syndrome that is associated with migraine with aura [96] and chronic migraine in females [97]. It is unclear whether metabolic derangements are risk factors for or consequences of migraine. Body mass index, for instance, was associated with frequency of attacks, but not with migraine prevalence [98].

Elevated cortisol levels have been found in episodic [90,99] and chronic migraine [100], as well as during migraine attacks [82], although a recent review concluded at overall mixed results [101]. Early morning migraine is also associated with increased catecholamine levels [102]. The fact that the increase in cortisol and catecholamines is not accompanied by an increase in glucose, might suggest that prior hypoglycaemia induced a stress reaction to counteract falling blood glucose levels. The body's physiologic reaction to hypoglycaemia involves indeed the secretion of cortisol, adrenalin and noradrenalin to protect cells by increasing gluconeogenesis and glycogenolysis [103], stimulating protein catabolism and blocking the action of insulin. Prolonged hypoglycaemia is avoided at all costs, even if this might require a constant elevation of stress-hormone levels. For this very reason and because it may take several hours before the attack occurs, hypoglycaemia may not be detectable, in particular at the time of migraine onset [104], unless blood glucose levels are longitudinally monitored over a long time period.

The body's response to hypoglycaemia also comprises the release of glucagon, the antagonist of insulin. Blood glucose increases in response to glucagon injection were significantly less pronounced in migraineurs than in controls [105]. A decreased energy compensation by glucagon could in part explain migraine attacks induced by fasting in susceptible individuals.

The data on other hormones involved in energy homeostasis, like leptin and adipocytokines, is conflicting [106]. Low leptin levels, found in one study of episodic migraineurs [107], could further exacerbate a cellular energy deficit. Another recent study, however, reported Increased levels of both leptin and adiponectin, which could enhance inflammation [106,108].

In summary, a large number of biochemical studies in migraine point towards a variety of different metabolic abnormalities, all related to energy homeostasis. It is likely that a combination of different metabolo-endocrinological abnormalities, possibly together with an abnormal cerebral responsivity, determines the migraine attack threshold in a given patient. The cumulative number of abnormalities, in combination with unfavourable environmental factors, is likely to determine disease severity [6].

3. Genetic studies

Genetic studies directly and indirectly support the assumption that migraineurs might have an increased vulnerability to oxidative stress, suboptimal mitochondrial functioning and / or an altered metabolism. They are listed in table 3.

Mutations in coding mtDNA

Migraine is about three times more prevalent in women than in men [109] and maternal transmission is more common [110], which suggests that either an X-linked form of inheritance could be involved or that mitochondrial DNA (mtDNA) transmission plays a role. Prevalence of migraine in mitochondrial disorders is more than doubled (29%-35.5% of patients) [111,112] and migraine-like attacks in MELAS are especially severe and prolonged [113]. The lifetime prevalence of migraine in normal carriers of the MELAS 3243A>G mutation in coding mtDNA is significantly higher than in the general population (58% vs. 18%; $P < 0.001$) [114]. 52% of patients with the MERRF m.8344A>G mutation in the MTTK gene also have migraine [115]. These findings suggest a clinical association between a monogenic inherited disorder of mitochondrial DNA and susceptibility to migraine.

Contrasting with these prevalence studies in miotchondriopathies, the majority of genetic studies failed to identify the classical mutations of the coding sequence of mtDNA in cohorts of migraine patients (see table 3) [116–122] but such mutations were reported in migraine with stroke episodes [123].

Common variants in non-coding mtDNA

Alternatively, mitochondrial function could be impaired in migraine due to common variants (Single Nucleotide Polymorphisms-SNP) in the non-coding portion of mtDNA, which may influence mitochondrial metabolism, or in nuclear-encoded mtDNA [124], which has received few attention up to now. Certain mitochondrial haplogroups defined by the combination of highly conserved polymorphisms in non-coding mtDNA, like the H Haplogroup, confer an advantage in OXPHOS performance [125,126] and are associated with a poorer response to the metabolic enhancing treatment with riboflavin compared to the other haplogroups (see table 3) [127]. Increased numbers of certain sequence variants were detected in non-coding mtDNA in migraineurs with occipital stroke associated with Haplogroup U [128] as well as in migraine without aura and in cyclic vomiting, a migraine equivalent of childhood [129]. In a similar patient population, Zaki et al. (2009) found an increased prevalence of the common 16519C→T polymorphism in non-coding mtDNA and an even greater difference with controls for the combination of this polymorphism and the less common 3010G→A polymorphism [130]. These two polymorphisms are also more frequent in chronic fatigue syndrome and depression [131]. Like for nuclear DNA, large cohort studies of cases and controls are needed to detect small- moderate associations between mtDNA variants and a complex disease like migraine, which explains that some results have not been replicated [132].

Variants in nuclear-encoded mtDNA

Given that the majority of proteins responsible for mitochondrial function are nuclear encoded, the role of Nuclear Encoded Mitochondrial Protein (NEMP) genes in relation to migraine susceptibility is a promising path to pursue. Recently a gene-centric association analysis of NEMP genes within the genetically isolated Norfolk Island population found an association with migraine in three NEMP genes that are involved in phosphorylation, fatty acid metabolism, and oxidative demethylation [133], thereby providing further evidence for a link between mitochondrial function and migraine susceptibility.

Other genes, oxidative stress and epigenetics

In contrast to nuclear DNA, mtDNA is particularly sensitive to reactive oxygen species (ROS) [134,135]. It remains to be determined whether accumulation of mtDNA damage due to oxidative stress (see table 2) over time could play a role in migraine chronification and increased vascular risk. Reduced anti-oxidant capacity or increased oxidative stress in migraine (see table 2) could be related to a genetic predisposition. A polymorphism (rs4880 TT - Val/Val genotype) in the SOD2 gene was associated with unilateral cranial autonomic symptoms in MA patients [136]. In paediatric migraine patients the SOD2 16 C/T genotype and C allele frequency as well as the catalase (CAT) -21 AA genotype and A allele frequency were significantly higher in both MA and MO patients compared to controls [137]. Further genetic support for altered metabolism in migraine comes from associations between polymorphisms in insulin-related genes and migraine [138–141]. GLUT1 deficiency syndrome, which impairs glucose transport to the brain (amongst others), has been linked to hemiplegic migraine and migraine with aura [142].

Migraine, in particular MA, has been associated with the methylenetetrahydrofolate reductase (MTHFR) gene mutation C677T [143,144] that diminishes the capacity to remethylate homocysteine to methionine [143,144]. Increased homocysteine levels favour vascular pathologies in humans and induce oxidative stress and reduced anti-oxidant capacity in rat [145]. Admittedly, in a Finnish MA population the association between MTHFR and migraine could not be replicated [146].

Next generation nuclear DNA sequencing in cyclic vomiting syndrome revealed a significant association with variants in the stress-induced calcium channel (RYR2) [147] that may increase mitochondrial calcium uptake and oxidative stress.

Functional enrichment analyses of migraine loci identified in GWAS

Gene set enrichment (also known as functional enrichment analysis) is a method to identify classes of genes or proteins that are over-represented in a large set of genes or proteins and may have an association with disease phenotypes. Genome-wide association studies (GWAS) have identified 38 genetic loci associated with migraine [148]. In a recent study, GWAS data was integrated with high-resolution spatial gene expression data of normal adult brains from the Allen Human Brain Atlas to identify specific brain regions and molecular pathways that are possibly involved in migraine pathophysiology [149]. Enrichment of a migraine GWAS signal was found for mitochondria in both subcortical areas and the cortex (amongst others), a finding that identifies a genetic link between mitochondrial function and common migraine [149]. A number of genes related to mitochondrial functioning have also been found to be differentially expressed in adolescent menstrual migraine compared to healthy controls [150].

In summary, genetic findings offer further support to the assumption that altered metabolism and mitochondrial functioning play a pivotal role in migraine pathogenesis. As pointed out in a recent review [151], epigenetic mechanisms, in particular mitochondrial methylation, may be a new avenue of interest to explore the underpinnings of mitochondrial dysfunction in migraine, since the mtDNA epigenetic status differs from healthy subjects in complex neurological disorders.

4. Therapeutic studies

While epigenetics may open new therapeutic prospects, a large number of metabolic treatments have already been performed in migraine and are shown in table 4. The fact that such treatments are effective in migraine does not prove that migraine is primarily a disorder of brain energetics, the more so that migraine is a multifactorial disorder where the predominant pathophysiological abnormality may vary between patients. It does strongly suggest nonetheless that in responders the metabolic treatments act via an improvement of brain energetics, although this still needs to be definitively proven by assessing brain metabolism before and after treatment.

Acute treatment

Only a few substances used in the abortive treatment of migraine have a proven link to energy metabolism. Corticosteroids are among the most efficacious drugs to abort prolonged migraine attacks and status migrainosus [152]. Caffeine (>100mg) is known to have a small but significant analgesic effect, at least when used in conjunction with common analgesics [153]. Apart from its suppressive effect on human TRPA1 activity [154], caffeine is known to stimulate cortisol secretion [155,156], and hence gluconeogenesis [157]. Additionally, it elevates FFA, while decreasing insulin responses [156], which is similar

to the metabolic changes described above during a migraine attack [82], thereby supporting the assumption that the ictal metabolic abnormalities rather reflect counter-regulatory effects. Long-term use of excess caffeine, however, is associated with insulin resistance [158] and migraine chronification [159], while caffeine discontinuation is associated with better efficacy of acute migraine treatment [160].

Prophylactic treatment – nutraceuticals, ketogenic diet, exercise

Several nutraceuticals [161] were shown to be migraine preventative [162] and most of them can be linked to energy metabolism and / or mitochondrial functioning [163]. The level of evidence, however, varies between them and not all of them are mentioned in international guidelines for migraine prevention. They are quasi devoid of adverse effects, which contrasts with most "classical" preventive drugs.

Riboflavin plays an important role in the metabolism of carbohydrates, proteins and fats, recycling of oxidized glutathione and is a precursor of flavin nucleotides necessary for the activity of flavoenzymes that participate in the electron transport chain [164,165]. Furthermore, it has neuroprotective properties, alleviating oxidative stress, mitochondrial dysfunction, neuroinflammation, and glutamate excitotoxicity [165,166]. Several studies demonstrate its efficacy at high dose (200-400mg/ day) in both adult and paediatric patients [167–170], but not at low dose (50mg/day) [171]. In a single blind comparative, parallel group (n=90) study riboflavin 400mg/d was as effective as sodium valproate 500mg/d for migraine prevention [172]. A recent systematic review showed that high dose riboflavin (400mg/ day) is well tolerated, inexpensive and has demonstrated efficacy in the reduction of migraine headache frequency [173]. Mitochondrial DNA haplogroups were shown to influence the therapeutic response to riboflavin in migraineurs [174]: Responders belong preferentially to the non-H haplogroups, but non-responders to the H haplogroup that has the better OXPHOS performance.

Coenzyme Q10 (CoQ10; ubiquinone in oxidised and ubiquinol in reduced form) is an essential cofactor of the electron transport chain with strong anti-oxidant properties [175,176]. CoQ10 (400mg capsules or 300mg liquid suspension) significantly reduced migraine frequency in adults in four placebo-controlled double-blind trials [177–180], and in two open-label studies [181,182]. A 100mg dose was not superior to placebo in another RCT on childhood and adolescence migraine (age 6-17yrs) [183], but the same group found beneficial preventive effects in an open study where Q10 was given to paediatric migraine patients with low blood Q10 levels [184].

Alpha-lipoic acid (ALA) (or thioctic acid) is a water- and fat-soluble antioxidant that can directly, by removing reactive species, and indirectly, by chelating transition metal ions, reduce oxidative stress [185,186]. A RCT with 600mg ALA showed a trend towards reduction of attack frequency, headache days and headache severity in patients treated for 3 months compared to placebo [187]. In migraine patients with insulin resistance, an open ALA trial showed 69% of 50% responders [188]. A 1-month combined treatment with topiramate and ALA was more effective for migraine prevention than either drug alone [189].

Folic acid or folic acid combined with B6 (25mg) and B12 (400µg) reduced migraine-related disability and frequency and severity of migraine with aura in double-blind RCTs at a 2mg daily dose [190,191], but not at 1mg [192]. In this trial, the C allele carriers of the MTHFR C677T variant and the A allele MTRR A66G (methionine synthase reductase) had a greater clinical effect and greater reductions in homocysteine levels [191].

Some preliminary evidence also supports the potential prophylactic effect of niacin (nicotinic acid or vitamin B3) [193].

Magnesium acts as a cofactor for as many as 300 enzymes and plays a vital role in energy metabolism. Its blood levels are significantly reduced in migraine [34,42,194] and a recent meta-analysis of randomized controlled trials supports a modest but significant effect of intravenous magnesium on acute attack and of oral magnesium on attack frequency and intensity [195]. A recent cross-over RCT showed that 500mg magnesium oxide had a similar preventive effect in migraine as 400mg sodium valproate [196].

A ketogenic diet (KD) promotes the hepatic production of an alternative energy substrate for the brain and to some extent mimics the state of fasting. Ketone bodies (KB: beta-hydroxybutyrate and acetoacetate) are not GLUT1 dependent and ketosis has a variety of other potentially beneficial effects for migraine pathophysiology, such as increased mitochondrial biogenesis, increased antioxidant capacity, upregulation of glucose (GLUT-1) and KB transporters, activation of inward rectifying potassium channels (metabolically sensitive K(ATP) channels) stabilizing neural excitability, increase of GABA but inhibition of glutamate transport and hence reduction of excitatory synaptic transmission, as well as of pain and inflammation (see review {Citation}). Several case studies have shown the migraine protective effects of ketosis [197–202]. A one month observational study of KD in 96 migraineurs as part of a weight loss program disclosed a reduction of up to 80% in attack frequency, severity and acute medication use [201]. The same intervention in 18 episodic migraineurs reduced migraine days by 62.5%, which was accompanied by a normalization of the interictal habituation deficit of visual evoked responses [202]. The same authors (Di Lorenzo et al., 2017, submitted) just completed a double-blind study in a population of 35 overweighed episodic migraine patients comparing two 1-month cross-over periods of a very low-calorie ketogenic diet and a very low-calorie non-ketogenic diet. The ketogenic diet was significantly superior to the non-ketogenic diet for reduction in monthly migraine days and 50% responder rate (74.3% and 8.6% respectively).
The potential preventive anti-migraine effect of supplementation with beta-hydroxybutyrate without a strict dietary change, is currently being examined in a RCT [203] .

Aerobic exercise is often recommended in migraine management. A recent open randomized study showed that regular aerobic exercise was associated with a decrease in migraine frequency comparable to that achieved with the level A prophylactic drug topiramate [204]. The metabolic effects of aerobic exercise mimic those of ketosis with upregulation of multiple proteins acting in brain energy metabolism, including enzymes involved in glucose catabolism, ATP synthesis and hydrolysis, and glutamate turnover [205]. A striking recent finding is that exercise training increases the number of mitochondria not only in muscle but also in the brain of mice [206].

Prophylactic treatment - pharmaceuticals

The precise mechanism of action of most “classical” drugs used in migraine prophylaxis is not known. Many of them down-regulate neural reactivity and might therefore reduce the energetic demands of the brain. Certain prophylactic agents can also have direct metabolic effects. Topiramate, for instance, a preventive drug with level A evidence for efficacy, protects against oxidative stress, inflammation [207] and mitochondrial membrane depolarization; it increases the activity of the mitochondrial respiratory chain complex, prolongs mitochondrial survival [208], slightly increases lipolysis in children [209] and has an insulin-sensitizing effect on adipocytes in female rats [210]. Amitriptyline (level B) also reduces markers of oxidative stress and increases antioxidant capacity [68]. Valproate (level A)

attenuates nitroglycerin-induced trigeminovascular activation by preserving mitochondrial function in a rat model of migraine [211] and increases mitochondrial biogenesis [212]. Amitriptyline and flunarizine significantly increase serum levels of leptin, insulin and BMI after 12 weeks of therapy in migraine patients [213]. Gabapentin, atenolol, verapamil, valproate, pizotifen and amitriptyline all increase weight in a substantial number of patients after 6 months of use [214]. Beta-blockers decrease whole-body metabolic rate, body fat and fat-free body [215], thereby leaving in theory more energy for the brain. Also, by regulating norepinephrine-mediated energy reserve consumption [103] during stress situations, beta blockers could reduce the rebound headache that often follows stressful events in migraineurs [216]. The double action of prophylactic drugs thus favours the equilibrium between metabolic needs and metabolic offers that is necessary in order to maintain cerebral homeostasis.

In summary, most therapeutic agents used in migraine prevention are able to influence metabolism and mitochondrial functioning (for potential mechanisms of action see figure 1). Nutraceuticals and dietary or pharmacological ketogenesis specifically target the metabolic facets of migraine pathophysiology and are a promising path to pursue because of their exquisite efficacy/adverse effect profile.

5. Link to migraine pathophysiology

In the previous sections we have highlighted the large body of clinical evidence favouring a crucial role of energy metabolism and mitochondrial functioning in migraine pathophysiology. In this section we will examine how such abnormalities may lead to a migraine attack and relate to other pathophysiological features of migraine. For this purpose, we will mainly draw upon experimental data obtained in rodents.

Hypothalamic and brain stem activation (thought to initiate or modulate the attack), cortical spreading depression (responsible for the aura) and trigeminovascular activation (causing the headache and associated symptoms) are the hallmarks of the pathophysiological migraine cascade.

Hypothalamic and brainstem activation

As mentioned above, the hypothalamus is activated early in the attack initiation, during the premonitory symptoms [78,79]. What activates the hypothalamus is not known: an intrinsic biorhythm, an environmental trigger or stimulation by a CNS centre highly connected to the hypothalamus, like the amygdala. Because of its role in metabolic homeostasis, the hypothalamus has the capacity to sense a metabolic disequilibrium in the brain thanks to the presence of chemosensitive neurons as well as in peripheral blood partly because some hypothalamic areas lack a proper blood-brain barrier. Chemosensitive neurons, notably to oxygen, form a network that extends from the thalamus to the brain stem [217]. With regards to the possible role of the amygdala, mitochondrial function and the sexual dimorphism of migraine, the following data is of interest. In the human and mouse basolateral amygdala, mitochondrial-related gene groups were identified as the top biological pathways associated with sexual dimorphism, featuring a regulatory cascade of mitochondrial function and circadian rhythm, potentially linked through sirtuins and hormone nuclear receptors. In females mitochondrial-related gene groups were down-regulated while genes regulating the circadian clock were up-regulated [218].

Cortical spreading depression (CSD)

CSD is the pathophysiological culprit for the migraine aura. CSD susceptibility is strongly modulated by metabolic factors. Cerebral glucose availability modulates extrinsically induced CSD in both directions [219,220]. On the one hand, hypoglycaemia significantly prolongs CSD duration [219] and inhibition of cerebral glycogen reduces CSD threshold in vivo [220]. On the other hand, hyperglycaemia protects the tissue from CSD induction [219]. Supplying the rat brain with an alternative energy substrate to glucose via both short- and long-term treatment with a middle chain triglycerides (MCT)-enriched ketogenic diet has a similar protective effect against CSD [221].

Hypoxia influences negatively energy metabolism and can trigger CSD [222,223]. Amongst others, it inhibits astroglial mitochondrial respiration, leading to mitochondrial depolarization, the production of free radicals, lipid peroxidation and release of calcium ions from the intracellular stores in mice and rats [224]. When hypoxia is preceded by pharmacological mitochondrial inhibition the occurrence of hypoxia-induced CSD in rat hippocampal slices is greatly facilitated [222], providing a mechanism by which a genetic or acquired mitochondrial dysfunction could exacerbate the impact of a metabolic stressor.

Trigeminovascular system activation

In experimental animals, *CSD* is able to activate the trigeminovascular system [225] . One mechanism by which this occurs can be the opening of Panx-1 large pore channel in neurons leading downstream to activation of the inflammatory pathway in astrocytes and via cytokines and prostanoids to sensitisation of meningeal nociceptors [226] (figure 2).

CSD itself and subsequent restoration of ion homeostasis are extremely energy demanding [227]. Moreover, CSD causes tissue hypoxia in mice [223,228] and increases mitochondrial uncoupling proteins, which decreases ATP synthesis and increases thermogenesis [229], creating a vicious circle. CSD also induces oxidative stress, such as hydrogen peroxide, in the trigeminal nociceptive system [230]. Hydrogen peroxide can activate transient potential (TRPA1) and acid-sensing (ASIC) channels and hence promote the release of CGRP by meningeal nociceptors, which is known to be pivotal in mediating the headache of the migraine attack [231–233] (figure 2).

Clinically, the majority of migraine patients never experience an aura. Other triggers for the trigeminovascular system must therefore exist. TRP channels, expressed in meningeal nociceptive nerve terminals, may contribute to the migraine attack generation [234] since they can be directly activated by a variety of exogenous and endogenous agents known to associated with migraine and induce the release of CGRP: they can sense reactive species either indirectly through second messengers or directly via oxidative modification of cysteine residues [235]; they are inhibited or desensitised by abortive migraine drugs [234]; the subchannel TRPA1 is particularly activated by oxidative, nitrosative and electrophilic stress [234,235], thereby providing a mechanism by which known migraine trigger factors that increase *oxidative stress* (see section 2.2) could lead to migraine pain.

Cerebral energy deficiency via inhibition of glycogen use in the brain was recently shown to cause CSD-independent Panx-1 large pore channel opening in neurons [220], a mechanism leading, as abovementioned, to activation of meningeal nociceptors [226]. That metabolic changes can also directly modify activity of central trigeminovascular nociceptors was recently demonstrated by a study where blood glucose changes after injections of insulin, glucagon or leptin were associated with a change in baseline firing of dural responsive nociceptive neurons in the spinal trigeminal nucleus [236].

Mitochondrial activity can be modulated by *nitric oxide* (NO) via a variety of different mechanisms [237], which might explain the delayed headache experienced by migraine patients after NO-donor administration [238]. For example, NO inhibits the mitochondrial respiratory chain in cultured astrocytes

[239] as well as glucose transport and metabolism, and lactate production in vivo [240]. By contrast, an increase of cortical lactate was found with ^{1}H-MRS in the nitroglycerin model of migraine in rats [241]. Higher NO concentrations were found to stimulate calcitonin-gene related peptide (CGRP) release [242].

Finally, there is scarce evidence from animal experiments that the migraine attack itself might impact on mitochondrial energy metabolism. In the rat migraine model of chronic migraine using dura mater applications of an inflammatory soup, abnormal mitochondrial dynamics and impaired mitochondrial biogenesis were demonstrated in the trigeminal ganglion [243,244] .

CGRP – A compensatory response to energy deficiency?

CGRP is known to play a role in both spontaneous and triggered migraine headache generation [231,232]. It is elevated in blood during attacks [245], triggers attacks in migraine patients [246] and blocking its action transiently (with CGRP antagonists) or durably (with monoclonal antibodies) respectively aborts the attack or reduces attack frequency [247]. As a consequence, CGRP is overwhelmingly considered the villain in migraine pathophysiology. We would like to challenge this perception by raising the question whether CGRP release in response to oxidative stress or cerebral energy disequilibrium might also be part of an adaptive response. In CGRP-triggered attacks, an aura occurs only in 28% of MA patients [248] and, contrary to PACAP, CGRP does not elicit premonitory symptoms [114], which suggests that it does not trigger a complete migraine attack, but rather represents the physiological correlate of the headache pain and the headache-related behaviour in migraine. Moreover, CGRP is widely distributed in the brain stem and diencephalon, including in a network involved in energy homeostasis comprising hypothalamic nuclei, locus coeruleus, area postrema, and nucleus tractus solitarius [249]. Several of these nuclei, in particular the circumventricular organs, do not have a functional blood-brain barrier and are thus amenable to systemic CGRP therapies, including the large monoclonal antibodies against CGRP or its receptor. CGRP has anti-oxidant and anti-inflammatory actions [250–253], which supports the assumption that its release may also mediate an adaptive response to potentially harmful oxidative stress levels and / or energy deficiency. That CGRP may contribute to increase endogenous energy availability for the brain is supported by rodent studies showing that CGRP can inhibit insulin stimulated glucose transport [254], decrease tolerance to glucose in the GTT without altering plasma insulin levels [255], inhibit muscle glycogen synthesis and cause insulin-resistance upon activation of skeletal muscle sensory nerves [256]. Intravenous injections of CGRP in the rat cause a significant increase in plasma glucose concentration [255], which can further contribute to restoring energy homeostasis.

Pituitary adenylate cyclase-activating peptide (PACAP), like CGRP, is released during migraine attacks and induces migraine-like headache in migraineurs; it is therefore another promising molecular target for migraine treatment. Besides its presence in the trigemino-parasympathetic viscero-autonomic circuit, where it contributes to the headache pain and associated autonomic symptoms, it has an important role in the hypothalamus where it modulates circadian rhythms and food anticipatory behaviour (review by [249]). In rat it was shown to stimulate glucose production via the sympathetic hepatic innervation [257].

In summary, cerebral energy deficiency and / or increased oxidative stress decrease CSD threshold and are able to activate TRP and ASIC channels that stimulate CGRP and PACAP release. These peptides are pivotal in eliciting the migraine headache and associated symptoms, but we hypothesize that they induce in parallel an antioxidant response and a variety of metabolic changes, which together with

energy-conserving behavioural changes, decrease oxidative stress levels and increase glucose and ketone body availability for the brain to help restore energy homeostasis (see figure 2).

6. Perspectives

Studies combining assessments of brain/mitochondrial metabolism and of sensory processing, similar to that by Lisicki et al (2018) [52], are necessary to disentangle the metabolico-functional conundrum. In such studies, age-related adaptive increases of glucose uptake in the brain stem and in visual areas like the fusiform gyrus have to taken into account [258]. By the same token, studies examining the role of specific alterations in mitochondrial functioning and / or energy metabolism in migraine subgroups are clearly needed. There is also insufficient data on the impact of improved energetics, e.g. via therapeutic interventions that are known to improve mitochondrial functioning such as ketosis, on sensory processing and cerebral energy availability using P-MRS and their correlation with treatment response.

Meanwhile, the findings described in this review could have several potential therapeutic implications. We would like to suggest a four-step approach to improve mitochondrial functioning and energy metabolism in migraine.

1) Individualizing supplementation of micronutrients: to ensure all micronutrients for proper mitochondrial functioning are available, individualized supplementation of insufficient minerals, hydrophobic and lipophilic vitamins and trace minerals based on lab tests may be a rewarding approach [184].

2) *Reducing oxidative stress and increasing antioxidants:* measuring oxidative / nitrosative stress levels and antioxidant status in individual patients could detect a potential mismatch between oxidative stress levels and antioxidant capacity and lead to therapeutic adjustments, but studies proving that such an approach improves migraine management are lacking. Strategies to reduce oxidative stress could include elimination or reduction of processed and high glycaemic foods and alcohol, use of green or blue light filtering glasses [259], interruption of hormone-based contraception, life-style changes and addition of antioxidants, such as polyphenols, CoQ10, alpha-lipoic acid or beta-hydroxybutyrate mineral salts.

3) Stabilizing blood glucose: an oral GTT should be performed in migraine patients with suggestive clinical features of glucose intolerance or family history. Patients with reactive hyperinsulinemia and reactive hypoglycaemia are likely to profit from stabilising blood glucose levels. This can often be achieved with dietary adjustments.

4) Providing an alternative energy substrate for the brain: in patients with a very compromised energy metabolism, an alternative fuel source for the brain in addition to glucose and lactate, might be beneficial, such as a ketogenic diet [198,201,202] and /or use of exogenous ketogenic substances like middle chain triglycerides or exogenous ketone body salts like beta-hydroxybutyrate. Further placebo-controlled trials are necessary validate ketogenic therapies in migraine.

Conclusion

This review indicates that commonly reported migraine trigger factors can be linked to an energy disequilibrium and oxidative stress, and that numerous biochemical and genetic studies point towards a variety of different metabolic abnormalities in migraine. Most preventive migraine treatments are able to enhance metabolic functioning, in addition to their possible effect on brain responsivity and excitability. A disruption of cerebral metabolic homeostasis is a plausible explanation

for the ignition of the brain's major alarm system: the trigeminovascular system and its limbic connections, via CSD induction, stimulation of chemosensitive brain stem neurons or direct activation of TRP/ASIC channels that stimulate CGRP and PACAP release by meningeal nociceptive fibers. The released neuropeptides are likely the culprit for the migraine headache and associated symptoms, but they could also participate in an antioxidant response and a variety of metabolic changes that help restore energy homeostasis.

The findings described in this review have several potential therapeutic implications, such as the individualized supplementation of micronutrients, reduction of oxidative stress and increase in antioxidants, stabilizing blood glucose levels and providing an alternative energy substrate for the brain. Some progress has been made in metabolic anti-migraine therapy in the past and novel strategies, such as ketone body supplementation, are being explored. More research is needed on different metabolic subtypes, a potential association between metabolic pheno-/ genotype and treatment response to metabolic agents, sensory–metabolic interactions and metabolic nutraceutical treatments in migraine.

From the metabolic perspective, migraine seems to be a conserved adaptive response [2] that helps reduce potentially harmful oxidative stress levels and restore brain energy homeostasis, a concept already sensed by Edward Living in 1873[260].

Proposed display items:

Boxes:

Box 1

What is the link between altered bioenergetics and trigeminovascular activation, and thus migraine headache?

Numerous studies using different approaches have repeatedly evidenced alterations in energy metabolism at the cortical level in migraine patients (this review). In a similar way, a vast line of research has reproducibly shown that migraineurs exhibit an increased cerebral reactivity to sensory stimuli for almost every sensory modality [261]. Put together, these lines of evidence suggest that the brain of migraine patients does not only have reduced energetic offers at the cortical level, but that it also has increased energetic needs. This possibility has been evaluated in one study in which the ratio between the magnitude of visually evoked cortical responses and cerebral glucose uptake in the visual cortex was compared between migraine patients and healthy controls [52]. Results from this study showed an almost three-fold higher ratio in migraine patients, which suggests that their metabolic reserves may be barely sufficient to cope with the increased energetic needs, a scenario that would eventually render them vulnerable to a disruption in cortical homeostasis. However, one piece of the puzzle would seem to be missing: *How can alterations in cortical homeostasis trigger migraine headache?* The most plausible answer involves Pannexin channels. These megachannels in neurons open under certain conditions of distress, acting like real sensors of cortical homeostasis. Once they are opened, a cascade of biochemical events involving molecules that belong to the alarmin family takes place. The final result of this cascade is trigemino-vascular activation and CGRP release in the extra-dural space [226]. In particular, reducing the metabolic substrates available for neurons in the cortex was shown to directly activate Pannexin 1 channels, which constitutes a possible explanation for the pathogenesis of migraine without aura [220] Similarly, metabolic distress lowers the threshold for cortical spreading depression [220], which can aggravate metabolic alterations and cause migraine with aura. Alternatively, diencephalic and brain stem chemosensitive neurons [217] could sense metabolic changes and sensitise directly or via descending pathways the trigeminovascular system. Therefore, Pannexin 1 channels, P2X7 receptors, and the subsequent cascade of molecular events that follows their activation constitutes a plausible 'missing link' between energy disequilibrium at the cortical level and the migraine attack.

Figures:

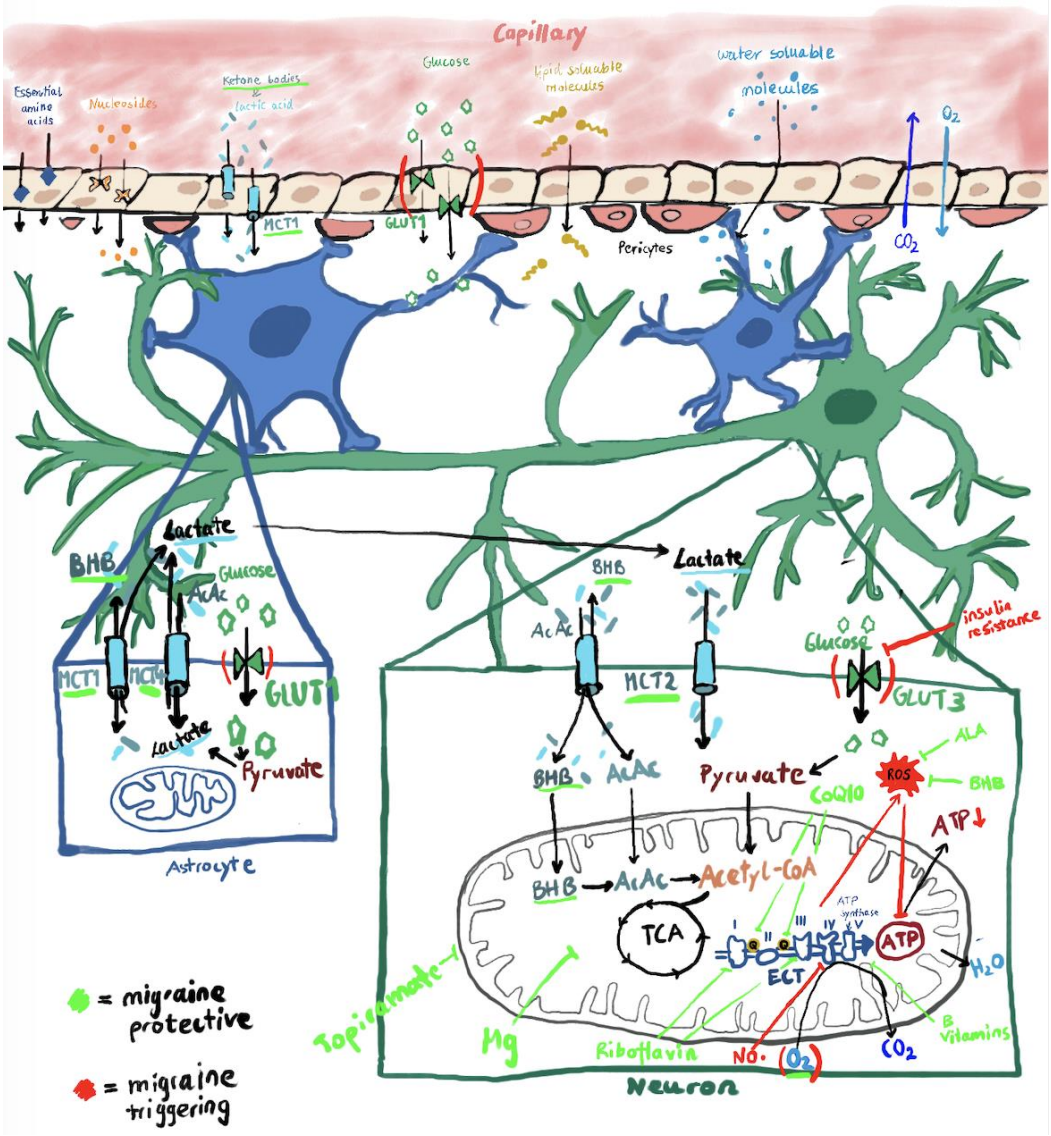

Figure 1

Cerebral metabolomics potentially involved in migraine pathogenesis and therapeutic targets

Glucose crosses the blood-brain barrier (BBB) via insulin-independent glucose transporter 1 (GLUT1), whose deficiency can contribute to migraine, as can insufficient glucose, oxygen, water or minerals. GLUT1 is expressed both by capillary endothelial cells and astrocytes. Non-oxidative glucose utilization produces pyruvate, which is converted to lactate and shuttled to neurons through monocarboxylate transporters (mainly MCT1 and MCT4 in astrocytes and MCT2 in neurons). In neurons this lactate can be used as an energy substrate following its conversion to pyruvate that can be converted into acetyl-CoA, fed in turn into the Krebs cycle (TCA). Neurons can also take up glucose via the neuronal glucose transporter 3 (GLUT3) that is insulin dependent and can thus be influenced by insulin resistance. Ketone bodies (beta-hydroxybutyrate, BHB and acetoacetate, AcAc) cross the BBB via MCT1 transporters, and penetrate astrocytes via MCT1/MCT4 and neurons via MCT2. BHB provides an alternative substrate to glucose for oxidative phosphorylation; it is converted to AcAc and subsequently in the mitochondria to acetyl-CoA, which feeds into the Krebs cycle (TCA) to produce ATP. BHB also has anti-oxidant properties and, as compared to glucose, its conversion to ATP produces less reactive oxygen species (ROS) per oxygen molecule (O_2) consumed. Increased ROS, nitric oxide (NO), lack of energy substrates or lack of necessary co-enzymes inhibit proper mitochondrial functioning and in turn reduce ATP levels. Antioxidants and co-enzymes, such as riboflavin, other B-vitamins, magnesium (Mg), alpha-lipoic acid (ALA), coenzyme CoQ10 (CoQ10), as well as the anticonvulsant topiramate, support mitochondrial functioning and have been shown to be migraine protective.

AcAc = acetoacetate, ALA = alpha-lipoic acid, AQP4 = aquaporin 4, ATP = adenosine triphosphate, BBB = blood-brain barrier, BHB = beta-hydroxybutyrate, CO_2 = carbon dioxide, CoQ10= coenzyme CoQ10, ECT = electron transport chain, Fe^{2+} = iron ions, H_2O = water, GLUT1/ 3 = glucose transporter 1/3, K^+ = potassium ions, MCT1/2/4 = monocarboxylate transporters, mg = magnesium, Mn^{2+} = manganese ions, Na^+ =sodium ions, NO= nitric oxide, O_2 = oxygen, ROS= reactive oxygen species, TCA = Krebs cycle, ZN^{2+} = zinc ions

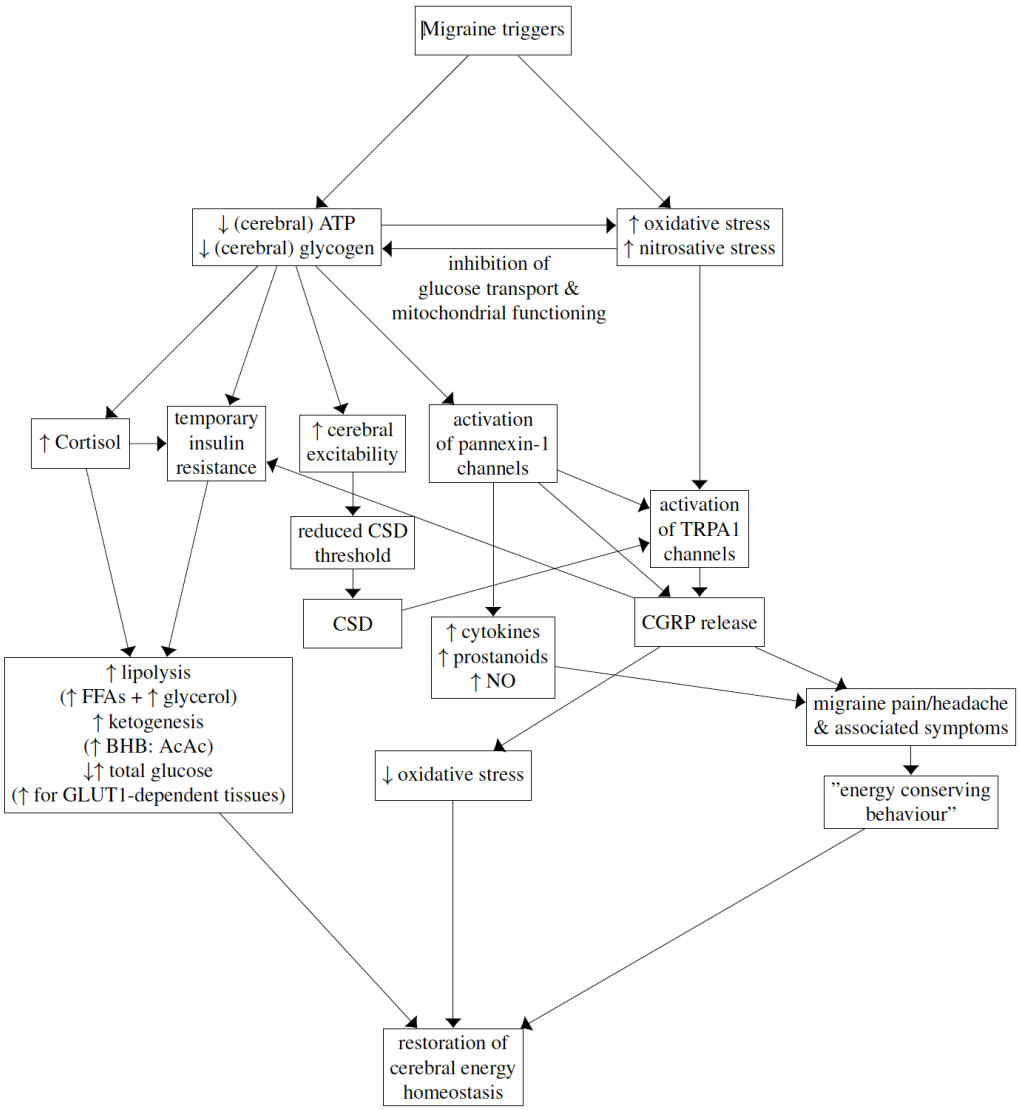

Figure 2

Metabolic facet of migraine attack generation and resolution

In genetically predisposed individuals, migraine triggers can lead to elevation of oxidative / nitrosative stress and/or a reduction in cerebral ATP and glycogen. This ignites a cascade of events that on the one hand favours CSD and results in trigeminovascular activation (via the activation of TRPA1 or ASIC and pannexin-1 channels, which subsequently release CGRP and PACAP), but on the other hand may also assist the restoration of cerebral energy homeostasis (via increased lipolysis and ketogenesis, reduction in oxidative stress and energy conserving behaviour, increased cortisol and temporary insulin resistance). The factors that are known to participate in migraine attack generation might also be involved in its resolution.

AcAc = acetoacetate, ASIC = acid sending ion channel, BHB = beta-hydroxybutyrate, CSD = cortical spreading depression, CGRP = Calcitonin gene-related peptide, FFA = free fatty acids, GLUT1 = glucose transporter 1, NO = nitric oxide, PACAP = pituitary adenylate cyclase-activating peptide, TRPA1 = transient receptor potential channels A1

Tables:

Table 1. Neuroimaging studies showing metabolic alterations in migraine patients.

Reference	Study Population	Methods	Findings
Welch et al. 1989 [29]	8 MA, 12 MO, 27 HC	^{31}P-MRS	↓ phosphocreatine to inorganic phosphate ratio during the attacks
Barbiroli et al. 1990 [39]	4 MA (prolonged aura), 4 MS, 15 HC	^{31}P-MRS	↓ phosphocreatine to inorganic phosphate ratio in all patients Mitochondrial metabolic defects also present in platelets and muscle
Barbiroli et al. 1992 [262]	12 MA, 12 HC	^{31}P-MRS	↓ phosphocreatine content in all patients ↓ phosphorylation potential ↑ ADP concentration
Montagna et al. 1994 [2]	22 MO, 18 HC	^{31}P-MRS	↓ phosphocreatine ↓ phosphorylation potential ↑ ADP concentration
Lodi et al. 1997 [38]	7 MA, 5 MA (brainstem aura), 3 MA (prolonged aura), 12 HC	^{31}P-MRS	↓ phosphocreatine and ↑ inorganic phosphate in all patients ↑ ADP concentrations and relative rate of mitochondrial oxidation in migraineurs
Schulz et al. 2007 [37]	10 MA, 11 HM, 16 HC	^{31}P-MRS	↓ phosphocreatine/inorganic phosphate ratio with increasing aura duration & in HM
Lodi et al. 2001 [34]	7 MS, 13 MA (prolonged aura), 37 MA or MA (basilar aura), 21 MO, 36 HC	^{31}P-MRS	↓estimated free energy released by ATP hydrolysis in migraineuers ↓↓ values of estimated free energy released by ATP hydrolysis in MS and highest in MO
Reyngoudt et al. 2011 [41]	19 MO, 25 HC	^{31}P-MRS	↓ in ATP (approximately 15%) ↓↓ATP concentrations in patients who had the highest attack frequency
Watanabe et al. 1996 [44]	3 MA , 1 MA (basilar aura), 2 M + infraction, 6 HC	^{1}H-MRS	↑ Lactate levels in patients with 'active' migraine (5)
Sándor et al. 2005 [43]	5 MA (visual), 5 MA or HM , 11 HC	^{1}H-MRS	↑ Lactate levels in migraine with visual aura
Schulz et al. 2007 [37]	10 MA, 11 HM, 16 HC	1H-MRS	No differences in lactate levels
Prescot et al. 2009[46]	10 EM, 8 HC	^{1}H-MRS	No differences in lactate levels
Reyngoudt et al. 2011 [41]	20 MO, 20 HC	^{1}H-MRS	No differences in lactate levels
Mohamed et al. 2013 [47]	22 MO, 10 HC	^{1}H-MRS	↑ Lactate levels in migraineurs in relation with disease duration and attack frequency
Arngrim et al. 2016[21]	15 MA, 14 HC	^{1}H-MRS	No differences in lactate levels
Becerra et al. 2016 [48]	17 MO, 15 MA, 33 HC	^{1}H-MRS	No differences in lactate levels
Kim et al.2010 [31]	20 MO, 20 HC	^{18}FDG-PET	↓ metabolism in several cerebral regions involved in pain processing
Lisicki et al. 2018 [52]	20 MO, 20 HC	^{18}FDG-PET	↓ glucose uptake in the visual cortex with respect to the magnitude of visually evoked responses

EM = episodic migraine, HC = Healthy controls, HM = hemiplegic migraine, M = migraine not otherwise specified, MA = migraine with aura; MO = migraine without aura, MS = migraine + stroke

Table 2. Human peripheral blood studies showing metabolic abnormalities in migraine.

Reference	Study population	Method	Finding
Mitochondrial functioning			
Littlewood et al., 1984 [53]	48 M (male) & 30 HC (male)	Platelet MAO & SDH activity ↓	↓ MAO & SDH activity in M compared to HC
Montagna et al., 1988 [55]	4 M, 5 with migrainous stroke	Mitochondrial energy metabolism markers in blood & muscle	↑ blood lactate during effort, ↓ activity of some respiratory chain enzymes, in 2 patients ↓ CCO, SCCR & NADH-CCR
Sangiorgi et al., 1994[54]	40 MA, 40 MO & 24 HC	Platelet mitochondrial enzyme activities	NADH-D & CS ↓in MA & MO compared to HC; ↓ NADH-CCR only in MA; no alteration in SDH
Okada et al., 1998[56]	14 M & 12 HC	Lactic & pyruvic acid levels in plasma	↑ Lactic & pyruvic acid levels in M compared to HC
Oxidative stress			
Shimomura et al., 1994 [66]	30 MO, 9 MA, 53 TTH, 30 HC	Concentrations of platelet SOD & activity of platelet SOD	↓ platelet SOD in MO & MA,↓ SOD activity in MA, but not MO or TTH
Tozzi-Ciancarelli et al., 1997 [67]	23 MA, 23 HC	Plasma TBARS, in vitro platelet behaviour resting & after thrombin stimulation	↑ TBARS in M, ↑membrane rigidity, ↓ cytosolic calcium in resting condition & after thrombin stimulation & ↓ aggregatory responses to ADP & collagen in MA platelets
Ciancarelli et al., 2003 [61]	30 MO/MA, 20 HC	24 h ictal & interictal urinary NOx & TBARS	Interictally: ↑ NOx & TBARS in M compared to HC & ↑ NOx compared to ictally or post-ictally, ictally: ↑ TBARS compared to interictally, no differences between MO & MA
Bolayir et al., 2004 [60]	11 MA, 17 MO, 32 TTH, 28 HC	GPx, catalase & SOD enzyme activities	Interictally: ↓ SOD & GPx enzyme activities in MA & MO than in TTH & HC
Ciancarelli et al., 2007 [62]	20 CM, 20 HC	Serum peroxides, NO & SOD	↓ NOx levels & SOD activity in CM than HC, ↑ peroxide levels in CM than HC
Yilmaz et al., 2007 [70]	22 MO, 14 MA, 20 HC	Platelets NOx, MDA, & thiol groups	Ictally: ↑ platelet levels of nitrate, nitrite & MDA in M compared to HC, Interictally: difference not sign.
Tuncel et al., 2008 [69]	37 MO, 19 MA, 25 HC	Erythrocyte SOD, catalase activity & MDA	↑ MDA levels in M compared to HC, ↑ SOD activity in MA compared to MO
Alp et al., 2010 [57]	75 MO, 65 HC	TAS, TOS of the plasma & OSI	↓ TAS, ↑ TOS & OSI in MO interictally compared to HC, OSI correlated with disease severity
Bernecker et al., 2011 [59]	48 MO, 17 MA, 48 HC (all female)	MDA, HNE, carbonylated proteins, parameters of associated NO stress, inflammation, lipid- & glucose-metabolism	Interictally: ↑ insulin, HOMA-indices, LDL, triglycerides, oxidized LDL, NOx, nitrate, HNE in M compared to HC, fasting BG, nitrite, nitrosylated proteins, MDA & carbonylated proteins levels not sign. different between M & HC
Aytaç et al., 2014 [58]	18 M with WMH, 14 M without WMH, 17 HC	MDA, SOD, GPx & catalase in serum	↓ catalase & ↑ MDA in all M, ↓ catalase in MWMH compared to M without WMH & HC
Eren et al., 2015 [63]	74 MO, 77MA, 70 HC	TAS, TOS & OSI, serum thiol levels	↓ thiol levels in M than compared to HC, which negatively correlated with M disability, no sign. difference in TAS, TOS, OSI between M & HC
Geyik et al., 2016 [64]	39 MO, 11 MA, 30 HC	TOS, TAS & OSI, & 8-OHdG in plasma	↑ 8-OHdG in M compared to HC; ↑ 8-OHdG was sign. more prominent in MO than MA, no sign. differences in TAS, TOS, & OSI between M & HC
Gumusyayla et al., 2016 [65]	63 MA&MO, 50 HC	Total thiol (-SH+-S-S-) & native thiol (-SH) levels in serum	↑ total & native thiol levels in M compared to HC
Tripathi et al., 2018 [68]	136MO, 14 MA, 100 HC	120 M rTMS & 30 M Amitriptyline, GSH GST & TAC before & after treatment	↓ GSH, GST & TAC levels in M compared to HC, ↑ GSH & TAC levels after treatment irrespective of modality & the increase correlated with treatment success
Glucose metabolism			
Blau & Cumings, 1966 [74]	12 M	19 hours water fast	50% developed migraine 11-14 h after fast onset, ↓ BG to 44 -65mg/dl in all of them
Pearce, 1971 [80]	20 MA & 10 HC	Insulin-induced hypoglycaemia	↓ BG to 20.4mg/dl in MA & 24.3mg/dl in HC. 2 of 20 migraine sufferers developed an aura (one with headache) within 2 hours after insulin
Hockaday et al. ,1971 [81]	10 M (with fasting /undue hunger associated migraine attacks)	50g oral GTT after 10h fast, 8 h observation	Migraine attacks in 6/ 10 (60%) within 4 h, ↑ FFAs & KB in all of them; no difference in glucose & glycerol levels between attack & no attack
Shaw et al., 1977 [82]	6 MA	Ictal & inter-ictal IV GTT	During attacks: ↑ glucose at 30,45 & 75 min, ↑ KB, FFAs, glycerol, cortisol, GH levels, & BHB:AcAc ratio, ↓ insulin; no sign. difference in lactate & pyruvate
Hsu et al., 1977 [102]	13 early morning onset M	Nocturnal blood analysis 3 hours prior to waking from migraine compared to non-migraine night	↑ Noradrenaline & adrenalin, glucose, FFA & insulin levels stable 3 h prior to awakening with migraine
Dexter et al., 1978 [83]	74 M (with fasting associated migraine attacks)	100g oral GTT	6 patients classified diabetic & 56 patients showed degrees of reactive hypoglycemia, i.e. BG of less than 65 mg/dl or a drop of 75 mg/dl within 1h
Cavestro et al., 2007 [87]	58 MO, 6 MA, 20 M + HT, 25 HT, 26 HC	75g oral GTT after 12h fast, 2h observation	↑ BG at baseline & up to 1 h post GTT in M, ↑ insulin levels at all time points compared to HC & other headache, 65% of migraineurs showed IR.
Sacco et al., 2014 [89]	50 MA, 50 MO, 50 HC	Fasted BG & insulin analysis, IR calculated with HOMA-IR, HOMA-B, QUICKI	Fasting BG ↑ in MA & MO, highest in MA, no differences in insulin & measures of IR
Kokavec 2015 [90]	12 M (7 f, 5 m), 8 HC	75g oral STT after 15h overnight fast, insulin, BG & cortisol over 150min period	↑ cortisol at baseline in M, no sign. difference in fasting insulin & BG, following STT: ↑ insulin in female M; ↑ cortisol in male M
Siva et al 2017 [91]	83 non-obese female migraine patients (EM & CM), 36 HC	75g oral GTT	↑ Fasting BG in M, ↑ IR in CM compared to EM or HC, ↑ neuropeptide Y in M
Wang et al., 2017 [84]	86 M, 95 HC	75g oral GTT, 2h observation, IR calculated with HOMA-IR, HOMA-B, QUICKI	No sign. differences in HbA1c, IR & IS between M & HC, only between prediabetic M and prediabetic HC, possible negative association between diabetes & M

8-OHdG = 8-hydroxy-2'-deoxyguanosine, AcAc = acetoacetate, BG = blood glucose, BHB = beta-hydroxybutyrate, CCO = cytochrome-c-oxidase activities, CCO = cytochrome-c-oxidase, CM = chronic migraine, CS = citrate synthase, EM = episodic migraine; FFA = free fatty acids, GH = growth hormone, GPx = Glutathione peroxidase, GSH =glutathione, GST = glutathione-S-transferase, GTT = glucose tolerance test, HC = healthy controls, HNE = 4-hydroxy-2-nonenal, HOMA-B = β-cell function, HOMA-IR = the homeostatic model assessment of insulin resistance, HT = different headache type to migraine, IR = insulin resistance, IS = insulin sensitivity, IV = intravenous, KB = ketones bodies, LDL = low-density lipoprotein, M = migraine not otherwise specified; MA = migraine with aura; MAO = monoamine oxidase, MDA = malondialdehyde, MO = migraine without aura, NADH-D = NADH-dehydrogenase, NADH-CCR= NADH-cytochrome-c-reductase, NO = nitric oxide, NOx = NO stable metabolites, OSI = Oxidative Stress Index, QUICKI = the quantitative insulin sensitivity check index, rTMS = repetitive transcranial magnetic stimulation therapy, SCCR = succinate-cytochrome-c-reductase, SDH= succinate dehydrogenase, sign. = statistically significant, SOD = superoxide dismutase, STT = sucrose tolerance test, TAC = total antioxidant activity, TAS = Total Antioxidant Status, TBARS = thiobarbituric acid-reactive substances, TOS = Total Oxidant Status, TTH= tension type headache, WMH = white matter hyperintensities

Table 3. Genetic studies supporting metabolic abnormalities in migraine.

Reference	Study population	Method	Finding
Mitochondrial DNA			
Klopstock et al., 1996[120]	23 MA	mtDNA mutation A3243G, G8344A, common deletion	Failed to detect any large-scale deletions or point mutations in MELAS/MERFF mutations of mtDNA
Russel et al., 1997[122]	30 MO, 30 MA, 30 HC	mtDNA mutation A11084G	11084 A to G base substitution of mtDNA not detected in Danes
Ojaimi et al., 1998[123]	36 MA, 34 unexplained ischaemic stroke, 6 HC	*Common mtDNA mutations* A3243G, G8344A, G3460A, T4160C, G11778A *Secondary LHON mtDNA mutations* A4136G, G5244A, G5460A, G7444A, G15257A, G15812A, T4216C, G13708A	None of the major disease- associated mutations in any of the patients or HC, of the secondary LHON mutations 8 MA harboured T4216C 8 & 3 G13708A
Majamaa et al., 1998 [128]	6 MS, 42 M, 48 HC	mtDNA mutations A3243G, G8344A, T8993C, G11778A, common deletions, mtDNA HGs	83% migraine-associated stroke, 19% M & 17% HC belonged to the mtDNA HG-U, none of these mtDNA mutations detected
Haan et al., 1999 [119]	7 MA, 9 MO, 10 SHM, 20 MS	mtDNA mutations A3243G, T3271C, A11084G, largescale deletions	No mutation found in these maternally transmitted M patients
Buzzi et al., 2000[116]	11 MO, 10 MA	mtDNA mutation A3243G	mtDNA A3243G MELAS mutation not associated with multigenerational female M
Takeshima et al., 2001[263]	123 MO, 43 MA, 483 HC	mtDNA mutation A11084G	frequency of A11084G 7.2% in Japanese M group & 7.3% in HC
Wang et al., 2004[129]	95 HC, 30 CVS, 32 MO, 18 MA, 35 HC with HG-H	homoplasmic nucleotide changes in hypervariable regions 1 of mtDNA control region	Within nt 16040–16188 segment, homoplasmic sequence variants were 3-fold more common relative to HC in CVS & MO, but not in MA
Rozen et al., 2004[121]	10 MA (prolonged aura)	*9 common mtDNA MELAS mutations:* A3243G, C3256T, T3271C, T3291C, A5814G, T8356C, T9957C, G13513A, & A13514G *3 secondary LHON mutations:* T4216C, A4917G, & G13708A	None of the mtDNA mutations found in MA
Zaki et al., 2009 [130]	30 CVS, 112 HG H MO, HG-H HC	Entire mitochondrial genome sequenced in 20 HG H CVS, polymorphisms of interest tested in 10 additional CVS & 112 HG-H MO	C16519T was highly disease associated: 70% of CVS subjects 52% of MO & 27% HC; G3010A was highly disease associated in subjects with C16519T: 29% of CVS & 26% of MO versus 1.6% of HC
Cevoli et al., 2010 [117]	2 maternal lineages with A3243G MELAS patients & M only	A3243G $tRNA^{Leu}$ mutational load in skeletal muscle & other somatic tissues	M-only subjects in MELAS maternal lineages lacked the MELAS mutation
Fachal et al., 2015 [118]	*First cohort:* 248 M & 310 HC *Second cohort:* 458 M & 384 HC	15 mtDNA SNPs	T4216C, G13708A & HG-J were associated with M in Spanish cohort, but this was not confirmed in second Spanish replication cohort
Others			
Kowa et al., 2000 [143]	52 MO, 22 MA, 47 TTH, 261 HC	MTHFR C677T polymorphism	Homozygous transition (T/T) in M (20.3%) was sign. higher than that in HC (9.6%) & especially high in MA (40.9%)
Lea et al., 2004 [144]	100 unrelated-MO, 168 unrelated-MA, 72 related-MO, 247 related-MA, 269 HC	MTHFR C677T polymorphism in related or unrelated migraine cases	677T allele over-represented in related M patients compared HC, specifically high in MA subtype (40% MA vs. 33% M), in unrelated Caucasians T/T genotype conferred a sign. increase in risk for MA, but not for MO
Kaunisto et al., 2006 [146]	898 MA, 800 HC	6 MTHFR polymorphisms	No differences in genotype distributions of SNPs C677T (MTHFR) in Finnish MA & HC
Hershey et al., 2012 [150]	18 MRM, 18 non-MRM, 20 HC	Genomic expression pattern was assessed from whole blood mRNA from MRM patients having an acute MRM or a non-MRM attack & HC	77 genes were identified that were unique to MRM, 61 genes were commonly expressed for MRM & non-MRM, & 127 genes were unique for non-MRM, many of those genes are related to mt functioning
Lee et al., 2015 [147]	75 CVS, 60 HC	Sequencing of over 1100 nuclear-encoded genes involved with energy production, metabolism, mitochondria, or ion channels	The only gene with sign. findings was RYR2, possibly involved in mt functioning
Palmirotta et al. 2015 [136]	246 MO, 107 MA, 137 CM, 246 HC	Polymorphisms of SOD1 gene (A/C substitution—rs2234694) & SOD2 gene (C/T transition—rs4880—Ala16Val).	The rs4880 TT (Val/Val) genotype was associated with presence of unilateral cranial autonomic symptoms & acute treatment type in MA
Eising et al., 2016 [149]	23,285 M, 95,425 HC	M GWAS data integrated with high-resolution spatial gene expression data of HC adult brains from the AHBA to identify brain regions & pathways	Enrichment of a migraine GWAS signal found for 5 modules that suggest involvement in migraine, two of which involve mitochondria in the cortex & subcortical areas
Stuart et al., 2017 [133]	*First cohort:* 80 related-MA+MO, 235 related-HC *Second cohort:* 554 unrelated-MA+MO, unrelated- 584 HC	Gene-centric association analysis of NEMP genes in most related Norfolk Island individuals; discovery phase: genes with 3 or more SNP associations ($P < 0.005$) to be investigated in replication phase in unrelated M–HC cohort.	3 NEMP genes shown to be associated with migraine in the replication cohort: CSNK1G3, ELOVL6 & SARDH, which are involved in phosphorylation, fatty acid metabolism, & oxidative demethylation, respectively

AHBA = Allen Human Brain Atlas, CM = chronic migraine, CVS = cyclic vomiting syndrome (a migraine-like variant), HC = healthy controls, HG = haplogroup, LHON = Leber's hereditary optic neuropathy, M = migraine not otherwise specified; MA = migraine with aura; MO = migraine without aura, MRM = menstrual-related migraine, MS = migrainous stroke, mt = mitochondrial, mtDNA = mitochondrial DNA, MTHFR = 5,10-methylenetetrahydrofolate reductase, NEMP=Nuclear Encoded Mitochondrial Protein, SHM = sporadic hemiplegic migraine, sign. =statistically significant, SNP = single nucleotide polymorphisms, SOD = superoxide dismutase

Table 4. Therapeutic studies supporting metabolic abnormalities in migraine.

Reference	Method	Dose	Study population	Finding
Riboflavin (B2)				
Schoenen et al., 1998[170]	3 months RCT double blind	400mg/ day	52 MO, 3 MA	↓ migraine attack frequency & migraine days
Boehnke et al., 2004 [167]	3/6 months open-label, uncontrolled	400mg/ day	17 MO, 6 MA	↓ migraine days & abortive migraine medication
Condo et al., 2009[168]	3/ 6 months retrospective	200 or 400mg/ day	41 paediatric & adolescent M	↓ migraine attack frequency
Bruijn et al., 2010 [171]	2X 16 weeks double-blind, crossover RCT	50mg/ day	42 paediatric & adolescent M	↓ migraine attack frequency
Rahimel et al., 2015	12 weeks RCT Double blind	B2 400mg/ day VAL 500mg / day	90 M	B2 = VAL in ↓ migraine attack frequency, but B2 ↓ side effects
Thompson and Saluja, 2017 [173]	Systematic review	50 – 400 mg/ day	11 B2 intervention studies	7 trials showed positive therapeutic effect of B2 4 studies did not find positive therapeutic effect of B2
Coenzyme Q10 (CoQ10)				
Rozen et al., 2002 [182]	3 months open-label	150 mg/ day	32 M	↓ migraine attack frequency
Sandor et al., 2005 [179]	3 months RCT	3 x 100mg/ day	42 M	↓ migraine attacks frequency, headache days & days-with-nausea
Hershey et al., 2007 [184]	Approx. 3 months open-label, uncontrolled	1-3mg/ kg/ day	252 paediatric / adolescent M with low CoQ10 levels	↓ migraine attack frequency &disability
Slater et al., 2011 [183]	RCT double-blind crossover	100mg/ day	120 paediatric and adolescent M	No signif. difference between Q10 and placebo after 32 weeks, (but signif. ↓ from baseline for Q10 at week 4)
Shoeibi et al., 2017 [181]	Open-label RCT	100mg/ day	80 M	↓ migraine attack frequency & severity
Dahri et al., 2017 [177]	12 weeks RCT double-blind	400mg/ day	84 M (female)	↓ migraine attack frequency, severity & duration
Dahri et al., 2018 [178]	3 months RCT double-blind	400mg/ day	46 M	↓ migraine attack frequency, severity & duration
Hajihashemi et al., 2019 [180]	8 weeks RCT double-blind	30mg/day + L-carnitine 500mg/d	56 M	↓ attack severity, duration, frequency ↓ serum lactate levels
Alpha-lipoic acid (ALA)				
Magis et al., 2007[187]	3 months RCT double-blind	600mg /day	43 MO, 11 MA	↓ migraine attack frequency, days & severity compared to placebo
Ali et al., 2010 [189]	1 month open-label	ALA 300mg/ day TPM 50mg/ day Both	40 adolescent M	↓ migraine days was sign greater in combined treatment TPM & ALA than TPM or ALA alone
Cavestro et al., 2018 [188]	6 months open-label, uncontrolled	400 mg/ day	32 M with IR	↓ migraine days & abortive migraine medication
Magnesium (Mg)				
Facchinetti et al., 1991[194]	2 months RCT double-blind	360 mg/ day	20 menstrual M	↓ number of migraine days & pain ratings
Chiu et al., 2016 [195]	Meta-analysis of RCTs	-	11 IV Mg acute studies (948 M patients) 10 oral Mg prevention studies (789 M patients)	IV Mg ↓ acute migraine within 15 – 45 minutes, 120 minutes, & 24 hours (ORs = 0.23, 0.20, and 0.25), Oral Mg ↓ frequency &intensity of migraine (ORs = 0.20 and 0.27)
Karimi et al., 2019 [196]	8 weeks cross-over RCT	500mg Mg / 400 mg VAL / day	63 M	Similar sign. ↓ migraine attack frequency, days, duration, severity & impact on quality of life with Mg versus VAL
Ketogenic diet (KD)				
Di Lorenzo et al., 2014 [201]	1 months RCT double-blind	Very low calorie KD; 30g carbs & 15 lipids / day	96 overweight M (female) (KD=45)	↓ migraine attack frequency, headache days & abortive M medication
Di Lorenzo et al., 2016 [202]	1 months open-label, uncontrolled	30g carbs/ day	18 MO, 4 MA	↓ migraine attack frequency & duration
Other B vitamins				
Prousky & Seely, 2005 [193]	Systematic review	25-200mg IV B3 50-500mg oral B3	9 B3 studies	B3 may have a migraine abortive and prophylactic effect, but RCTs are still lacking
Lea et al., 2009 [190]	6 months RCT double-blind	2mg B9, 25mg B6, 400µg B12	52 MA	↓ migraine attack frequency, severity & disability

ALA = alpha-lipoic acid / thioctic acid, B2= riboflavin, B3 = niacin, B9 = folic acid, carbs = carbohydrates, CoQ10 = coenzyme Q10 (ubiquinol or ubiquinone), IR = insulin resistance, IV = intravenous, KD = ketogenic diet, M = migraine not otherwise specified; MA = migraine with aura; Mg = magnesium, MO = migraine without aura, OR = odds ratio, RCT = randomised controlled trial, sign. = statistically significant, TPM = topiramate, VAL = sodium valproate,

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2.2 Manuscript 2: Mitochondrial Function and Oxidative Stress Markers in Higher-Frequency Episodic Migraine

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Mitochondrial function and oxidative stress markers in higher-frequency episodic migraine

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E.C.G. participated in the design of the study and its organisation, conduct and data acquisition, and was responsible for the main composition of the manuscript. NP participated in the conduct of the study. DRV covered all statistical aspects of the study and the manuscript. PS and DF participated in the study design, its organisation, and edited the manuscript. J.S. provided additional text and citations and in-depth editing of the manuscript. All authors proofread the final manuscript prior to submission.

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Mitochondrial function and oxidative stress markers in higher-frequency episodic migraine

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Abstract:

Introduction

Increasing evidence points towards the role of mitochondrial functioning, energy metabolism, and oxidative stress in migraine. However not all previous research has been conclusive and some mitochondrial function / oxidative stress markers have not yet been examined.

Methods

To this end, alpha-lipoic acid (ALA), total thiols, total plasma antioxidant capacity (TAC), lipid peroxide (PerOx), oxidised LDL (oxLDL), HbA1c and lactate were determined in the serum of 32 higher frequency episodic migraineurs (5-14 migraine days/ months, 19 with aura, 28 females).

Results

The majority of patients had abnormally low ALA and lactate levels (87.5% and 78.1%, respectively). 46.9% of the patients had abnormally high PerOx values, while for thiols and TAC over one third of patients had abnormally low values (31.2% and 37.5%, respectively). 21.9% of patients had abnormally low HbA1c and none had an HbA1c level above 5.6%. oxLDL was normal in all but one patient.

Discussion

This study provides further evidence for a role of oxidative stress and altered metabolism in migraine pathophysiology, which might represent a suitable therapeutic target. ALA, being too low in almost 90% of patients, might represent a potential biomarker for migraine. Further research is needed to replicate these results, in particular a comparison with a control group.

Keywords: migraine, oxidative stress, mitochondrial functioning, energy metabolism, mitochondria, antioxidants, alpha-lipoic acid

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Author Contributions: E.C.G. participated in the design of the study and its organisation, conduct and data acquisition, and was responsible for the main composition of the manuscript. NP and ALO participated in the conduct of the study. DRV covered all statistical aspects of the study and the manuscript. PS and DF participated in the study design, its organisation, and edited the manuscript. J.S. provided additional text and citations and in-depth editing of the manuscript. All authors proofread the final manuscript prior to submission.

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Key Findings:

- The majority of patients had abnormally low alpha-lipoic acid and lactate levels (87.5% and 72%, respectively).
- 46.9% of the patients had abnormally high lipid peroxide values
- For thiols and total antioxidant capacity over one third of patients had abnormally low values (31.2% and 37.5%, respectively).
- 21.9% had abnormally low HbA1c and no patient exceeded the healthy reference range.
- oxidized LDL was normal in all but one patient.

Abbreviations

ALA = alpha-lipoic acid

ATP = adenosine triphosphate

CGRP = Calcitonin gene-related peptide

CM = chronic migraineurs

EM = episodic migraineurs

HbA1c = Glycosylated hemoglobin

ICHD-3 = international classification of headache disorders version 3

IQR = interquartile range

KD = ketogenic diet

PerOx = lipid peroxide

MA = migraine with aura

MDA = malondialdehyde

MIDAS = migraine disability assessment

MO = migraine without aura

NSAIDs = non-steroidal anti-inflammatory drugs

oxLDL = oxidised LDL

H- MRS = proton magnetic resonance spectroscopy

H2O2 = hydrogen peroxide

HNE = 4-hydroxynonenal

OXPHOS = oxidative phosphorylation

ROS = reactive oxygen species

SOD2 = superoxide dismutase 2

TAC = total plasma antioxidant capacity

TRP = transient receptor potential channels

1. Introduction

Migraine is a complex, common and debilitating neurological disorder (1) and yet its primary pathogenic mechanisms are not completely understood. Despite being referred to as a “hypoglycemic headache” in 1935 already (2), the focus of clinical and basic research has shifted towards (neuro-)vasculature, cerebral excitability and neurotransmission for several decades. In recent years, metabolism and mitochondrial (dys-)function have regained interest and various lines of evidence – much of it clinical – are suggesting that migraine is - at least partially - an energy deficit syndrome of the brain.

For example, magnetic resonance spectroscopy (MRS) studies in migraine consistently show abnormalities of mitochondrial oxidative phosphorylation (OXPHOS), such as hypometabolism or decreased ATP levels (3–14). These findings are supported by early studies showing metabolic changes induced by fasting, glucose or insulin administration, which can even trigger migraine attacks in susceptible patients (15–21).

Further support for a link to energy metabolism and / or mitochondrial functioning comes from the migraine preventative effect of several nutraceuticals (22), such as riboflavin at high dose (200-400mg/ day) (23–29); coenzyme Q10 (400mg capsules or 300mg liquid suspension) (30–35), magnesium (36) and alpha-lipoic acid (ALA; 600mg) (37–39). Dietary approaches, such as a ketogenic diet (KD), which promotes the hepatic production of an alternative energy substrate for the brain and to some extent mimics the state of fasting, have been shown to be migraine protective (40–45) (see (46) for potential mechanism of ketosis in migraine). Moreover, elevated plasma lactate and pyruvate levels have been reported, but mostly in severely affected patients, such as migrainous stroke (47,48).

Reactive oxygen species (ROS), such as hydroxyl radicals, hydrogen peroxide and superoxide radical anions, are produced as by-products of normal metabolic processes, such as electron transport in mitochondria, host defence or enzymatic reactions (49). In healthy organisms, antioxidant defence systems protect the cells and tissues against these species (50,51). When the generation of ROS exceeds the body's antioxidant capacity, oxidative stress, i.e. damage to cellular constituents, such as proteins, lipids, DNA and sugars, occurs (49,51). Oxidative stress could be the common denominator of most migraine trigger and aggravating factors (52). While for some of the more "metabolic" triggers, such as fasting /skipping a meal, physical exercise, stress and relaxation thereafter a direct link to energy homeostasis seems obvious, most of the seemingly unrelated triggers, such as ovarian hormone changes, weather changes, alcohol, strong odours, intense light and loud noises, also have a potential common denominator: changes in mitochondrial metabolism and/or oxidative stress (see reviews (52,53) for further details). Mechanistically, transient receptor potential (TRP) channels, expressed in meningeal nociceptive nerve terminals, can be activated by oxidative, nitrosative and electrophilic stress (54,55), thereby providing a mechanism by which known migraine trigger factors that increase oxidative stress could lead to migraine pain.

Increased oxidative / nitrosative stress and / or decreased anti-oxidant capacity have directly been found in migraine patients (56–69), however the results were not always consistent. Of all biomarkers examined, superoxide dismutase activity seemed to be the one consistently reduced in migraine patients, also interictally (70). The inconsistent results for the other markers could be due to differences in methodology, patient selection and variations depending on the migraine cycle. Regarding the latter, nitrosative and

oxidative stress (69) and nitric oxide (70) were significantly elevated during migraine attacks, but not interictally.

The aim of this study was to analyse peripheral markers of mitochondrial functioning / energy metabolism that have either not previously been looked at (to the best of our knowledge) or previously produced inconsistent results, in order to further decipher the metabolic face of migraine, with a particular focus on oxidative stress markers. Through the measurement of ALA, thiols, total plasma antioxidant capacity (TAC), lipid peroxide (PerOx), oxidised LDL (oxLDL), HbA1c and lactate in the serum of 32 medium to high frequency migraineurs the present study aimed to provide peripheral markers of mitochondrial functioning / energy metabolism that could easily be analysed by most practitioners to potentially assist with individualised treatment. Taking into account previous findings, we hypothesised that the three-month average plasma glucose concentration (Hba1c) would be reduced, the oxidative stress markers ALA, thiols and TAC would be reduced, PerPx and oxLDL would be increased and that there would be no difference in lactate levels in our episodic migraine patient population.

2. Materials and Methods (ca. 1400 words)

2.1. Patients

After receiving ethical approval from the local ethics committee (EKNZ PB 2016-00497), we recruited patients from the Neurology Out-patient Departments at the University Hospitals of Basel, Bern, Zurich, the University of Basel, using internet announcements and by advertising in local busses and trains. Patients were part of the MigraKet trial (71) (ClinicalTrials.gov Identifier: NCT03132233). Written informed consent was obtained from each patient. The diagnosis of migraine was made by a trained neurologist based on the criteria according to the International Headache Society (72).

A German version of the migraine disability assessment (MIDAS) was used to assess migraine related disability (73).

2.1.2. Inclusion criteria

Patients were included, if they were previously diagnosed with migraine (with or without aura) in accordance with the ICHD-3 (International Classification of Headache Disorders version 3 Beta) Classification criteria (72), were between the ages of 18 and 65 years, experienced between 5 and 14 migraine days per month (over the last 4 months), had an age of onset of migraine less than 50 years old and had not changed the type, dosage or frequency of any prophylactic medication (exclusive of medications taken for acute relief of migraine symptoms) for at least 3 months prior to study onset.

2.1.3. Exclusion criteria

Patients were excluded, if they had a history of any significant neurological, psychiatric or other medical condition or a known history of suspected secondary headache, if they were taking simple analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) more than 14 days per 4 weeks or triptans on more than 10 days per 4 weeks for headaches or other body pain or any prescription opioids, if they had a previous diagnosis of medication overuse headache, which has reverted to episodic migraine within the last 6 months or met ICHD-3 Beta Classification criteria (72) for chronic migraine (> 15 headache days per month), if they had had a surgery for migraine prevention, if they had received botulinum toxin injections within the last 6 months or if they were pregnant.

2.2. Laboratory procedures

The following mitochondrial function markers were examined (normative values from local laboratory (Ganzimmun Diagnostic AG, Mainz, Germany or University Hospital Basel, Basel Switzerland) indicated in brackets):

- Total anti-oxidative capacity (TAC; >280 μmol/l)
- Oxidated LDL (OxLDL; <235 ng/ml)
- Alpha-lipoic acid (ALA; >0.52 μg/l)
- Lipid-peroxide (PerOx; <180 μmol/l)
- Thiols (complete; >55 μmol/l)
- HbA1c (4.8–5.9 %)
- Lactate (1.1–2.0 mol/l)

Venous blood samples were drawn from an antebrachial vein following overnight fasting. After 30-60min at room temperature the serum was separated from the rest of the blood by centrifugation at 1300G for 10 mins. Aliquots of serum were stored at −80°C. Blood samples for HbA1C and lactate were not stored, but immediately cooled and sent to the inhouse laboratory for immediate analysis.

2.2.1 Total antioxidant capacity

TAC was measured using an ImAnOx-assay (Ganzimmun Diagnostic AG, Mainz, Germany) (inter-assay variation: 2.43%; intra-assay variation: 2.33%). This photometric test reflects the sum of all antioxidant components by measuring hydrogen peroxide (H2O2) degradation by the serum antioxidants. Please refer to (74) for further details.

2.2.2. Peroxides

Serum total peroxide concentrations were determined photometrically by the peroxide concentration assay (Ganzimmun Diagnostic AG, Mainz, Germany) (inter-assay variation: 3.5-3.6%; intra-assay variation: 2.0-3.6%), which is based on the reaction of horseradish peroxidase with plasma peroxides using tetramethylbenzidine as a chromogen substrate (450-nm wavelength). Please refer to (75) for further details.

2.2.3. Oxidised LDL

The measurement of serum oxidised LDL was performed using a sandwich ELISA method (ox-LDL ELISA kit, Ganzimmun Diagnostic AG, Mainz, Germany) (inter-assay variation: 9–11%; intra-assay variation: 3.9–5.7%). No antioxidants were added to the plasma samples before collection. Please refer to (76) for further details.

2.2.4. Thiols

Thiols were determined in serum using the ImmuChrom HPLC assay (Ganzimmun Diagnostic AG, Mainz, Germany) (inter-assay variation: 5.5-5.9%; intra-assay variation: 5.2-5.8%), where thiols present in serum proteins are precipitated with 80% saturated ammonium sulfate. Please refer to (77) for further details.

2.2.5. Alpha-lipoic acid

ALA was determined in serum using the HPLC method (Ganzimmun Diagnostic AG, Mainz, Germany). The standards and the solutions were sourced from Merck KGaA. In brief, 100 µl of the serum sample was diluted in 1.9 ml acetone. The sample was mixed thoroughly for 5 sec. After that, the sample was centrifuged for 10 min at 3500 U/min. 800 µl of the supernatant was evaporated under air at 45 °C for 10 min. The dry residue was dissolved in 400 µl 30/70 0.1 % acetic acid/ acetone mix. The sample was mixed thoroughly for 5 sec. 300 µl of the solution was transferred in a HPLC vial. A calibration curve in empty serum was prepared with five different standard concentrations. The highest standard was 200 µg/l and the lowest 12.5 µg/l. The preparation of the standard was equal to the sample preparation. The concentration of ALA was determent by LC-MS/MS with a Varian 320 in negative mode. For the HPLC method an Atlantis T3, 3µm, 150x2.1 mm column from Waters GmbH was used. The isocratic gradient was 30 % 0.1% acetic acid and 70 % acetone with a flowrate of 0.3 ml/min and an injection volume of 40 µl. The runtime was 4 minutes with a retention time of ALA at 2.1 min.

2.2.6 Glycosylated hemoglobin (HbA1c) and lactate

HbA1c and lactate were analysed using the Cobas 8000 c502 (Roche Diagnostics) at the laboratory of the University Hospital Basel. HbA1c was determined using the HbA1c Turbidimetric Immunoassay (Tina-quant, 3rd generation) from haemolysed full blood EDTA samples. Lactate was analysed from EDTA / fluorid blood samples using an enzymatic colour assay.

2.3. Statistical Analyses

Summary statistics (mean, median, interquartile range, minimum and maximum) and the number and percentage of patients with non-normal values are indicated for each biomarker. Individual measures are both shown separately in the original units and on a common, standardized scale for better comparison. For endpoints with normal ranges (HbA1c and lactate), the original values were scaled such that the minimum and maximum of the normal range correspond to 0 and 1. Values < 0 are thus abnormally low and values > 1 abnormally high. For the other endpoints with a single normal cut-off, the original values were centered, such that the cut-off corresponds to 0, and scaled by dividing by the standard deviation, such that 1 corresponds to one standard deviation. For TAC, ALA and thiols, values < 0 are considered abnormal, while for oxLDL and PerOx, values >0 are considered abnormal. Boxplots were drawn as follows. The Boxes contain the 25% through 75% quantiles (spanning the interquartile range), the thick horizontal line is the median. Whiskers indicate the most extreme values lying within the box-edge and 1.5 * the interquartile range. All eventual further values (outliers) are plotted as individual points.

Several post-hoc analyses were performed. Correlations of biomarkers and migraine intensity were examined visually and Spearman's rank correlation coefficient was calculated. As measure of migraine intensity, the number of migraine days and the MIDAS score at baseline were considered. Subgroup comparisons were performed between patients with and without migraine prophylaxis, between patients with and without acute migraine attack at baseline ± 2 days and between MA and MO. Subgroups were

tested for a difference using Wilcoxon's rank sum test (continuous outcomes) and Fisher's exact test (frequencies).

According to the exploratory nature of the analyses, p-values should be considered as a continuous, hypothesis-generating measure of evidence and not be interpreted as confirmative.

All analyses were conducted using the statistical software package R (78).

3. Results

3.1. Study population

Thirty-two patients were included in the study (28 female, 4 male). The mean age was 34±10.8 years. Twelve patients had migraine without aura (MO) and 20 migraine with aura (MA). Patient characteristics and demographics' information are shown in Table 1. Eleven patients were using at least one stable migraine prophylaxis (no changes within at least 3 months prior to study onset) (see table 2 for the migraine preventatives used).

Table 1: Summary statistics of patient characteristics.

Table 2: Types and frequencies of migraine prophylactic treatments.

3.2. Summary statistics

Summary statistics of all endpoints are given in Table 3. Single observations are visualized in their original units (Figure 1) and scaled (Figures 2 and 3).

For ALA and lactate, the majority of patients had abnormally low values (28/32 (88%) and 23/32 (72%) respectively). Only two patients' lactate levels were too high. For one patient, an extremely high level of ALA (13.25) was measured. For PerOx half of the patients (46.9%) had abnormally high values. For thiols and TAC about one third of patients had abnormally low values (31.2% and 37.5%, respectively). For HbA1c about 20% of patients (21.9%) had abnormally low values and no one had an HbA1c above 5.6%.

For oxidated LDL, a very high, abnormal level was measured in one patient, while for all other patients, the levels were in the normal range.

Table 3: Summary statistics of mitochondrial function markers.

Figure 1: Baseline values of mitochondrial function markers for each patient. Dashed lines indicate the normal-range cut-off(s). Green dots indicate values within normal range, red dots indicate values outside the normal range. For alpha lipoic acid one very extreme value (13.25) has been removed for better visualisation.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Figure 2: Standardized (= .std) baseline values of mitochondrial function markers with a single cut-off. Values are standardized such that zero (the dashed line) indicates the normal cut-off, 1 indicates one standard deviation, 2 indicates two standard deviations,etc. Green dots indicate values within normal range, red dots indicate values outside the normal range. For alpha lipoic acid one very extreme value (5.6) has been removed for better visualisation.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Figure 3: Standardized (= .std) baseline values of mitochondrial function markers with two cut-offs. Values are standardized such that zero (the dashed line) indicates the lower cut-off of the normal range and 1 indicates the upper cut-off of the normal range. Green dots indicate values within normal range, red dots indicate values outside the normal range.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

3.3. Correlations of mitochondrial function biomarkers and migraine severity

We found no indication for a correlation of the 7 mitochondrial function biomarkers with MIDAS score or number of migraine days per month at baseline (Figures 4 and 5 and Table 4). Corresponding correlation coefficients and p-values are given in Table 4.

Figure 4: Mitochondrial function biomarkers vs MIDAS score at baseline.
Alpha lipoic acid: outlier data for one patient with an extremely high value (>10) is not shown. Smoothingcurves, using locally estimated scatterplot smoothing (LOESS) with span 1.0, are shown.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Figure 5: Mitochondrial function biomarkers vs number of migraine days per month at baseline.
Alpha lipoic acid: outlier data for one patient with an extremely high value (>10) is not shown. Smoothingcurves, using locally estimated scatterplot smoothing (LOESS) with span 1.0, are shown.

ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Table 4: Correlations of mitochondrial function markers with migraine severity at baseline.

3.4. Comparison between patients with and without migraine prophylaxis

Summary statistics of absolute levels of the mitochondrial function biomarkers and the frequencies of patients with abnormal values according to migraine prophylaxis are presented in Table 5. Our data provide no evidence for an effect of migraine prophylaxis.

Table 5: Comparison of mitochondrial function markers between patients with migraine prophylaxis and without.

3.5. Comparison between patients studied during or outside of an attack

Summary statistics of absolute levels of the mitochondrial function biomarkers and the frequencies of patients with abnormal values according to acute migraine attack at baseline (baseline visit ±2 days) are presented in Table 6. Most patients presented with acute migraine at baseline; for one patient this information is missing. Our data provide no evidence for any difference between these two groups.

Table 6: Comparison of mitochondrial function markers between patients with and without acute migraine at baseline (±2 days).

3.6. Comparison between patients with and without aura

Summary statistics of absolute levels of the mitochondrial function biomarkers and the frequencies of patients with abnormal values according to aura are presented in Table 7. We found no evidence for differences between patients with or without aura, neither in the absolute values of the biomarkers nor in the proportions of patients with abnormal values.

Table 7: Comparison of mitochondrial function markers between patients with aura and those without.

4. Discussion

We have shown that apart from oxLDL and HbA1c most other markers of mitochondrial functioning showed abnormalities in a significant proportion (>30%) of the patients examined.

ALA

To the best of our knowledge ALA levels have not previously been determined in migraine. Almost 90% of patients in this sample had abnormally low values of ALA. ALA, also known as thioctic acid, is an eight-carbon, sulfur-containing compound that functions as a water- and fat-soluble antioxidant (79,80). It can directly (by removing reactive species) and indirectly (by chelating transition metal ions) reduce oxidative stress (79,80). The human body can synthesize small amounts of ALA (79). ALA also plays an important role as co-enzyme in energy metabolism (79–81). Furthermore, it is able to regenerate other antioxidants, such as vitamin C and E, CoQ10, it increases intracellular glutathione and activates endogenous antioxidant systems (82–84). Apart from its anti-oxidant action, ALA seems to assist weight loss (85), increase insulin sensitivity and decrease blood lipids (86). All of these mechanisms are probably migraine relevant. Interestingly, ALA supplementation (300-600mg) per day has been shown to significantly reduce migraine attack frequency, severity and duration (37–39), which seems to align with our findings. Further research is needed to see, whether this finding is specific to our medium-high frequency episodic migraine population or a general characteristic of migraine or even a general characteristic of other (neurological) diseases with a mitochondrial / oxidative stress component. Should this finding be replicated and migraine specific, ALA might represent a potential biomarker.

TAC

Serum (or plasma) concentrations of different antioxidants can be measured separately, but since the measurement of different antioxidant molecules individually is impractical and costly and their antioxidant effects are additive, the total antioxidant capacity of a sample is typically measured, and this is typically referred to as total antioxidant capacity (TAC), total antioxidant status (TAS) or other synonyms, which will be used interchangeably.

Almost 40% of our patients had abnormally low TAC being in line with results of previous research. A study on 75 MO patients demonstrated that the levels of total antioxidants were decreased and the levels of total oxidants and the oxidative stress index were increased (56). Another study found TAC to be significantly reduced in migraineurs compared to controls (67). TAC levels increased after successful prophylactic treatment compared to the baseline, irrespective of treatment modality (rTMS versus amitriptyline) and the increase correlated with treatment success (67).We assume higher TAC with lower migraine severity, less recent oxidative stress exposure, and increased distance to previous and future migraine attack. These assumptions would have to be validated in future research.

PerOx

Lipid peroxidation is the oxidative degradation of lipids via free radical damage of the lipids in cell membranes, polyunsaturated fatty acids in particular. The end products of lipid peroxidation are reactive aldehydes, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA). Free radicals cause increased accumulation of these lipid peroxidation by-products in the blood. About half of the patients had abnormally high total PerOx levels, being in line with previous research. Several studies have found serum levels of MDAs to be significantly elevated in migraine patients (57)(68), even in the interictal phase (87).

oxLDL

Oxidized low-density lipoprotein (LDL) is a harmful type of cholesterol that is produced when normal LDL cholesterol is damaged by chemical interactions with ROS. All but one patient had normal levels for oxLDL, which is in contrast to the study of Bernecker et al. that found highly significantly elevated levels oxLDL in female migraineurs (58). This result could be due to differences in study population, as the migraineurs of the Berecker et al. study tended to have metabolic syndrome and had generally higher BMIs as our migraine patient population.

Thiols

The term “thiol” refers to organic compounds containing sulfur (in form of the functional group -SH, the thiol group). Thiol groups are able to destroy ROS and other free radicals by enzymatic as well as nonenzymatic mechanisms (88). Total thiol levels have previously been used to evaluate excess free radical generation, both in physiological and pathological conditions (89). Protein thiol levels in serum have been shown to be a direct measure of the in vivo reduction/oxidation (redox) status in humans, because thiols react readily with ROS to form disulfides (77). Thiol redox homeostasis plays an important role in neurogenerative diseases (90) and in nine other categories of human disorders serum protein thiols have been found to be significantly reduced compared to healthy controls (77).

Only about one third of patients had abnormally low serum thiol levels, but this seems to be in line with previous research. A larger study found significantly reduced thiol levels in 151 migraine patients (74 MO, 77MA) compared to 70 healthy controls and there was a negative correlation with migraine disability (62). A negative correlation between the levels of total thiols and the duration of the headaches has also been demonstrated (56). However, others studies found no significant difference in thiol groups between patients and controls, even during attacks (69) and one study even found higher total (-SH+-S-S-) & native thiol (-SH) levels in serum of migraineurs, but this did not correlated with disease severity or migraine type (64). Recent exposure to oxidative stress, migraine severity, time in the migraine cycle and similar aspects could explain the different results.

HbA1c

HbA1c (glycated hemoglobin) is an indication of the average blood glucose levels over the last two to three months. Just over 20% of patients had abnormally low HbA1c levels and none of them had HbA1c levels that were above 5.6%. To the best of our knowledge HbA1c has rarely been looked at in migraine. One study found no significant difference in HbA1c levels between CM, EM and healthy controls (20)

However, magnetic resonance spectroscopy (MRS) studies in migraine have consistently shown abnormalities of mitochondrial oxidative phosphorylation (OXPHOS), such as hypometabolism between (3–10) and during migraine attacks (11), in the resting brain and in the muscle following exercise (3,12,13). A 16% decrease of absolute ATP levels in migraine without aura patients was also demonstrated interictally using 31P-MRS (14). These findings are supported by early studies showing that metabolic changes induced by fasting, glucose or insulin administration can trigger migraine attacks; e.g. a 50g glucose tolerance test (GTT) after a 10-hour fast triggered a migraine in 6 out of 10 migraine patients reporting attacks associated with fasting (15). Abnormal metabolic responses were also reported in GTT studies (15,16) and interictal impaired glucose tolerance and insulin resistance has been reported in various other studies (17–21). While only 20% of our migraineurs had abnormally low HbA1c levels, all levels tended to be on the lower side, despite reported higher carbohydrate diets. As HbA1c levels correspond to an average blood sugar measurement, low average values despite probable highs after carbohydrate rich meals could be an indication that there might be lows as well. This would be in line with previous neuroimaging and GTT research results, but it is speculation only and these assumption need to be confirmed by future research.

Lactate

Lactate is typically measured to assess tissue oxygenation, arising from either decreased oxygen delivery or a disorder in oxygen use, both of which lead to increased anaerobic metabolism and increases in lactate levels. In certain types of migraine, especially migrainous stroke, elevated serum lactate and pyruvate levels have previously been reported (47,48). In contrast to this, only 2 patients had abnormally high serum lactate levels in our cohort and over 70% of patients serum lactate levels were abnormally low.

While there is little data on serum lactate levels in migraine, data on brain lactate analysed with ^{1}H-MRS have also been shown to vary due to patient selection (see review by Reyngoudt et al (2012) for details (9)). Elevated brain lactate levels were found in some studies of MA (91,92), but not in MO (93–96). Occipital baseline lactate levels were increased in patients with visual auras, but not in those having complex neurological auras. By contrast, during photic stimulation lactate increased significantly in the latter, but not in the former (91). Stimulus-induced lactate increases are physiological (97) and can be explained by the neuron-astrocyte lactate shuttle (98). Hence, their absence in migraine patients, whose

neuronal activation is energetically more demanding (99), could be considered pathological and might be contributing to an energetic crisis.

To the best of our knowledge, no recent studies have looked at baseline serum lactate levels in episodic migraine patients or subgroups thereof. More research is needed to replicate this finding; in particular a study combining lactate level quantification in the cortex with that of the periphery and with brain energetics seems warranted. We can only speculate as to why lactate levels were predominantly low in the majority of our patients. They all came rested, but fasted overnight to the trial site. Decreased baseline lactate levels might be a sign of increased cerebral lactate consumption and an indicator of an increased cerebral energy demand of the migraine brain, as in addition to ketone bodies, lactate constitutes the only other major alternative brain energy substrate from glucose and is used especially during times of high metabolic demands or hypoglycemia (100). A study using ^{13}C-L-lactate and magnetic resonance spectroscopy suggested that the contribution of plasma lactate to brain metabolism can be up to 60% (101), which is very similar to ketone bodies. It could also be a sign of decreased lactate synthesis as demonstrated with 1H-MRS (91).

In summary, we have shown that apart from oxLDL and HbA1c most other markers of mitochondrial functioning are abnormal in at least >30% of the patients examined. As oxidative stress is a complex mechanism including different sources of ROS and various pathways, differing results in previous research may at least be partially caused by different oxidative stress parameters examined, e.g. MDA versus HNE, as well as by different study groups investigated, e.g. adults versus children, MA versus MO, females versus males, and differences in migraine severity, recent oxidative stress exposure and the time within the individual migraine cycle, where measurements were taken. Genetic research examining oxidative stress related genes in larger homogenous migraine cohorts could be interesting future research that would hardly be influenced by these factors.

Our data provide no evidence for correlations between any of the seven mitochondrial function / oxidative stress markers and migraine severity. This could be due to our sample population being fairly homogenous or the sample size being too small. In addition, we found no evidence for an effect of migraine prophylaxis. This is not surprising, since patients were still suffering from a substantial number of migraine days / months despite the prophylactic treatment (5-14 days / months), suggesting that the critical migraine pathophysiological mechanisms remained active. Furthermore, no evidence for an effect of a preceding or subsequent migraine attack has been found. This might be due to only 5 patients being migraine attack free within 2 days before and after the venous puncture, making an analysis of the potential impact of an attack difficult. We also found no evidence for a difference between MA and MO patients. For a randomly selected migraine cohort mainly recruited via public advertisements, the number of MA patients was unusually high (62.5%) in our study population. We can only speculate as to why this

might be the case. Since participants were part of the 9 months MigraKet intervention trial (71), it seems plausible that MA patients might have been more motivated to take place in such a lengthy trial and this led to the observed over-representation.

While we found no correlation between these mitochondrial function / oxidative stress markers and disease severity, differences in methodologies used and patient characteristics, recent oxidative stress exposure and also time in the respective migraine cycle is likely to play a role. Future research examining these markers at different time points during the migraine cycle and in different migraine types would be interesting.

The most important limitation of this study is the absence of a matched control group. While abnormally low levels in 90% of patients in the case of ALA are likely to be of importance, we cannot be sure that PerOx, TAC and thiol level findings would have been significantly different from controls. Future research is needed to replicate these findings in the presence of a control group. Secondly, the sample size was fairly small, in particular with regards to the correlation analyses. In addition, one third of patients was using a migraine prophylaxis. While our data provide no evidence for an effect of migraine prophylaxis, the inclusion of patients who are using a prophylaxis is not ideal.

In conclusion, this study provides further support for metabolic abnormalities in migraine, in particular the role of increase oxidative stress and decreased anti-oxidant capacity respectively in migraine pathophysiology. The peripheral markers assessed here could easily be examined in most doctor's offices and might assist personalised migraine treatment that targets oxidative stress and mitochondrial functioning; however, further research is needed to replicate these findings, ideally in the presence of a control group.

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Table 1: Summary statistics of patient characteristics.

SD= standard deviation, m = meter, kg = kilogram

Variables	All patients
Age, mean (SD)	34 (10.8)
Female, N (%)	28 (87.5)
Male, N (%)	4 (12.5)
Height (m), mean (SD)	1.7 (01.)
Weight (kg), mean (SD)	67.3 (13.9)
Migraine days / months, mean (SD)	8.6 (2.1)
Migraine without aura, N (%)	12 (37.5)
Migraine with aura, N (%)	20 (62.5)
MIDAS, mean (SD)	31 (19.9)
Stable migraine prophylaxis, N (%)	11 (34.4)
No migraine prophylaxis, N (%)	21 (65.6)

Table 2: Types and frequencies of migraine prophylactic treatments.

Note that more than one type of prophylactic drug could be used.

Type	N
Antidepressants	2
Anticonvulsants	2
Beta-blockers	5
Cefaly™ neurostimulator	1
Calciumantagonists	2
Magnesium	7
Riboflavin/Vitamin B2	1

Table 3: Summary statistics of mitochondrial function markers.

Marker	Normal range	Minimum	Q1	Median	Mean	Q3	Maximum	Non-normal
ALA	>0.52 µg/l	0.13	0.24	0.29	0.72	0.41	13.25	28 (87.5%)

TAC	>280 μmol/l	264.0	276.00	284.00	286.88	295.25	348.00	12 (37.5%)
PerOx	< 180 μmol/l	6.0	75.50	166.00	283.31	306.50	1315.00	15 (46.9%)
Ox-LDL	< 235 ng/ml	41.3	45.10	62.15	106.35	136.38	593.90	1 (3.1%)
Thiols	> 55 μmol/l	41.0	52.75	62.50	63.62	72.25	99.00	10 (31.2%)
HbA1c	4.8–5.9 %	4.1	4.80	4.95	4.93	5.10	5.60	7 (21.9%)
Lactate	1.1–2.0 mol/l	0.5	0.73	0.85	1.05	1.15	2.66	25 (78.1%)

Q1 – Q3: interquartile range; non-normal: number (%) of patients with values outside normal range.

ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Table 4: Correlations of mitochondrial function markers with migraine severity at baseline.

ϱ (rho) = Spearman's rank correlation coefficient.

Migraine intensity	Biomarker	ϱ (rho)	p-value
MIDAS score	ALA	-0.009	0.961
MIDAS score	HbA1c	0.05	0.787
MIDAS score	Lactate	-0.13	0.491
MIDAS score	oxLDL	0.17	0.361
MIDAS score	PerOX	0.15	0.399
MIDAS score	TAC	-0.0061	0.974
MIDAS score	Thiols	-0.054	0.771
Migraine days per month	ALA	0.2	0.279
Migraine days per month	HbA1c	0.11	0.566
Migraine days per month	Lactate	-0.25	0.163
Migraine days per month	oxLDL	0.22	0.228
Migraine days per month	PerOX	-0.073	0.691
Migraine days per month	TAC	-0.21	0.249
Migraine days per month	Thiols	0.28	0.127

ALA = Alpha-lipoic acid; MIDAS = migraine disability assessment; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Table 5: Comparison of mitochondrial function markers between patients with migraine prophylaxis and without.

Marker	With prophylaxis (n=11)	No prophylaxis (n=21)	p
ABSOLUTE LEVELS: MEDIAN [IQR]			
ALA, median [IQR]	0.28 [0.23, 0.44]	0.29 [0.24, 0.39]	0.858
TAC, median [IQR]	286.00 [278.00, 293.00]	284.00 [276.00, 296.00]	0.721
PerOX, median [IQR]	114.00 [52.50, 222.50]	241.00 [100.00, 381.00]	0.126
oxLDL, median [IQR]	74.00 [46.55, 170.10]	54.90 [45.60, 128.50]	0.498
Thiols, median [IQR]	69.00 [60.00, 72.50]	58.00 [50.00, 70.00]	0.159
HbA1c, median [IQR]	5.00 [4.85, 5.00]	4.90 [4.70, 5.10]	0.920
Lactate, median [IQR]	0.78 [0.75, 1.55]	0.86 [0.71, 1.06]	0.691
NUMBER (%) OF PATIENTS WITH ABNORMAL VALUES			
ALA.abnorm, N (%)	10 (90.9)	18 (85.7)	1.000
TAC.abnorm, N (%)	4 (36.4)	8 (38.1)	1.000
PerOX.abnorm, N (%)	3 (27.3)	12 (57.1)	0.217
oxLDL.abnorm, N (%)	0 (0.0)	1 (4.8)	1.000
Thiols.abnorm, N (%)	2 (18.2)	8 (38.1)	0.452
HbA1c.abnorm, N (%)	1 (9.1)	6 (28.6)	0.415
Lactate.abnorm, *N (%)*	8 (72.7)	17 (81.0)	0.933

.aborm = abnormal value (outside of the normal range); ALA = Alpha-lipoic acid; IQR = interquartile range; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

Table 6: Comparison of mitochondrial function markers between patients with and without acute migraine at baseline (±2 days).

	Acute attack (n=26)	No attack (n=5)	p
ABSOLUTE LEVELS: MEDIAN [IQR]			
ALA, median [IQR]	0.29 [0.23, 0.42]	0.27 [0.24, 0.28]	0.667
TAC, median [IQR]	283.00 [276.00, 297.25]	286.00 [284.00, 287.00]	0.914
PerOX, median [IQR]	194.00 [89.50, 307.50]	114.00 [74.00, 283.00]	0.554
oxLDL, median [IQR]	55.50 [41.88, 134.05]	69.70 [68.20, 199.20]	0.280
Thiols, median [IQR]	62.50 [52.50, 72.75]	59.00 [53.00, 68.00]	0.610
HbA1c, median [IQR]	4.95 [4.80, 5.10]	4.90 [4.80, 5.20]	0.828

Lactate, median [IQR]	0.87 [0.72, 1.20]	0.78 [0.76, 1.08]	0.809
NUMBER (%) OF PATIENTS WITH ABNORMAL VALUES			
ALA.abnorm, N (%)	23 (88.5)	4 (80.0)	1.000
TAC.abnorm, N (%)	11 (42.3)	1 (20.0)	0.662
PerOX.abnorm, N (%)	13 (50.0)	2 (40.0)	1.000
oxLDL.abnorm, N (%)	1 (3.8)	0 (0.0)	1.000
Thiols.abnorm, N (%)	8 (30.8)	2 (40.0)	1.000
HbA1c.abnorm, N (%)	6 (23.1)	1 (20.0)	1.000
Lactate.abnorm, N (%)	20 (76.9)	4 (80.0)	1.000

.aborm = abnormal value (outside of the normal range); ALA = Alpha-lipoic acid; IQR = interquartile range; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; perTAC = total antioxidant capactity

Table 7: Comparison of mitochondrial function markers between patients with aura and those without.

	With aura (n=19	Without aura (n=13)	p
ABSOLUTE LEVELS: MEDIAN [IQR]			
ALA, median [IQR]	0.26 [0.21, 0.42]	0.32 [0.26, 0.39]	0.284
TAC, median [IQR]	284.50 [278.00, 292.00]	281.00 [272.50, 298.25]	0.471
PerOX, median [IQR]	150.50 [70.00, 395.50]	194.00 [105.50, 284.25]	0.876
oxLDL, median [IQR]	50.30 [43.03, 102.85]	118.90 [65.73, 149.60]	0.100
Thiols, median [IQR]	64.50 [55.50, 73.00]	60.00 [50.00, 69.00]	0.508
HbA1c, median [IQR]	5.00 [4.80, 5.10]	4.90 [4.77, 5.03]	0.223
Lactate, median [IQR]	0.26 [0.21, 0.42]	0.32 [0.26, 0.39]	0.284
NUMBER (%) OF PATIENTS WITH ABNORMAL VALUES			
ALA.abnorm, N (%)	17 (85.0)	11 (91.7)	1.000
TAC.abnorm, N (%)	6 (30.0)	6 (50.0)	0.451
PerOX.abnorm, N (%)	9 (45.0)	6 (50.0)	1.000
oxLDL.abnorm, N (%)	0 (0.0)	1 (8.3)	0.793
Thiols.abnorm, N (%)	5 (25.0)	5 (41.7)	0.555
HbA1c.abnorm, N (%)	4 (20.0)	3 (25.0)	1.000
Lactate.abnorm, N (%)	15 (75.0)	10 (83.3)	0.912

.aborm = abnormal value (outside of the normal range); ALA = Alpha-lipoic acid; IQR = interquartile range; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; perTAC = total antioxidant capactity

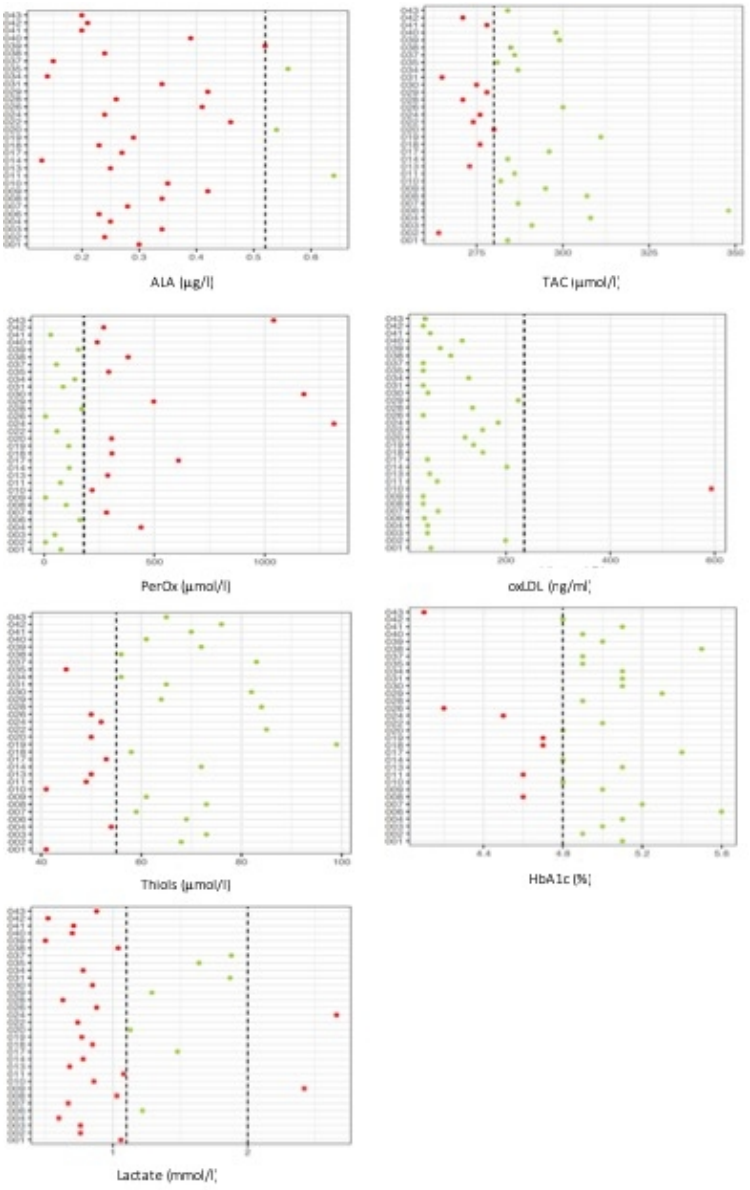

Figure 1: Baseline values of mitochondrial function markers for each patient. Dashed lines indicate the normal-range cut-off(s). Green dots indicate values within normal range, red dots indicate values outside the normal range. For alpha lipoic acid one very extreme value (13.25) has been removed for better visualisation.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

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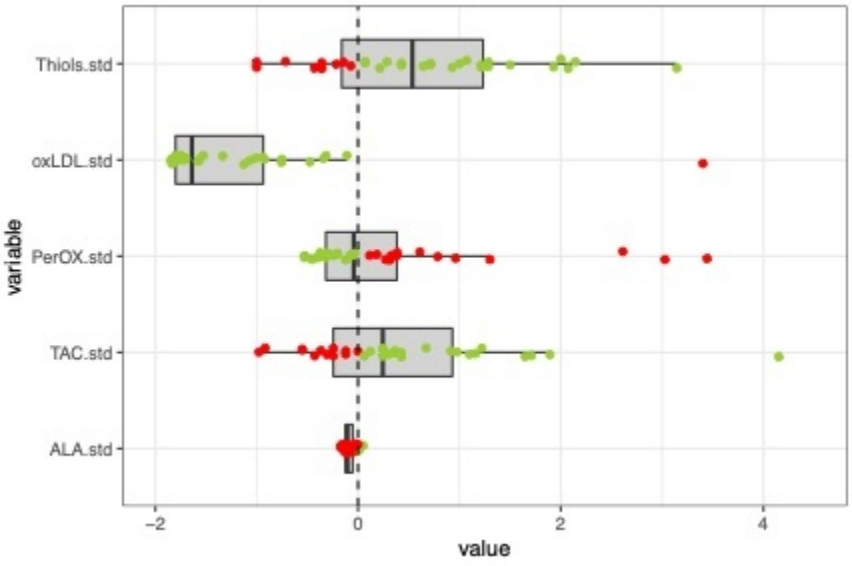

Figure 2: Standardized (= .std) baseline values of mitochondrial function markers with a single cut-off. Values are standardized such that zero (the dashed line) indicates the normal cut-off, 1 indicates one standard deviation, 2 indicates two standard deviations,etc. Green dots indicate values within normal range, red dots indicate values outside the normal range. For alpha lipoic acid one very extreme value (5.6) has been removed for better visualisation.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

152x101mm (72 x 72 DPI)

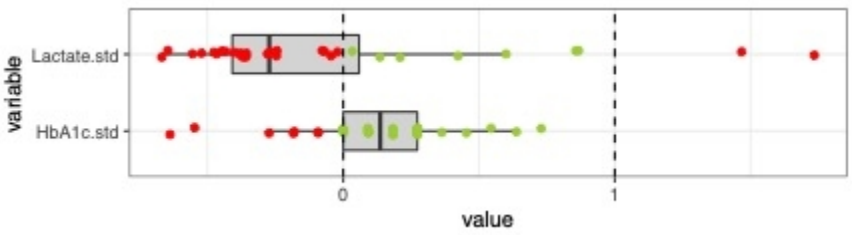

Figure 3: Standardized (= .std) baseline values of mitochondrial function markers with two cut-offs. Values are standardized such that zero (the dashed line) indicates the lower cut-off of the normal range and 1 indicates the upper cut-off of the normal range. Green dots indicate values within normal range, red dots indicate values outside the normal range.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

152x43mm (72 x 72 DPI)

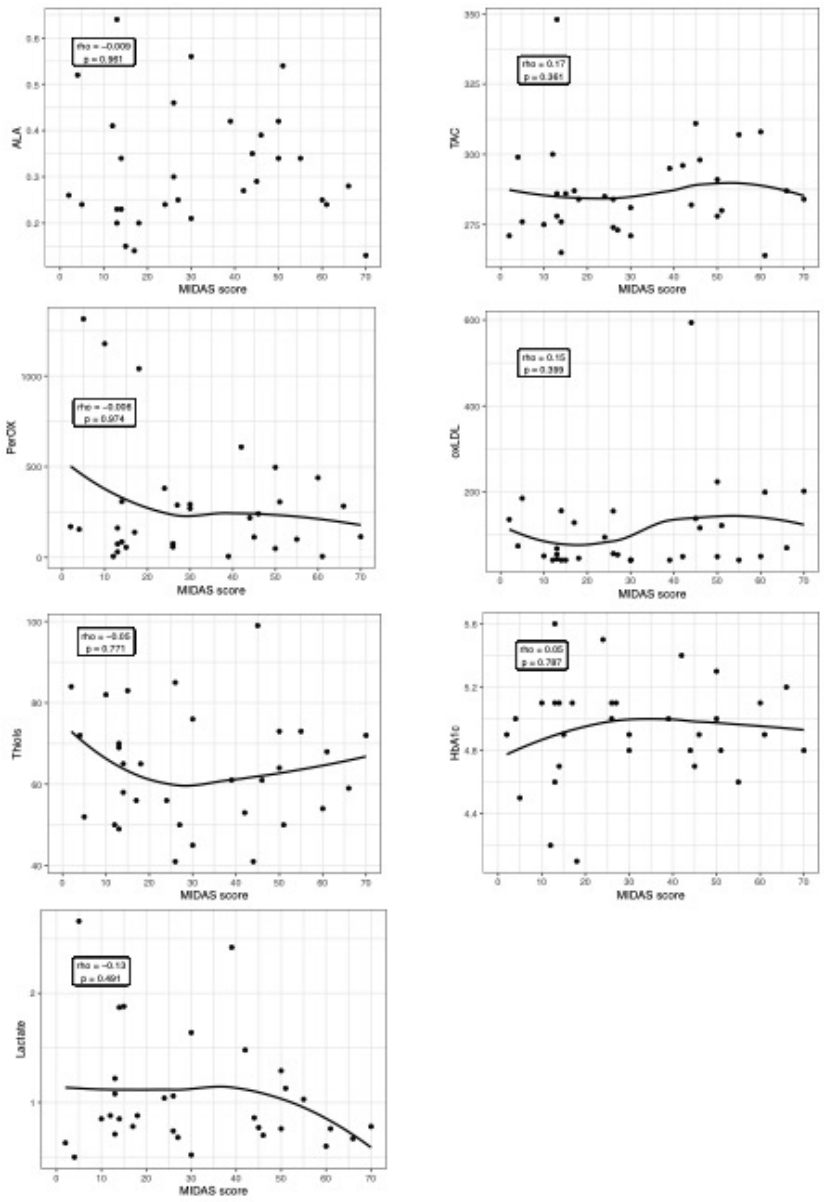

Figure 4: Mitochondrial function biomarkers vs MIDAS score at baseline.
Alpha lipoic acid: outlier data for one patient with an extremely high value (>10) is not shown.
Smoothingcurves, using locally estimated scatterplot smoothing (LOESS) with span 1.0, are shown.
ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

156x227mm (72 x 72 DPI)

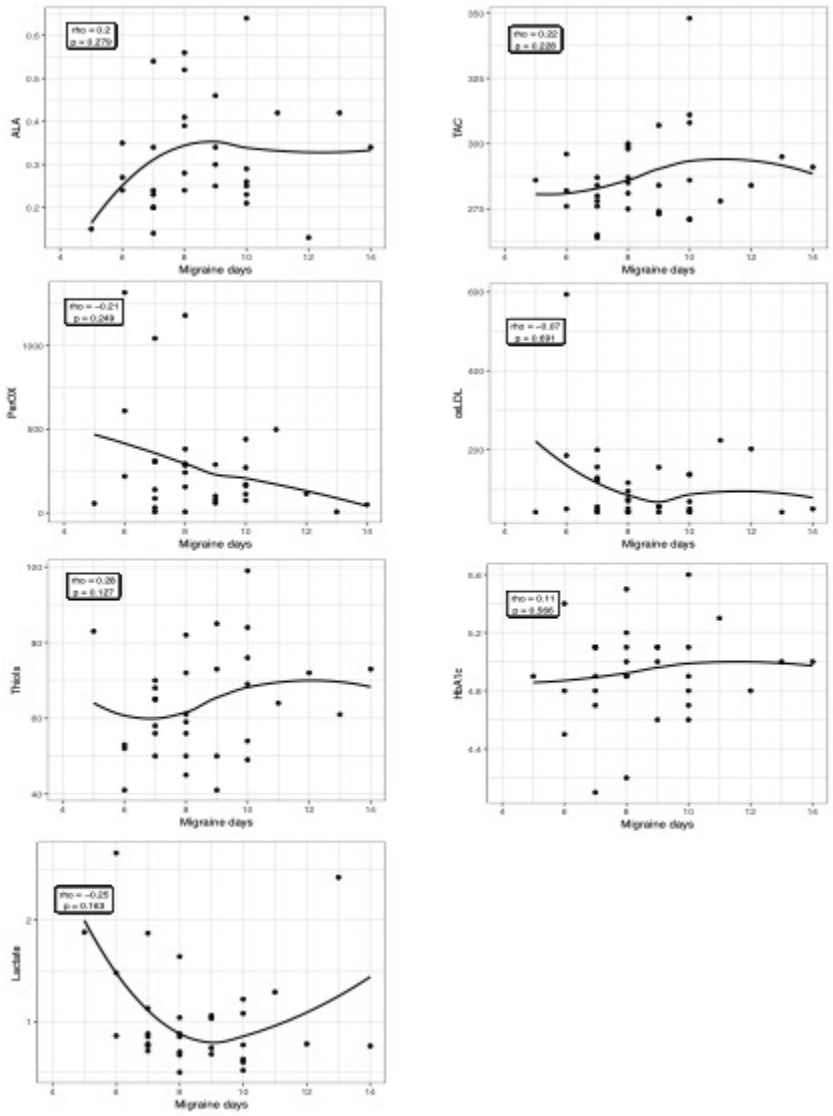

Figure 5:Mitochondrial function biomarkers vs number of migraine days per month at baseline. Alpha lipoic acid: outlier data for one patient with an extremely high value (>10) is not shown. Smoothingcurves, using locally estimated scatterplot smoothing (LOESS) with span 1.0, are shown. ALA = Alpha-lipoic acid; ox-LDL = oxidised LDL; PerOx= total lipid peroxide; TAC = total antioxidant capactity

146x196mm (72 x 72 DPI)

2.3 Manuscript 3: Potential protective mechanisms of ketone bodies in migraine prevention

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Potential protective mechanisms of ketone bodies in migraine prevention

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Author Contributions:

E.C.G. was responsible for literature search and the main composition of the manuscript, including display items. R.K. and J.S. edited the manuscript and provided additional text and citations. D.P.D and D.F. edited the manuscript. All authors proofread the final manuscript prior to submission.

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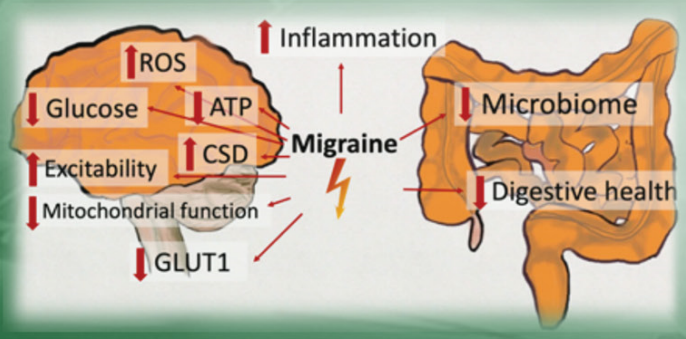

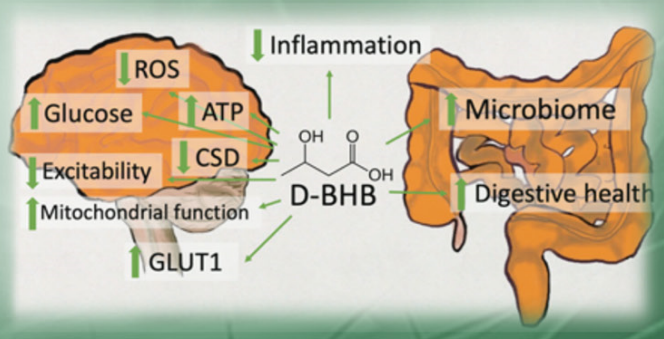

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Review

Potential Protective Mechanisms of Ketone Bodies in Migraine Prevention

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Abstract: An increasing amount of evidence suggests that migraines are a response to a cerebral energy deficiency or oxidative stress levels that exceed antioxidant capacity. The ketogenic diet (KD), a diet mimicking fasting that leads to the elevation of ketone bodies (KBs), is a therapeutic intervention targeting cerebral metabolism that has recently shown great promise in the prevention of migraines. KBs are an alternative fuel source for the brain, and are thus likely able to circumvent some of the abnormalities in glucose metabolism and transport found in migraines. Recent research has shown that KBs—D-β-hydroxybutyrate in particular—are more than metabolites. As signalling molecules, they have the potential to positively influence other pathways commonly believed to be part of migraine pathophysiology, namely: mitochondrial functioning, oxidative stress, cerebral excitability, inflammation and the gut microbiome. This review will describe the mechanisms by which the presence of KBs, D-BHB in particular, could influence those migraine pathophysiological mechanisms. To this end, common abnormalities in migraines are summarised with a particular focus on clinical data, including phenotypic, biochemical, genetic and therapeutic studies. Experimental animal data will be discussed to elaborate on the potential therapeutic mechanisms of elevated KBs in migraine pathophysiology, with a particular focus on the actions of D-BHB. In complex diseases such as migraines, a therapy that can target multiple possible pathogenic pathways seems advantageous. Further research is needed to establish whether the absence/restriction of dietary carbohydrates, the presence of KBs, or both, are of primary importance for the migraine protective effects of the KD.

Keywords: migraine; beta-hydroxybutyrate; ketone bodies; ketosis; migraine prevention; ketogenic diet; exogenous ketone bodies

1. Introduction

Migraine is a complex, common and debilitating neurological disorder [1]. Its episodic form is characterized by recurrent moderate to severe, typically throbbing and unilateral headache attacks that last between 4–72 h, which are aggravated by any kind of physical activity and accompanied by either photo-, phono-, or osmophobia, nausea, or a combination of these. Migraine affects approximately 17% of women and 8% of men in Europe [2], and with a peak incidence during the most productive

years of life, migraine not only causes a huge amount of suffering, but also inflicts a substantial number of costs on society: approximately €18.5 billion per year in Europe alone [3,4]. Current migraine treatment options have limited efficacy and many—often intolerable—side-effects [5,6], with the potential exception of the very recent addition of monoclonal Calcitonin gene-related peptide (CGRP) antibodies [7]. Despite migraine's primary pathogenic mechanisms being still largely unknown [8], accumulating evidence suggests that migraines could be—at least partially—an energy deficit syndrome of the brain, and the migraine attack a response to increased oxidative stress and/or (cerebral) hypometabolism [9]. Therapeutic approaches targeting cerebral metabolism may be warranted.

Ketone bodies (KBs: D-β-hydroxybutyrate (D-BHB), acetoacetate (AcAc), and to a lesser extent acetone) are mainly produced by the liver, but also other tissues, such as astrocytes [10], when glycogen storage is deprived, to serve as energetic substrates in the absence or severe reduction of dietary glucose, in particular for the heart and the brain. Mimicking this state of fasting, the ketogenic diet (KD) promotes the hepatic production of KBs with a high fat, low carbohydrate and moderate protein content. It was developed about 100 years ago after the observation that prolonged fasting has anticonvulsive properties [11]. Within recent years, the KD has received renewed interest, in particular since KBs might be beneficial for a variety of other neurological disorders [12–14]. All brain cells have the capacity to use KBs as respiratory substrates [10].

Out of the three physiological KBs, D-BHB constitutes up to 70% of KBs produced during ketosis [15] and is of particular interest, since it is not only a glucose transporter protein, i.e., a (GLUT)-independent alternative metabolite, but also a vital signalling molecule [16]. Many of these collateral effects make it a molecule of interest for therapeutic purposes. During a standard Western diet, the blood concentration of D-BHB is very low (<0.2 mmol/L) compared to glucose (≅5 mmol/L) [17]. During fasting or the KD D-BHB concentrations typically rise to levels between 0.5–5 mmol/L and up to 8 mmol/L during starvation [18]. Elevated KB levels have been shown to be well tolerated for extended periods of time (up to several years [19–32]).

Several case studies have demonstrated the potentially migraine protective effects of ketosis [22,33–37]. A one-month observational study of KD in 96 migraine patients as part of a weight loss program found a reduction of up to 80% in migraine frequency, severity and acute medication use [37]. The same intervention in 18 episodic migraineurs induced a 62.5% reduction in migraine days, which was accompanied by a normalization of the interictal habituation deficit of visual evoked responses [36]. The reduction in migraine attack frequency, severity and the use of acute anti-migraine medication during ketosis had effect sizes ranging from a total absence of attacks [33] to a reduction to 1/5th of the run-in period [37]. In addition, preliminary evidence suggests that the protective effect may outlast the duration of ketosis [33], as is often the case in pediatric epilepsy patients, and could be the result of longer-lasting gene-expression changes [12,38].

This review will describe the mechanisms by which the presence of ketone bodies, D-BHB in particular, could influence migraine pathophysiology (see Figure 1). To this end, common abnormalities in migraine (such as abnormalities in glucose metabolism and transport, mitochondrial functioning, oxidative stress, cerebral excitability, inflammation and the gut microbiome) are summarised with a particular focus on clinical data, including phenotypic, biochemical, genetic and therapeutic studies. Experimental animal data will be discussed to elaborate on the potential therapeutic mechanisms of elevated KBs in migraine pathophysiology with a particular focus on the actions of D-BHB. Please note that there is not enough research at present to disentangle the potentially differential effects of D-BHB within the scope of a KD (i.e., endogenous KBs via restriction of dietary carbohydrates) versus D-BHB added to a standard Western diet (i.e., exogenous BHB in addition to dietary carbohydrates). Research studies using either method will be cited, but not contrasted.

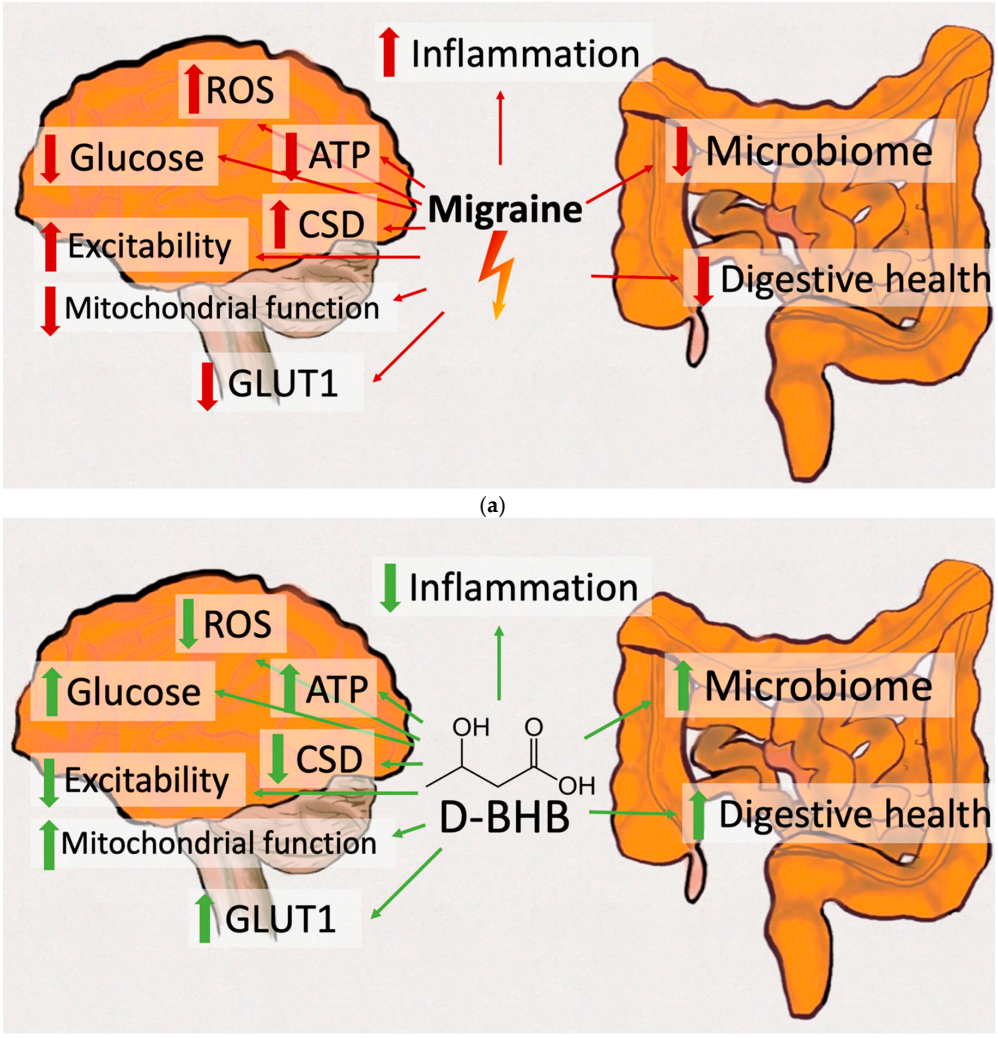

Figure 1. Potentially migraine relevant mechanisms of ketosis. (**a**) Amongst key migraine pathophysiological mechanisms are hypometabolism, decreased glucose transport (including glucose transporter 1 (GLUT1) deficiency), reduced mitochondrial functioning, increased cerebral excitability, increased cortical spreading depressions (CSD) incidence, increased oxidative stress (reactive oxygen species (ROS)), increased inflammation, microbiome abnormalities and reduced digestive health. (**b**) D-β-hydroxybutyrate (D-BHB; with or without the context of a ketogenic diet) has been shown to positively influence each of these mechanisms: increasing cerebral metabolism, increasing glucose transport (including glucose transporter 1 (GLUT1) deficiency), increasing mitochondrial functioning, reducing cerebral excitability, decreasing cortical spreading depressions (CSD) incidence, reducing oxidative stress (reactive oxygen species (ROS)), decreasing inflammation, improving the microbiome and increasing digestive health. ATP = adenosine triphosphate; CSD = cortical spreading depressions; D-BHB = D-β-hydroxybutyrate; GLUT1 = glucose transporter 1; ROS = reactive oxygen species.

2. Potentially Migraine Relevant Mechanisms of Ketosis

2.1. Hypoglycemia/Hypometabolism

Hypoglycaemia has been associated with migraine for almost a century [39–41] and fasting/skipping a meal is not only amongst the most commonly cited migraine triggers [42–44], but it can also be used experimentally to elicit migraine attacks in susceptible patients [39]. Due to very limited glycogen stores and high energy demands, the human brain is highly dependent on fuel sources from the circulation that can pass the blood-brain barrier and especially vulnerable to their

potential short-comings. A simple comparison between migraine associated symptoms, premonitory symptoms in particular, and symptoms of hypoglycaemia [45] show several similarities: for example, dizziness, pale skin, cold hands and feet, binge eating/sugar cravings, yawning, nausea, low blood pressure, shaking, cognitive difficulties, tiredness, fatigue, visual dysfunction and slurred speech. Increased migraine frequency has also been observed during Ramadan [46] and migraine prevalence in type 2 diabetics was shown to proportionally increase with the number of hypoglycaemia attacks [47].

Further support for the role of energy deficiency and/or glucose metabolism in migraine comes from neuroimaging studies. Using 31P-MRS, an impairment of brain oxidative phosphorylation (OXPHOS) was detected first during migraine attacks [48] and thereafter between attacks [49–56]. OXPHOS abnormalities in patients with migraine were found both in the resting brain and in the muscle following exercise [49,56,57] (review by [54]), where a reduced glycolytic flux could be demonstrated [49,51]. More recently, a 16% decrease of absolute ATP levels in migraine without aura patients was demonstrated interictally using 31P-MRS [58]. This hypometabolism is generally found to moderately correlate with attack frequency [52,56,58]. A recent study comparing resting cerebral glucose uptake using 18-fluorodeoxyglucose-PET and visual cortex activation using visual evoked potentials showed that visual neuronal activation exceeded glucose uptake in visual areas in 90% of interictal migraine without aura patients, but in only 15% of healthy controls [59]. This supports the concept that a mismatch between brain activity and glucose metabolism may be a cornerstone of migraine pathophysiology.

KBs are known to be able to counteract some of the negative effects of hypoglycemia and/or hypometabolism or prevent it all together. D-BHB has been shown to efficiently prevent neuronal death in the cortex of hypoglycemic animals and in vitro it was found to stimulate ATP production in glucose deprived cortical cultures [60]. Glycolysis is reduced in the presence of D-BHB and ketosis proportionally spares glucose utilization in the brain [61,62]. When present in sufficient concentration to saturate metabolism, D-BHB provides full support of all basal (housekeeping) energy needs and up to approximately half of the activity-dependent oxidative needs of neurons in 36h fasted rats [63]. Not only is D-BHB an alternative fuel source for the brain, it also seems to be more efficient. When catabolized for the synthesis of ATP in mitochondria, D-BHB produces more ATP per oxygen molecule consumed than many other respiratory substrates [64] and general "positive" shifts in energy balance have been observed [65–67].

There is circumstantial evidence from early experimental studies that oral glucose tolerance tests after an overnight fast can elicit migraine attacks in susceptible patients [68,69]. Interestingly, the metabolic responses in patients who developed an attack differed substantially from those that did not: free fatty acid (FFA) and KB levels increased significantly in the former, already before headache onset, and kept increasing despite similar food intake [68,69]. This can be interpreted as a counter-regulatory response to a cerebral energy deficit. Since KBs are an efficient alternative fuel for the brain, when glucose availability is low, their elevation would be expected to restore brain energy homeostasis, if present in sufficient quantity.

2.2. Glucose Transport

GLUTs are a wide group of membrane proteins that facilitate the transport of glucose across a plasma membrane. GLUT1 is an insulin-independent glucose transporter responsible for transporting glucose under basal conditions in all cells. This is especially the case in endothelial cells of the blood brain barrier as well as astrocytes and oligodendrocytes. In addition to glucose utilization, glucose transport might also play a role in migraine. GLUT1 deficiency syndrome has been linked to hemiplegic migraine and migraine with aura [70].

GLUT4 (adipose tissue and striated muscle) and GLUT3 (neuronal and glial cells) are the major insulin-mediated glucose transporters [71]. Insulin is the main anabolic hormone of the body and the key regulator of glucose homeostasis. It promotes the absorption of glucose from the blood and simultaneously blocks carnitine transporters and thus the penetration of FFA into the cells.

Interictal impaired glucose tolerance and insulin resistance in migraine has been reported in various studies [72–76], but the evidence is not always conclusive [77]; see also the review by [78]. Some genetic support for a potential role of insulin in migraine comes from associations between polymorphisms in insulin-related genes and migraine [79–82].

KBs are taken up in to the brain via the monocarboxylate transporters (MCTs) and are hence completely GLUT and insulin independent. This KB transport mechanism allows the body and brain access to fuel even when glucose transport (GLUT1 or GLUT3/4) is compromised. Furthermore, KBs can also be produced endogenously in brain by astrocytes that have the capacity to metabolize free fatty acids and ketogenic amino acids L-Lysine and L-Leucine [10,83,84]. A strict KD can lead to complete remission in GLUT1 deficiency syndrome [85]. Additionally, a KD has been shown to lead to a marked upregulation of both GLUT1 and MCTs [86], thereby further enhancing available energy to the brain in a not completely GLUT1 compromised individual.

2.3. Mitochondrial Functoning

Several lines of evidence point towards a role of mitochondrial functioning in migraine. The prevalence of migraine in mitochondrial disorders is more than doubled (29–35.5% of patients) [87,88] and migraine-like attacks in Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) are especially severe and prolonged [89]. Maternal transmission in migraine is more common [90], which suggests that either an X-linked form of inheritance could be involved or that mitochondrial DNA (mtDNA) transmission plays a role, since mtDNA derives exclusively from maternal origin. Furthermore, enrichment of a migraine genome-wide association study (GWAS) signal was found for mitochondria in both subcortical areas and the cortex (amongst others), a finding that identifies a genetic link between mitochondrial function and common migraine [91].

Further support for a generalized metabolic dysfunction in migraine comes from the reduced activity of mitochondrial enzymes, such as monoamine-oxidase, succinate-dehydrogenase, NADH-dehydrogenase, cyclooxygenase (COX) and citrate-synthetase in the platelets of migraine patients with and without aura [92,93]. Interestingly, these biochemical changes are restricted to enzymes of the respiratory chain that are encoded by mtDNA. In contrast to nuclear DNA, mtDNA is particularly sensitive to ROS because it lacks protection from histones [94,95].

Therapeutic studies also support mitochondrial dysfunction in migraine. Most nutraceuticals that have been demonstrated to be migraine preventative can directly be linked to energy metabolism and/or mitochondrial functioning, such as: high dose riboflavin (200–400 mg) [96–100], coenzyme Q10 (400 mg capsules or 300 mg liquid suspension) [101–106], alpha-lipoic acid (or thioctic acid) [107–109], B vitamins [110–112] and magnesium [113]. Even pharmaceutical prophylactic agents used against migraine are able to influence mitochondrial functioning and metabolism. For example, Topiramate prolongs mitochondrial survival, increases the activity of the mitochondrial respiratory chain complex [114], protects against oxidative stress, inflammation [115] and mitochondrial membrane depolarization, and has an insulin-sensitizing effect on adipocytes in female rats [116]; Amitriptyline also increases antioxidant capacity and reduces markers of oxidative stress [117] and Valproate preserves mitochondrial function in a rat model of migraine [118] and increases mitochondrial biogenesis [119].

KBs have been shown to enhance mitochondrial function [120–122] p. 20, [123] and stimulate mitochondrial biogenesis in the rat [65,124,125]. Furthermore, D-BHB may bypass complex I deficits due to its effects on complex II (succinate dehydrogenase) [123], thereby maintaining mitochondrial respiration and ATP production even in the presence of a complex I inhibitor (rotenone), but not a complex II inhibitor (malonate) [126].

2.4. Oxidative Stress

While reactive oxygen species (ROS) and reactive nitrogen species (RNS) are necessary for certain signalling pathways, their unregulated production is deleterious, if it exceeds the anti-oxidant capacity

of the organism. All common migraine triggers (stress/relaxation thereafter, fasting/skipping a meal, sleep changes (too much or too little), hormonal changes (including menses or oral contraceptives), weather changes (including hypoxia and high altitude), physical exercise (including sexual activity), alcohol, strong odours (especially perfume or cigarette smoke), intense light (especially bright or blue light) and loud noises [42,43,127]) are likely to negatively impact the balancing of oxidative stress levels, either directly or indirectly via negatively impacting mitochondrial functioning or energy metabolism [9,128].

Free iron is highly pro-oxidant and accumulates in the brain stem nuclei of migraine patients proportionally to disease duration [129]. Other heavy metals with pro-oxidant properties may also be increased in migraine [130]. Increased oxidative/nitrosative stress and/or decreased anti-oxidant capacity have also directly been found in migraine patients [117,131–143]. Of all biomarkers examined, superoxide dismutase (SOD) activity seems to be consistently reduced in migraine patients, also interictally [144]. Reduced anti-oxidant capacity or increased oxidative stress in migraine could be related to a genetic predisposition. A polymorphism in the SOD2 gene was associated with unilateral cranial autonomic symptoms in migraine with aura patients [145], and in paediatric migraine patients' polymorphisms in the SOD2 and catalase gene were significantly higher in both migraine with and without aura patients compared to controls [146].

Further support for the role of oxidative stress in migraine is that as aforementioned antioxidants, such as Coenzyme Q10 (400 mg capsules or 300 mg liquid suspension) [101–106] and alpha-lipoic acid (or thioctic acid) [107–109] have been shown to have migraine protective effects.

The elevation of KBs, D-BHB in particular, seems to be an effective method for combating the negative consequences of elevated ROS/RNS. Systemic administration of D-BHB has been shown to reduce ROS production in distinct cortical areas and subregions of the hippocampus and efficiently prevented neuronal death in the cortex of hypoglycemic animals [60]. KBs themselves (AcAc, D-BHB and the non-physiological isomer L-BHB) have scavenging capacity [147].

Hydroxyl radicals (*OH) were effectively scavenged by D- and L-BHB, but only the administration of D- or L-BHB, but not of AcAc, was able to prevent the hypoglycemia-induced increase in lipid peroxidation in the rat hippocampus [147]. Furthermore, the metabolism of KBs results in a more negative redox potential of the NADP antioxidant system, which is a terminal destructor of ROS [148]. By increasing NADH oxidation, KBs have also been shown to be able to inhibit mitochondrial production of ROS following glutamate excitotoxicity [149].

D-BHB is a natural inhibitor of class 1, 2a, 3 and 4 histone deacetylases that repress transcription of the FOXO3a gene; this epigenetic action results in transcription of the enzymes of the antioxidant pathways, such as mitochondrial superoxide dismutase (MnSOD), catalase and metallothionein [150–154]. This has been shown to lead to significantly reduced markers of oxidative stress, such as lipid peroxidation and protein carbonylation, in the kidneys of D-BHB-treated compared to control mice [153]. BHB was also shown to increase FOXO3a activity through direct AMPK phosphorylation [155]. Furthermore, up-regulated glutathione and lipoic acid biosynthesis, enhanced mitochondrial antioxidant status, and protection of mtDNA from oxidant-induced damage in rats fed a KD for 3 weeks compared to controls have also been demonstrated [156]. One possible mechanism by which glutathione biosynthesis may be increased is through the activation of the Nrf2 transcription factor pathway [121]. Hence, KBs may increase antioxidant capacity via several mechanism [121,150].

To summarise, KBs, D-BHB in particular, generate lower levels of oxidative stress and increase antioxidant protein levels in combination with a higher cellular energy output [16,121,150]. D-BHB has previously been shown to be elevated as a compensatory response against oxidative stress in failing mice hearts [152]. It can be hypothesized that the aforementioned increased KB levels during a migraine attack [68,69] could represent an analogous reaction in response to increased oxidative stress.

2.5. Cerebral Excitability

The KD is known to be effective as treatment for refractory epilepsy [157]. There is comorbidity and shared genetic susceptibility between migraine and epilepsy [158] and hence partially similar pathophysiologic mechanisms are not unlikely. This assumption is supported by the overlap in certain pharmacological agents used to treat both disorders. One of the most reproducible and ubiquitous interictal abnormalities of the migraineurs' brain is a lack of habituation in neuronal information processing [159]. While the exact underlying mechanisms of this phenomenon are uncertain, an imbalance between neuronal activation and inhibition is likely to be a pathogenic cornerstone in migraine, as it is in epilepsy [160–164]. The genetic association between migraine and epilepsy is illustrated by their co-occurrence in the hitherto known three subtypes of familial hemiplegic migraine (FHM), a rare autosomal dominant monogenic form of migraine. The three mutated genes are responsible for increased glutamate release (FHM1—CACNA1A) [165,166], reduced glutamate re-uptake (FHM2—ATP1A2) [167] or a decreased excitation of inhibitory interneurons (FHM3—SCN1A) [168]. In the common forms of migraine with and without aura, GWAS have identified 38 susceptibility loci [169–171]. These loci point towards genes involved in a variety of functions including glutamate release and re-uptake, and generation of action potentials [172], which may lead to a "generalized" neuronal hyperexcitability of the migraine brain.

The observation that increased KB metabolism produces seizure protection suggests that fuel utilization and neuronal excitability are linked, however, the mechanisms underlying this link are poorly understood [173]. In addition to providing an alternative and more effective energy substrate, its positive effect on oxidative stress levels and mitochondrial functioning, some additional mechanisms have been proposed to underlie the effects of ketosis on cerebral excitability:

A higher synthesis of the inhibitory neurotransmitter GABA from glutamate [174] and a reduction of neuronal firing in GABAergic neurons of the substantia nigra pars reticulata have been demonstrated in response to KBs; this reduction being greater in faster-firing neurons [175]. Another effect of KBs on neural excitability seems to be mediated by an inhibition of glutamate transport and reduction in glutamate release [176], which in turn affects excitatory synaptic transmission [176]. Further reduction in excitability might be achieved via adenosine signalling through A1 purinergic receptors [177] and regulation of excitability by the activity of lactate dehydrogenase [178]. D-BHB has also been shown to be an agonist at FFA receptor GPR41, directly modulating the activity of N-type Ca^{2+} channels [179]. Furthermore, a KD was shown to activate inward rectifying potassium channels (metabolically sensitive K(ATP) channels) that in turn stabilize central neural excitability [38,175,180,181]. D-BHB specifically can bind to BAD (BCL-2 agonist of cell death) that, in addition to being pro-apoptotic, disrupts glucose metabolism KBs and hence opens K(ATP) channels [181]. An increase in inhibition as well as a reduction in excitability could be migraine protective.

2.6. Cortical Spreading Depression

Cortical spreading depression (CSD) denotes a wave of cellular depolarization of the neurons and neuroglia within the cerebral cortex and has been implicated as the underlying pathophysiological mechanism in migraine aura. CSD susceptibility is strongly modulated by metabolic factors. Hypoxia can trigger a CSD [182,183] and cerebral glucose availability modulates extrinsically induced CSD in both directions [184,185]. Hypoglycaemia significantly prolongs CSD duration and hyperglycaemia protects the tissue from CSD induction [184]. Supplying the rat brain with an alternative energy substrate to glucose via both short- and long-term treatment with a middle chain triglyceride enriched ketogenic diet has a similar protective effect against CSD [21].

2.7. Inflammation

Inflammation is a localised response designed to protect tissues against disease, infection or injury. Even though the involvement of neurogenic inflammation in migraine remains controversial [186],

and migraine has not classically been considered an inflammatory disease, possibly because it is not obviously associated with redness, heat and swelling, several lines of evidence point towards the involvement of pro-inflammatory peptides or a "sterile neurogenic inflammation" in migraine pain (review [187,188]). Most importantly, calcitonin gene related peptide (CGRP) [189–193] see review [194], but also substance P, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), nitric oxide (NO) [135,195–198] and to some extent cytokines [199], are all molecules associated with migraine both in animal and human studies review [187,200]. Cytokine Polymorphism (TNF-α and IL-1β gene polymorphisms) in patients with migraine without aura provide some more suggestive evidence for a possible contribution of inflammation in migraine [201]. Meningeal mast cell activation has been discussed to play a role in meningeal nociceptor activation in migraine [202].

D-BHB was reported to reduce inflammation generated by macrophages via a mechanism independent from any of those reported above [203]. The NLR family, pyrin domain-containing 3 (NLRP3) inflammasome, which is expressed mainly in immune cells, is activated by a shift to low cytoplasmic K+ levels [204]. Youm et al. noted that D-BHB prevented the decline in K+ levels and prevented activation of NLRP3 [203]. A reduction in pain and inflammation has been observed in rats fed the KD [205–207], which could further be migraine protective.

2.8. Gut Microbiome

The potential role of the microbiome in migraine pathophysiology is not yet fully established. Two recent reviews report an increased frequency of gastrointestinal (GI) symptoms or disorders in migraine patients compared to the general population, such as increased rates of nausea, cyclic vomiting syndrome, inflammatory bowel syndrome, irritable bowel syndrome, celiac disease, gastroparesis, hepatobiliary disorders, helicobacter pylori infection, gastric stasis, and alterations in the microbiota [208–210]. While there was no evidence for an added benefit of probiotics in an RCT with 63 episodic migraineurs [211] an uncontrolled observational study with 1020 patients using a multispecies probiotic showed a significant reduction in migraine days, headache intensity and the use of painkillers [212]. Additionally, an immunoglobulin-G based elimination diet among migraine patients with irritable bowel syndrome was associated with significant reductions in attack frequency, duration, severity and medication use [213]. Possible underlying mechanisms of migraine and GI diseases could be inflammation, alterations in the gut microbiota and its metabolites (such as neurotransmitters and neuropeptides) as well as increased gut permeability.

The role of inflammation in migraine as well as the anti-inflammatory effects of ketosis have already been reviewed above. In addition, it has very recently been shown that a KD in children with severe epilepsy alters the gut microbiome [214,215]. Recent experiments in mice suggest that the KD seems to mediate some of its anti-seizure effects [216]. Mice treated with antibiotics or reared germ free were resistant to KD-mediated seizure protection otherwise found. Enrichment of and co-colonization with the KD-associated *Akkermansia* and *Parabacteroides* was shown to restore seizure protection. Even in mice fed a control diet, transplantation of the KD gut microbiota and treatment with *Akkermansia* and *Parabacteroides* each conferred seizure protection [216].

In support of this, in infants with refractory epilepsy a KD decreased seizure frequency in the majority of cases and significantly changed the gut microbial composition towards that of healthy controls: *Bacteroides* and *Prevotella* increased, while *Cronobacter* levels decreased by approximately 50% [217]. Other studies show a normalization of the microbiota in two other neurological disorders, namely autism [218] and multiple sclerosis [219]. In the BTBRT+tf/j mouse model of autism spectrum disorder, a KD significantly increased the Firmicutes/Bacteroidetes ratio which is typically low in autism spectrum disorder and also normalized the overabundance of the mucin-degrading bacterium *Akkermansia muciniphila* [218]. In patients with multiple sclerosis who were characterized by a reduced mass and diversity of microbial species, a KD initially decreased total gut bacterial concentrations

during the first weeks, but, when maintained over six months, was able to restore the microbial mass to levels similar as in healthy controls [219].

However, these findings could not be replicated in all studies. For example, a small study on children with severe epilepsy found that the relative abundance of *bifidobacteria* as well as *E. rectale and Dialister* was significantly diminished during the KD intervention while an increase in relative abundance of E. coli was observed instead [214]. In a small study on GLUT1 deficiency syndrome a significant increase in *Desulfovibrio spp.*, a bacterial group supposed to be involved in the exacerbation of the inflammatory condition of the gut mucosa, was found [220]. The differences observed might be due to the kind of KD employed, as different forms of the KD can vary tremendously in micro- and even macronutrient content, depending on the ratio of fat to protein/carbs, the use of processed meal replacements or processed vegetable oils, source of protein and carbohydrates and other factors. The children with severe epilepsy were mostly on a very strict classical KD with 4:1 ratio that allows for little fibre content. In addition, the use of pro-inflammatory processed vegetable oils and instant ketogenic formulas or meal replacements may be suboptimal for the gut microbiome. Lastly, given the structural and functional similarity between butyrate and BHB, it could be hypothesized that higher systemic concentrations of the latter in case of the higher KD rations (4:1) could decrease the importance of microbial butyrate production [221].

In sum, an alteration of the microbiome and its downstream beneficial effects on gut permeability, synthesis of metabolites and neuropeptides, as well as inflammation could thus be another potential disease modifying mechanism of ketosis in migraine.

3. Discussion and Conclusions

Migraine is a very heterogeneous disease, with most probably a multitude of fairly common genetic polymorphisms and in turn pathophysiological mechanisms contributing to the migraine phenotype. We have reviewed the potential contribution of eight such pathophysiological mechanisms and their possible exploitation through dietary ketosis (KDs and/or D-BHB supplementation): (1) hypoglycaemia/hypometabolism, (2) glucose transport, (3) mitochondrial functioning, (4) oxidative stress, (5) cerebral Excitability, (6) CSD, (7) inflammation and (8) the microbiome. Which mechanisms contribute to the migraine phenotype in a given individual are likely to vary. Further research is needed to confirm these mechanistic hypotheses and their translational relevance for patients. Furthermore, the scope and importance of these metabolic mechanisms within the broad range of migraine pathophysiology remains to be determined.

With migraine being such a diverse and multigenic disease, finding the one treatment target seems a nearly impossible endeavour. Exciting recent technological advances in the field of genetics and induced pluripotent stem cells are paving the way for future more personalised treatment approaches. Until those reach the clinic, an elevation of KBs, D-BHB in particular, which have been shown to potentially influence all of the aforementioned migraine pathophysiological mechanisms, might offer a long-needed relatively side-effect free remedy for at least a proportion of migraine sufferers.

It remains to be determined whether the absence/restriction of dietary carbohydrates, the presence of KBs, or both, are of primary importance for the potentially migraine protective effects of the KD that has previously been demonstrated. Additionally, third factors, such as increased fatty acids, amino acids, supplementation with medium-chain triglycerides [222] or other dietary changes as well as alteration of the microbiome could also be disease modifying. The potential preventative anti-migraine effect of supplementation with BHB without a strict dietary change is currently being examined in an RCT [223] and could help answer some of these questions.

Moreover, a lot of the mechanistic effects of ketosis and/or presence of BHB have been examined in animals and more clinical research is needed to validate those effects. Such future clinical research could additionally help determine whether, and to what extent all the aforementioned potentially disease-modifying effects of ketosis are actually also occurring in migraine patients.

Patents: E.C.G. and D.F. are the inventors of the patent WO 2018/115158 Al) held by the UKBB and University of Basel on the use of beta-hydroxybutyrate in migraine prevention. D.P.D. is inventor on International Patent # PCT/US2014/031237, University of South Florida, "Compositions and Methods for Producing Elevated and Sustained Ketosis". D.P.D. is co-owners of the company Ketone Technologies LLC. These interests have been reviewed and managed by the University in accordance with its Institutional and Individual Conflict of Interest policies.

Author Contributions: E.C.G. was responsible for literature search and the main composition of the manuscript, including display items. R.J.K. and J.S. edited the manuscript and provided additional text and citations. D.P.D and D.F. edited the manuscript. All authors proofread the final manuscript prior to submission.

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2.4 Manuscript 4: Migraine Prevention and Treatment

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Abstract:

The invention relates to a compound for use in a method of treatment or prevention of migraine and/or symptoms thereof. The compound is selected from beta-hydroxybutyric acid (βHB) or a pharmaceutically acceptable salt thereof, acetoacetate (AcAc) or a pharmaceutically acceptable salt thereof, a metabolic precursor of βHB or AcAc 1,3- butanedio and a compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety. The invention further relates to a pharmaceutical composition comprising βHB, AcAc or a pharmaceutically acceptable salt thereof.

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(54) **Title:** MIGRAINE PREVENTION AND TREATMENT

(57) **Abstract:** The invention relates to a compound for use in a method of treatment or prevention of migraine and/or symptoms thereof. The compound is selected from beta-hydroxybutyric acid (βHB) or a pharmaceutically acceptable salt thereof, acetoacetate (AcAc) or a pharmaceutically acceptable salt thereof, a metabolic precursor of βHB or AcAc 1,3- butanedio and a compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety. The invention further relates to a pharmaceutical composition comprising βHB, AcAc or a pharmaceutically acceptable salt thereof.

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Migraine prevention and treatment

The present invention relates to the use of D beta-hydroxybutyric (βHB) acid or a metabolic precursor for the prevention or treatment of migraine or symptoms of migraine.

Background

Migraine

Migraine is a complex, genetically heterogeneous, common and debilitating neurological disorder that affects approximately 15% of the world population. With a peak incidence during the most productive years of life, migraine not only causes much suffering, but also inflicts substantial costs on society: approximately € 18.5 billion per year in Europe alone. It is characterized by recurrent moderate to severe, typically throbbing and unilateral headache attacks that last between 4 - 72 h, which are aggravated by any kind of physical activity and accompanied by either photo-, phono-, or osmophobia, nausea or a combination of these. It is a very heterogeneous disorder, divided into two major subgroups, based on the presence (migraine with aura (MA)) or absence (migraine without aura (MO)) of an aura, a phase of transient and reversible visual, sensory or motor disturbances that typically occurs up to one hour before the attack itself in one third of migraineurs. Migraines are much more than the headache (ictal) phase, as they are typically accompanied by neurological symptoms during a premonitory phase preceding the headache by up to 12 hours and a postdromal phase, which follows the migraine and can last hours or days. To date, the primary migraine pathogenic mechanisms are still largely unknown.

Migraine therapy

Current migraine treatment options are limited and their mechanisms of action are also not completely understood. While the primary goals of preventative migraine treatment include reducing headache frequency and restoring function, an additional important goal may be the prevention of progression to chronic migraine. None of the prophylactic agents licensed to date (such as beta-blockers, anticonvulsants or antidepressants) are migraine-specific and most are associated with significant - often intolerable - side-effects. Furthermore, their migraine-preventive properties are moderate at most (<25% average reduction in migraine frequency). Hence, there is a huge medical need for developing alternative anti-migraine therapies. The objective of the present invention is to provide novel therapeutic agents for the treatment of migraine, which exhibit improved efficiency and decreased side-effects.

Ketogenic diet / Endogenous elevation of KB and reduction in glucose

The ketogenic diet (KD) was developed about 100 years ago after the observation that prolonged fasting has anticonvulsive properties. With its high fat, low carbohydrate and protein content it simulates the metabolic effects of starvation. KD has been shown to be an effective alternative when treating refractory epilepsy and albeit its mechanisms are still poorly understood, there is mounting experimental evidence for its broad neuro-protective mechanisms and its potential use in multiple neurological disease states, for example metabolic defects, such as mitochondrial disorders, neurodegenerative disorders, such as Parkinson's Disease and Alzheimer's Disease (AD), trauma and ischemia, narcolepsy and maybe even depression or autism. Nevertheless, clinical evidence on the benefit of ketosis is still mostly confined to refractory epilepsy. Here elevated KB levels achieved via a KD have been shown to be well tolerated for extended periods of time (up to several years). However, a strict KD is unlikely to provide a feasible long-term solution for many patient populations, because it can be difficult to implement in an ambulatory setting and patient adherence may be limited.

Exogenous KB

An alternative means to induce a state of mild to medium nutritional ketosis, irrespective of dietary carbohydrate and protein intake, is the dietary supplementation with exogenous ketogenic substances, such as middle chain triglycerides (MCTs), ketogenic amino acids, βHB or AcAc supplements and more recently keto esters (βHB and/or AcAc esterified with one another). Dietary supplementation of KB themselves does not require the limitation of carbohydrate and protein, thus increasing the chance of compliance, particularly since carbohydrate diets are common in most cultures.

In comparison to the KD itself, the therapeutic efficacy of KB supplementation is less established to date. Studies in humans using MCTs suggest that those are safe, but in higher therapeutic doses not well tolerated due to strong gastrointestinal upset. Ketone esters have the problem of a very foul taste and while high blood ketone concentrations can be reached, most research has been conducted on gavaged animals. A direct administration of ketogenic acids is potentially dangerous, due to the possibility of acidosis following rapid absorption in the gastrointestinal tract.

Based on the above mentioned state of the art, the objective of the present invention is to provide a new treatment option for migraine by exogenously raising blood KB levels in a safe way with improved palatability and reduced gastrointestinal distress, thereby increasing patient compliance. This objective is attained by the claims of the present specification.

Description of the invention

Terms and definitions

In the context of the present specification, "KB" refers to ketone bodies. Ketone bodies are endogenous metabolites, which are produced by the liver from fatty acids released from adipose tissue in times of starvation, fasting, glucose deprivation or caloric restriction. They can be used as an alternative energy substrate to glucose by most tissues of the body, most notably the brain, which cannot metabolise any other energy substrate apart from glucose and KB. Endogenous KB include beta-hydroxybutyrate (βHB; also known as 3 betahydroxybyturate) and acetoacetate (AcAc). There are some natural exogenous substances that are also ketogenic, such as middle chain triglycerides (MCTs). More recently, other exogenous ketogenic substances have become available, such as βHB mineral salts or keto esters.

In the context of the present specification, the term "ketogenic amino acid" refers to an amino acid that can be degraded to Acetyl-CoA, the precursor of ketone bodies. Leucine and lysine are ketogenic amino acids that are exclusively ketogenic. Isoleucine, phenylalanine, tryptophan and tyrosine are ketogenic amino acids that are also glucogenic.

In the context of the present specification, "βHB" refers to beta-hydroxybutyric acid or beta-hydroxybutyrate, CAS No. 300-85-6.

In the context of the present specification, "D-βHB" refers to the D enantiomer of βHB.

In the context of the present specification, "AcAc" refers to acetoacetate, CAS No. 541-50-4.

In the context of the present specification, "LL" refers to L-leucine.

In the context of the present specification, "LY" refers to L-lysine.

In the context of the present specification, the term "ketogenic diet (KD)" refers to a diet with high fat content, low carbohydrate and medium protein content.

In the context of the present specification, the term "mild to medium nutritional ketosis" refers to a concentration of blood ketone bodies of 0.4-4 mmol/l, which is achieved by a suitable nutrition.

In the context of the present specification, the term "triglyceride" refers to an ester derived from glycerol (CAS No. 56-81-5) and three fatty acids.

In the context of the present specification, the term "fatty acid" refers to an aliphatic monocarboxylic acid comprising a chain of 4 to 28 carbon atoms. The chain can be saturated or unsaturated. The term "free fatty acid" refers to a fatty acid that is not bound to another molecule, e.g. glycerol.

In the context of the present specification, the term "middle chain fatty acid (MCFA)" refers to an aliphatic monocarboxylic acid comprising a chain of 6 to 12 carbon atoms. The chain is saturated.

In the context of the present specification, the term "middle chain triglyceride (MCT)" refers to an ester derived from glycerol (CAS No. 56-81-5) and three MCFA.

In the context of the present specification, "triacetin" refers to 1,2,3-triacetoxypropane, CAS No. 102-76-1.

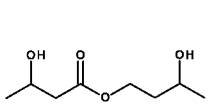
Detailed description of the invention

According to a first aspect, the invention provides a compound for use in a method of treatment or prevention of migraine and/or symptoms thereof. The compound is selected from

a. beta-hydroxybutyric acid (βHB),
b. acetoacetate (AcAc),
c. a metabolic precursor of βHB or AcAc,
d. a compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety.

The metabolic precursor is selected from 1,3-butanediol (CAS No. 107 88 0) and triacetin (CAS No. 102-76-1).

The compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety is described by any one of formulae (Ia) to (Ve)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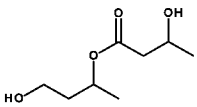
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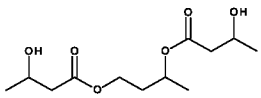
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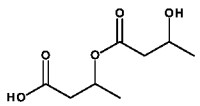
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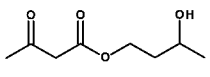
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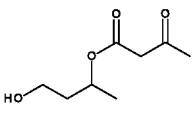
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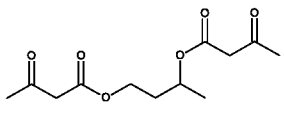
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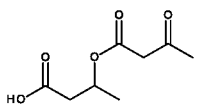
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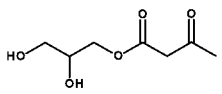
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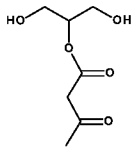
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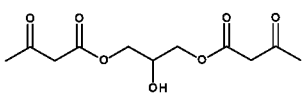
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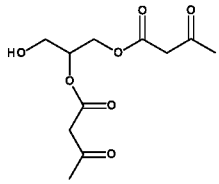

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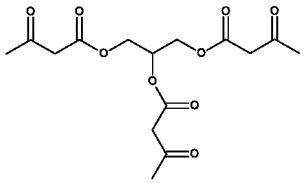

(Ve).

Formula (Ia) specifies 3-hydroxybutyl 3-hydroxybutanoate.

Formula (Ib) specifies (3-hydroxy-1-methyl-propyl) 3-hydroxybutanoate.

Formula (Ic) specifies 3-(3-hydroxybutanoyloxy)butyl 3-hydroxybutanoate.

Formula (II) specifies 3-(3-hydroxybutanoyloxy)butanoic acid.

Formula (IIIa) specifies 3-hydroxybutyl 3-oxobutanoate.

Formula (IIIb) specifies (3-hydroxy-1-methyl-propyl) 3-oxobutanoate.

Formula (IIIc) specifies 3-(3-oxobutanoyloxy)butyl 3-oxobutanoate.

Formula (IV) specifies 3-(3-oxobutanoyloxy)butanoic acid.

Formula (Va) specifies 2,3-dihydroxypropyl 3-oxobutanoate.

Formula (Vb) specifies [2-hydroxy-1-(hydroxymethyl)ethyl] 3-oxobutanoate.

Formula (Vc) specifies [2-hydroxy-3-(3-oxobutanoyloxy)propyl] 3-oxobutanoate.

Formula (Vd) specifies [3-hydroxy-2-(3-oxobutanoyloxy)propyl] 3-oxobutanoate.

Formula (Ve) specifies 2,3-bis(3-oxobutanoyloxy)propyl 3-oxobutanoate.

βHB, AcAc, the metabolic precursor of βHB or AcAc and the compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety can be in the form of a pharmaceutically acceptable salt.

In certain embodiments, the compound is a pharmaceutically acceptable ester of βHB.

In certain embodiments, the compound is a pharmaceutically acceptable ester of AcAc.

In certain embodiments, the metabolic precursor of D-βHB is an esterified molecule synthesised using D-βHB.

In certain embodiments, the compound is an ester of βHB or AcAc with a monohydric, dihydric or trihydric alcohol.

In certain embodiments, the compound is a pharmaceutically acceptable amid of βHB.

In certain embodiments, the compound is a pharmaceutically acceptable amid of AcAc.

In certain embodiments, the compound is selected from βHB, AcAc, or pharmaceutically acceptable salt thereof.

In certain embodiments, the compound is selected from βHB, a metabolic precursor of βHB and a compound comprising a 3-hydroxybutyrate moiety and a pharmaceutically acceptable salt of said compounds.

In certain embodiments, the compound is βHB or a pharmaceutically acceptable salt thereof.

In certain embodiments, the compound is D-βHB or a pharmaceutically acceptable salt thereof.

In certain embodiments, the D-βHB is administered in a form which also supplies AcAc. In certain embodiments, the D-βHB is administered as a metabolic precursor, which when administered to a human or animal body is metabolised, e.g. by liver, to produce D-βHB and AcAc, preferably in a physiological ratio.

A physiologically acceptable salt of the compound according to the invention, in particular in combination with ketogenic amino acids and a mineral mix, offers a way to improve palatability of the compound.

In certain embodiments, the pharmaceutically acceptable salt is selected from a potassium salt, a sodium salt, a calcium salt, a magnesium salt, an arginine salt, a lysine salt, a histidine salt, an ornithine salt, a creatine salt, an agmatine salt, a citrulline salt, a methyl glucamine salt and a carnitine salt, possibly in conjunction with other ketogenic substances. The salt may also be a more complex pharmaceutically acceptable salt.

In certain embodiments, the pharmaceutically acceptable salt is a combination of several of the aforementioned salts. In order to avoid undesirable consequences of some products (e.g. pure sodium salts), it is preferred to use a combination of several mineral salts or a combination of a lysine salt and several mineral salts. By increasing the number of different mineral salts, the total tolerated dose can be increased.

In certain embodiments, the combination of salts comprises a mixture of a lysine salt and a calcium, potassium, magnesium and sodium salt. In certain embodiments, the combination of salts comprises a mixture of a lysine salt and a calcium, potassium, magnesium or sodium salt. In certain embodiments, the combination of salts comprises a lysine salt, a calcium salt, a potassium salt and/or a magnesium salt and/or a sodium salt.

In certain embodiments, the combination of salts is a combination of a lysine salt and a mineral salt. In certain embodiments, the combination of salts is a combination of a lysine, a calcium and a sodium salt.

In certain embodiments, the combination of salts is a combination of a calcium and a sodium salt.

The salts may contain the isomer D-βHB or the racemic DL- βHB.

The compound may be provided alone or in combination with other ketogenic substances.

In certain embodiments, the compound is provided for

(i) decreasing migraine attack frequency;

(ii) decreasing migraine attack severity;

(iii) reducing any of the neurological symptoms associated with migraine, such as phono-, photo-, and/or osmophobia, visual, sensory or motor disturbances, allodynia;

(iv) reducing any of the other features known to accompany, precede or follow a migraine attack, such as fatigue, nausea, cognitive difficulties, tiredness, ravenous hunger or thirst, muscle ache, reduced libido, depression, mania, mood swings;

(v) reversing, retarding or preventing structural or functional nerve cell damage, such as white matter lesions or disturbances in functional connectivity, associated with migraine;

(vi) preventing, retarding or reversing the transition of acute migraine to chronic migraine.

In certain embodiments, the treatment or prevention has the effect of decreasing migraine attack frequency; decreasing migraine attack severity; decreasing the severity of migraine symptoms; preventing disease progression and/or preventing disease chronification.

In certain embodiments, the symptoms of migraine include at least two of the following symptoms: medium to strong predominantly unilateral headache, light, noise and/or smell sensitivity, nausea or sickness, facial pain, sore eyes, balance disturbance, word finding difficulties, other neurological symptoms, such as sensory or motor disturbances, allodynia or any other of the features known to accompany, precede or follow a migraine attack, such as fatigue, nausea, cognitive difficulties, tiredness, ravenous hunger or thirst, reduced libido, depression, mania, mood swings, as well as changes in brain structure and function, such as white matter lesions or disturbances in functional connectivity.

In certain embodiments, the compound is to be administered before symptoms of a migraine attack, in particular those recited in the previous paragraph, occur.

In certain embodiments, the daily dose to be administered is 0.05 g/kg to 1 g/kg body weight (=3.5-70 g / 70 kg). In certain embodiments, the daily dose to be administered is 0.1 g/kg to 0.7 g/kg body weight (=7-49 g / 70 kg). In certain embodiments, the daily dose to be administered is 0.2 g/kg 0.4 g/kg body weight (=14-28 g / 70 kg).

In certain embodiments, the daily dose to be administered is 3.5 g to 70 g. In certain embodiments, the daily dose to be administered is 5 g to 50 g. In certain embodiments, the daily dose to be administered is 10 g to 40 g. In certain embodiments, the daily dose to be administered is 10 g to 40 g.

In certain embodiments, the daily dose to be administered is 10 g. In certain embodiments, the daily dose to be administered is 20 g.

The inventors have examined the effect of various ketogenic substances, such as LL, LY, racemic and D-βHB on blood KB levels (pharmokokinetic), tolerability and migraine attack frequency. MCTs were not used due to known problems with tolerability and palatability.

LL, but not LY was shown to lead to a very small increase (up to 0.35 mmol/l) in blood βHB over approximately 4 hours. The ketogenic amino acids were not well tolerated, it was impossible for the patients to consume 26 g of amino acids per day. The bitter taste further contributed to the problem.

In comparison, βHB was well tolerated and had a strong effect on blood KB levels.

Surprisingly, the D-βHB isomer led to a more than threefold elevation in blood βHB levels (up to 1.94 mmol/l) as compared to the racemic version. Levels remained elevated for over 4 hours. In addition, there was no concomitant drop in blood glucose, as observed with the racemic mix. Participants reported the taste was improved (less foul) and fewer gastrointestinal side-effects were observed, even with 2 months consumption. Efficacy data suggest that surprisingly as little as 10 g of D-βHB daily might match the efficacy of 40 g of the racemic mix, with an average of 68.5% reduction in migraine days with 10 g of D-βHB compared to 72% reduction with 40 g racemic βHB.

Exogenous KB in quantities much lower than produced by the liver during a KD or fasting (20 g instead of around 150 g) were found to have a migraine preventive effect. Racemic βHB salts led to an increase in βHB blood levels approximately double of LL (up to 0.62 mmol/l), but the half-life was very short, with levels dropping back to baseline after 2 hours. In addition, a substantial drop in blood glucose levels was observed. Tolerability and palatability of the racemic βHB was problematic, in particular gastrointestinal upset and nausea. 20 g daily were found to reduce average migraine day frequency by 51%. This reduction ranged from 25-80%. Despite fairly good efficacy only 2 out of 5 patients continued to take the racemic βHB salts. An increased dose of 40 g racemic βHB lead to a further reduction of 72% in migraine days. Nevertheless, this increased dose exacerbated the side-effects.

In certain embodiments, the daily dose is divided into one to six doses. In certain embodiments, the daily dose is divided into two doses. In certain embodiments, the daily dose is divided into three doses.

In certain embodiments, the daily dose is to be administered over a time period of at least one month. In certain embodiments, the daily dose is to be administered over a time period of at least 6 months. In certain embodiments, the daily dose is to be administered over a time

period of at least one year. In certain embodiments, the daily dose is to be administered over a time period of 2 years.

In certain embodiments, the administration of said compound to a subject causes elevation of blood ketone body (KB) levels to 0.3 mM to 6 mM. In certain embodiments, the administration of said compound to a subject causes elevation of blood ketone body (KB) levels to 0.4 mM to 4 mM. In certain embodiments, the administration of said compound to a subject causes elevation of blood ketone body (KB) levels to 1 mM to 4 mM.

According to a second aspect of the invention, a pharmaceutical composition is provided for use in the treatment or prevention of migraine and/or symptoms thereof comprising the compound according to the first aspect of the invention. The pharmaceutical composition can be a medicament or a nutritional aid.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is a formulation or dosage form. In certain embodiments, the dosage form is a powder, tablet, gas or a solution.

In order to mask the potentially bitter taste of lysine, the pharmaceutical composition may comprise stevia and/or other artificial sweeteners (saccharin, acesulfame, suclarose), menthol, citrus, berry or other flavours.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is a combination medicament further comprising an amino acid selected from the group comprising leucine, lysine, isoleucine, tryptophan, tyrosine and phenylalanine. The components of the combination medicament can be administered simultaneously or one after another.

In certain embodiments of this aspect of the invention, the content of the compound according to the first aspect of the invention in the pharmaceutical composition is at least 25% (w/w). In certain embodiments of this aspect of the invention, the content of the compound according to the first aspect of the invention in the pharmaceutical composition is at least 35% (w/w). In certain embodiments of this aspect of the invention, the content of the compound according to the first aspect of the invention in the pharmaceutical composition is 50% to 100% (w/w).

In certain embodiments of this aspect of the invention, the pharmaceutical composition is to be administered to a subject diagnosed with migraine suffering from 1 to 31 migraine days per months.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is to be administered before symptoms of a migraine attack occur.

In certain embodiments of this aspect of the invention, the daily dose to be administered of said compound comprised in the pharmaceutical composition is 0.05 g/kg to 1 g/kg body weight, preferably 0.1 g/kg to 0.7 g/kg body weight, more preferably 0.2 g/kg to 0.4 g/kg body weight (depending on disease severity).

In certain embodiments of this aspect of the invention, the daily dose is divided into one to six doses, particularly into two or three doses.

In certain embodiments of this aspect of the invention, the daily dose is to be administered over a time period of at least one month, preferably at least 6 months, most preferably over 2 years.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is formulated for oral administration. In certain embodiments of this aspect of the invention, the pharmaceutical composition is formulated for parenteral administration. In certain embodiments of this aspect of the invention, the pharmaceutical composition is formulated for any other form of conventional administration.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is formulated as a powder for oral administration. The powder is dissolved in water prior to comsumption.

In certain embodiments of this aspect of the invention, the pharmaceutical composition is a drink.

In certain embodiments of this aspect of the invention, the administration of said pharmaceutical composition to a subject causes elevation of blood ketone body (KB) levels to 0.3 mM to 6 mM, particularly to 0.4 mM to 4 mM, more particularly to 1 mM to 4 mM.

The inventors' results suggest that between 5 g to 70 g of KB, particularly 5 g to 40 g of βHB, more particularly 10 g to 20 g of βHB per patient per day are required to achieve this. The necessary elevation for migraine freedom will depend on disease severity (i.e. number of migraine days per months).

According to another aspect of the invention, a method of treatment or prevention of migraine and/or symptoms thereof is provided, comprising administration of the compound according to the first aspect of the invention or the pharmaceutical composition according to the second aspect of the invention to a subject in need thereof.

In certain embodiments, the subject in need is suffering from 1 to 31 migraine days per month.

In certain embodiments, the subject in need shows manifestation of at least two of the following symptoms of migraine: medium to strong predominantly unilateral headache, light,

noise and/or smell sensitivity, nausea or sickness, facial pain, sore eyes, balance disturbance, word finding difficulties, other neurological symptoms, such as sensory or motor disturbances, allodynia or any other of the features known to accompany, precede or follow a migraine attack, such as fatigue, nausea, cognitive difficulties, tiredness, ravenous hunger or thirst, reduced libido, depression, mania, mood swings, as well as changes in brain structure and function, such as white matter lesions or disturbances in functional connectivity.

Wherever alternatives for single separable features are laid out herein as “embodiments”, it is to be understood that such alternatives may be combined freely to form discrete embodiments of the invention disclosed herein.

The invention is further illustrated by the following examples and figures, from which further embodiments and advantages can be drawn. These examples are meant to illustrate the invention but not to limit its scope.

Brief description of the figures

Fig. 1 shows the pharmacokinetics of 13 g L-leucine (LL) ante cibum in 2 migraine patients (grey) compared to 10 g βHB (black). Depicted are βHB blood levels in mmol/l (y-axis) before (baseline = 0 h) and after (0.5, 1, 2, 3, 4 h) 13 g of LL (grey) and 10 g βHB consumption (black) respectively in 2 migraine patients. Substances were given in powdered form, dissolved in water on an empty stomach. Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 2 shows the pharmacokinetics of 10 g racemic beta-hydroxybutyrate (βHB) ante cibum on 5 migraine patients and its effect on glucose levels. (A) Depicted are βHB blood levels in mmol/l (y-axis) before (Baseline=0 h) and after (0.5, 1, 2, 3 h, 4 h) 10 g of βHB consumption in 5 migraine patients. βHB was given in powdered sodium-calcium-salt form dissolved in water on an empty stomach. Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM). (B) Blood glucose levels in mmol/l (y-axis) before (Baseline value = 0 h) and after the consumption of 10 g βHB ante cibum in 5 migraine patients. The rise in blood glucose from 1 h onwards corresponds to the intake of a mixed food breakfast (food intake indicated by the arrow) one hour after consumption of βHB, which was given to prevent potential hypoglycemia. Blood glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 3 shows the effect of 20 g βHB daily on days with migraine. Average number of migraine days (y-axis) at baseline (black) in a group of 4 medium-high frequency migraineurs (6-22

migraine days/month) and the reduction in migraine days after 4 weeks of intervention with 20 g βHB daily (grey). Similar to the average, the median at baseline is 18 migraine days and at 7 migraine days after the intervention. Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 4 A shows the dose dependent effect of 20 g βHB or 40 g βHB on days with migraine. Average number of migraine days (y-axis) at baseline (black) in 2 chronic migraineurs (20 or 22 migraine days/month) and the reduction in migraine days after 4 weeks of intervention with 20 g βHB (grey) and 40 g βHB (white dotted). Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 4 B shows the dose response curve to 0, 20 and 40 g βHB daily (percent reduction in migraine days from baseline). Percent in reduction in migraine days from baseline (y-axis) after 4 weeks of 0 g βHB daily (0 g), after 4 weeks of 20 g βHB daily (20 g) and after 4 weeks of 40 g βHB daily (40 g) in 2 chronic migraineurs (20 or 22 migraine days/month). Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 5 shows the pharmacokinetics of 10 g D-beta-hydroxybutyrate (βHB) ante cibum on 5 participants and its effect on glucose levels. (A) Depicted are βHB blood levels in mmol/l (y-axis) before (Baseline=0 h) and after (0.5, 1, 2, 3 h, 4 h) 10 g of βHB consumption. D-βHB was given in powdered mixed mineral and lysine-salt form dissolved in water on an empty stomach. Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM). (B) Blood glucose levels in mmol/l (y-axis) before (Baseline value = 0 h) and after the consumption of 10 g βHB ante cibum. Blood glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 6 shows the comparison of the effect of a one-time dose of either 10 g racemic beta-hydroxybutyrate (βHB) or 10 g D-βHB on blood glucose levels (in mmol/l; y-axis) before (Baseline value = 0 h) and after the consumption of either 10 g racemic βHB (black) or D-βHB (grey). The rise in blood glucose from 1 h onwards in the case of the racemic βHB corresponds to the intake of a mixed food breakfast (food intake indicated by the arrow) one hour after consumption of βHB, which was given to prevent potential hypoglycemia. Blood glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig.7 shows the comparison of pharmacokinetics of 10 g racemic DL-beta-hydroxybutyrate (βHB) (black) and 10 g D-βHB (grey) ante cibum on 5 participants at baseline. Depicted are βHB blood levels in mmol/l (y-axis) before (Baseline = 0 h) and after (0.5, 1, 2, 3, 4 h) 10 g of βHB consumption. Racemic βHB was given in powdered sodium-calcium-salt form, D-βHB was given in powdered mixed mineral and lysine-salt form, both dissolved in water on an empty stomach. Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes. Error bars depict the standard error of the means (SEM).

Fig. 8 shows the effect of 10 g racemic βHB versus 10 g D-βHB daily on migraine frequency. Average number of migraine days (y-axis) at baseline (white) in 2 high-frequency migraineurs (17 and 10 migraine days/month) and the reduction in average monthly migraine days after 8 weeks of intervention with 10 g racemic βHB (grey) versus 10 g D-βHB (black). Blood βHB concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes.

Examples

1. Pilot experiments

Patients diagnosed with medium-high frequency or chronic migraine according to International Headache Society Classification version 3 by an experienced neurologist were included. They were excluded if they had any significant other neurological, psychiatric or medical disorder. A minimum average of 6 migraine days / month was required during the last 3 months. Ten migraine patients (age range: 25-61 years, 1 male, attack frequency range: 6-24 migraine days/months) were included in the pilot study and randomly assigned to four conditions: 1) L-leucine (LL), 2) L-lysine (LY), 3) racemic βHB, 4) D-βHB.

1.1. Preliminary pharmacokinetics on 13 g L-leucine and 13 g L-lysine in 4 migraine patients

L-leucine (LL) and L-lysine (LY) are the two completely ketogenic amino acids. Via various steps, unused ketogenic amino acids (i.e. leucine or lysine) are metabolised into KB. While this is commonly known, to the best of our knowledge no data exists on the extent and time frame of such ketogenic amino acids to raise blood βHB levels. For the pharmacokinetics, four migraine patients were instructed to ingest either 13 g LL or 13 g LY on an empty stomach. Blood βHB and glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes at 5 points in time: 1) Baseline (directly before consumption), 2) 0.5 h after consumption, 3) 1 h after consumption, 4) 2 h after consumption, 5) 3 h after consumption and 6) 4 h after consumption. 13 g roughly correspond to the same number of particles (mmol) as 10 g βHB. Highest average concentrations of βHB were found after 2 and 3 h (mean=0.35 mmol/l; SEM=0.05) and

remained levels to remain elevated for over 4 hours (see Figure 1). With LY no blood βHB elevations could be measured at all.

Preliminary results of the L-leucine (3 patients) and L-lysine (2 patients) intervention:

Monthly migraine attack frequency was summarized over the last 3 months and the average was used for baseline comparison. Patients were either instructed to take 26 g LL or 26 g LY in two daily doses (one hour before breakfast and one hour before dinner, respectively) for the duration of 4 weeks. They were instructed to refrain from any other changes in medication or food habits for the duration of that period. The primary outcome measure was changes in days with migraines from baseline. Days with migraine were recorded using a mobile app (myheadache.ch) or a pen and paper diary and averaged across participants.

Adverse events occurred in all patients from the beginning of the trial, such as diarrhoea or nausea when 26 g of LL or LY daily were consumed (13 g twice a day) and the bitter taste of the powder was intolerable. One patient in the LL group already dropped out during the pharmacokinetic part of the trial. The dose had to be drastically reduced and long-term use of a high dose of those ketogenic amino acids is unlikely to be feasible due to palatability and feasibility issues. In addition, the dose reduction made the data incomparable and the very slight or non-measurable increases in blood βHB levels would have made the results hard to interpret with regards to mechanisms of action.

1.2. Preliminary pharmacokinetics on 10 g racemic βHB in 5 migraine patients

For determining pharmacokinetics, the patients were given 10 g racemic beta-hydroxybutyrate (βHB) orally dissolved in water in 3 different conditions: (1) post cibum (after meal) (2) ante cibum (before meal) (3) 1 hour before meal. Blood βHB and glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes at 5 points in time: 1) Baseline (directly before consumption), 2) 0.5 h after consumption, 3) 1 h after consumption, 4) 2 h after consumption, 5) 3 h after consumption and 6) 4 h after consumption. Greatest elevations of βHB blood levels were demonstrated when βHB was consumed fasted (results depicted in figure 2 A). Highest average concentrations of βHB were found after approximately 1 h (mean=0.62 mmol/l; SEM=0.08), which is approximately double the amount of LL. The levels dropped to near baseline after 2 hours.

Glucose levels were measured at the same time as βHB, in order to examine the effect of βHB on glucose levels. Figure 2 B shows that the increase of βHB blood levels after 0.5 and 1 h is accompanied by a concomitant substantial drop in blood glucose levels. In order to prevent hypoglycaemia, a mixed meal was provided and measurements continued.

1.3. Preliminary efficacy of 20 g racemic βHB in 5 patients (open-label intervention on 5 patients)

Monthly migraine attack frequency was summarized over the last 3 months and the average was used for baseline comparison. Patients were either instructed to take 20 g βHB, 26 g LL or 26 g LY in two daily doses (one hour before breakfast and one hour before dinner, respectively) for the duration of 4 weeks. They were instructed to refrain from any other changes in medication or food habits for the duration of that period. The primary outcome measure was changes in days with migraines from baseline. Days with migraine were recorded using a mobile app (myheadache.ch) or a pen and paper diary and averaged across participants.

Intolerable adverse events occurred in one patient, who reported severe nausea and vertigo after consumption and dropped out 8 days after intervention onset. The other 4 patients also experienced gastrointestinal upset, which got a bit better the powder was taken with or after dinner. The palatability remained an issue, with a foul taste being reported. In sum, tolerability and palatability of the racemic βHB was problematic, in particular gastrointestinal upset and nausea, which might be further exacerbated by the accompanying drop in blood glucose levels after consumption.

There was an average reduction of 51% in migraine days compared to baseline (mean baseline = 16.25 days, SEM= 3.71; mean after βHB= 8 days, SEM= 2.92; see Figure 3). This reduction ranged from 25-80%. Despite fairly good efficacy only 2 out of 5 patients continued to take the racemic βHB salts, after the 4 weeks were completed.

Dose response data (20 g versus 40 g βHB*):*

Those two chronic migraineurs (20 or 22 migraine days/month) where instructed to take 20 g racemic βHB daily for 4 weeks and after a 1 week washout period double the dose to 40 g βHB for the following 4 weeks. Migraine days were recorded for the duration of the intervention and patients were instructed to refrain from any other life-style or medication changes. The mean baseline attack frequency was 21 migraine days (SEM=1) and dropped to 11.5 days after 4 weeks of 20 g βHB daily and 6 days after 4 weeks of 40 g βHB daily (see figure 4 A). This preventive effect is roughly proportional to the increase in dose, with 20 g βHB leading to 46% reduction in migraine days from baseline (SEM=18.86) and 40 g βHB to a 72% reduction (SEM=12.95; see figure 4 B). This preliminary observation suggests that the migraine preventive effect of βHB is likely to be dose dependent. Nevertheless, the increased dose also increased side-effects further in both patients and 40 g of foul tasting powder per day also seemed difficult to consume in the longer term.

1.4. Preliminary pharmacokinetics on 10 g D-βHB in 5 participants

Participants were given 10 g D-beta-hydroxybutyrate (βHB) orally dissolved in water in a fasted state. Blood βHB and glucose concentrations were measured using a portable point-of-care blood ketone meter (Precision Xtra®) and matching test stripes at 5 points in time: 1) Baseline (directly before consumption), 2) 0.5 h after consumption, 3) 1 h after consumption, 4) 2 h after consumption, 5) 3 h after consumption and 6) 4 h after consumption (see Figure 5A). As with racemic βHB, highest average concentrations of βHB were found after approximately 1 h (mean=1.94 mmol/l; SEM=0.48). However, to the inventors' surprise the peak levels were more than triple the amount achieved with the racemic βHB (see Figure 7). In addition, the βHB levels remained elevated even after 4 hours (Figure 5A).

Glucose levels were measured at the same time as βHB, in order to examine the effect of D-βHB on glucose levels. To the inventors' surprise, the high average elevation of βHB blood levels to almost 2 mmol/l is not accompanied by a concomitant change in blood glucose levels, which seem to stay completely stable throughout the 4 hours (see Figure 5B). This is in contrast to the drop in blood glucose patients experienced with racemic βHB (see Figure 6); it did not seem to occur when consuming the same amount of D-βHB.

1.5. Preliminary efficacy of 10 g racemic βHB versus 10 g D-βHB on 2 patients (open-label intervention)

Monthly migraine attack frequency was summarized over the last 3 months and the average was used for baseline comparison. Patients were instructed to take 10 g racemic βHB for the duration of 8 weeks, followed by one week wash-out period and then 8 weeks of 10 g D-βHB daily. They were instructed to refrain from any other changes in medication or food habits for the duration of that period. The primary outcome measure was changes in days with migraines from baseline. Days with migraine were recorded using a mobile app (myheadache.ch) or a pen and paper diary and averaged across participants.

During the 8 weeks of 10 g racemic βHB, an average reduction of 18.5% in migraine days compared to baseline (mean baseline = 13.5 days, SEM= 2.86; mean after βHB= 11 days, SEM= 1.15; see Figure 8) was observed. During the 8 weeks of 10 g D-βHB, an average reduction of 68.5% in migraine days compared to baseline (mean baseline = 13.5 days, SEM= 2.86; mean after βHB= 4.25 days, SEM= 0.43; see Figure 8) was observed. This reduction in migraine days is comparable to the effect observed with 40 g racemic βHB (both approximately 70% reduction in days with migraine).

Palatability and side-effect profile were much improved with the D-βHB and the reduced dose. No side-effects were reported at the given dose. And both patients are continuing to take D-βHB on a daily basis.

These very preliminary findings suggest that surprisingly D-βHB not only seems to be able to raise blood βHB levels much higher than other ketogenic substances in the human, but also leads to a pronounced reduction in migraine frequency (up to 70%) with only a fraction of the dose the human body produced during a KD (10 g versus approx. 150 g). In addition, it seems to be much better tolerated and more palatable than other ketogenic substances and does not seem to lead to a potentially unwanted drop in blood glucose levels. This finding suggests that D-βHB might not only act as a metabolite in the migraine patient, but also a signalling molecule, which positively impacts migraine relevant pathophysiological mechanisms.

2. Clinical trial

Clinical trial description: Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A cross-over randomised, placebo-controlled, double-blind study.

2.1. Clinical trial synopsis

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Title of clinical trial	Long title: Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A cross-over randomised, placebo-controlled, double-blind study Short title: Exogenous ketone bodies in migraine prevention (MigraKet)
Clinical trial type and phase	A cross-over double blind randomized placebo-controlled phase 2 safety and efficacy trial
Objective(s)	To test if exogenous beta-hydroxybutyrate treatment, compared to placebo, reduces migraine frequency in episodic migraineurs (5-14 migraine days/month) by 25% at least (using change in days with migraine as assessed by a headache diary as primary clinical endpoint).
Intervention(s)	Experimental intervention: 18 g beta-hydroxybutyrate in mineral salt form per day (taken orally) Control intervention: Matched placebo Duration of intervention per patient: 2x12 weeks Duration of wash-out period per patient: 8 weeks
Key inclusion and exclusion criteria	*Key inclusion criteria:* Diagnosis of episodic migraine (5-14 migraine days/month, 18-65 years old, stable preventive treatment for >3 months *Key exclusion criteria:* Any significant neurological, psychiatric or other medical condition, Botox within 6 months of study onset, anti-inflammatory drug (NSAIDs) or triptan use greater than 10 days per

	month, current participation in other migraine trial
Endpoint(s)	*Primary endpoint:* Change from baseline in days with migraine during last 4 weeks of intervention *Secondary endpoint(s):* Change in migraine Disability Assessment, change in migraine intensity (VAS 0-10), change in days with medication use, change in headache days of any severity *Exploratory endpoint(s):* Change in markers of markers of oxidative stress, gene expression changes, genetic profile, peak KB and blood glucose level change from baseline
Sample size	Number of patients to be assessed for eligibility: 54 Number of patients to be allocated to the trial: 45 Number of patients to be analysed: 32
Statistical analysis	Efficacy: ANCOVA of primary endpoint, intention-to-treat set of patients Description of the primary efficacy analysis and population: ANCOVA, intention-to-treat set Safety: ANCOVA, intention-to-treat set Secondary endpoints: ANCOVA, intention-to-treat set
Trial duration	First patient in to last patient out (months): 27 Recruitment period (months): 21 Duration of the entire trial (months): 36
Participating centres	Single Centre, University Children's Hospital Basel, Switzerland
Key words	Migraine, prevention, double blind randomised placebo-controlled clinical trial, exogenous KB, genetics

2.2. Study medicament

In order raise blood ketone levels exogenously, the inventors propose the use of D-βHB, or a metabolic precursor thereof, alone or in combination with other ketogenic substances, in the manufacture of a medicament or nutritional aid for the treatment of prevention or migraine or symptoms thereof.

For feasibility reasons and mineral load, patients in the phase 2 trial described below are dosed with 18 g βHB in mineral salt form. In the following the study medicament will be referred to as verum.

2.3. Study design

The study is a double-blind, randomised, placebo-controlled, safety, tolerability and efficacy trial with one active intervention (βHB) and one placebo group. 45 medium- to high-frequency migraineurs (5-14 migraine days/months) aged between 18 and 65 years are included. Participants are required to keep a detailed headache diary (www.myheadache.ch), for the entire duration of the study.

The study period will begin with a 4 week run-in period, during which there is no investigational treatment (see figure 1). The purpose of the run-in period will be observation for baseline comparison. The run-in period will be followed by a 12 week intervention period,

when the subjects will receive the study medicament or matched placebo (three times a day). The first intervention period will be followed by an 4 week wash-out period and a second 4 week run-in period during which there will be no further intervention. This will be followed by the second intervention period, when the participants will “cross-over”, i.e. received the alternative treatment of the first intervention (if they received verum the first 12 weeks, they will receive placebo the second 12 weeks or the other way round).

Table 1 Detailed study schedule.

STUDY PERIOD	*Screening*	*Baseline*	*Intervention 1*		*Wash-out*	*Baseline 2*	*Intervention 2*	
VISIT	*V1*	V2	*V3*	*V4*		*V5*	*V6*	*V7*
TIMEPOINT (in weeks)	*-4 (+/-2)*	*0 (+/-2)*	*4 (+/-1)*	*12 (+/-1)*		*20 (+/-2)*	*24 (+/-1)*	*32 (+/-1)*
ENROLLMENT:								
Demographics	X							
Medical History	X							
Pregnancy test	X							
Informed consent	X	X						
Inclusion/Exclusion	X	X						
Randomisation		X						
INTERVENTIONS:								
Observational run-in	◆—	—◆				◆—	—◆	
Treatment/ Placebo 1		◆—	—	—◆				
Wash-out					◆—◆			
Treatment/ Placebo 2							◆—	—◆
Dispensing of study medication		X	X	X		X	X	X
Collection of study medication			X	X			X	X
ASSESSMENTS:								
Adverse Events			X	X		X	X	X
Vital Signs[1]	X	X	X	X		X	X	X
Physical examination	X							
Migraine Diary[2]	X	X	X	X		X	X	X
MIDAS & HIT-6 questionnaire[3]		X		X		X		X
Blood ketone & glucose level[4]		X	X	X		X	X	X
Blood draw for safety analyis[5]		X	X	X		X	X	X
Blood draw for genetic analysis[6]		X		X		X		X
Blood draw for markers of oxidative / nitrosative stress and cytokines [7]		X	X	X		X	X	X

[1] Blood pressure, heart rate, weight, height

[2] Pen and paper headache dairy

[3] Migraine Disability Questionnaire and Headache Impact Test, German versions, standard questionnaires for assessing the extent of migraine related disability

[4] Blood beta-hydroxybutyrate and glucose levels, measured with a portable ketone meter (precision xtra by Abbot)

[5] Routine laboratory (renal and liver function tests, electrolytes, full blood count, C reactive protein, serum cholesterol, triglycerides, serum proteins, albumin, glucose, Hba1c, insulin, cortisol, lactate, TSH, FT4 and FT3)

[6] Blood draw (1 x EDTA, 1 x PAXgene) at each time point for genetic profiling and gene expression analysis using microarrays.

[7] Blood draw at each time point for oxidative and nitrosative stress markers (malondialdehyde (MDA), carbonylated proteins, nitrite, nitrotyrosine) and serum cytokine measurements (including, but not limited to: IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, MCP-1, TNFα& β, TGF-β1).

2.4. Targeted Outcomes

2.4.1. Primary efficacy outcome measure

The primary objective is to show in moderate- to high-frequency episodic migraineurs the superiority of the verum to placebo with regard to the reduction in migraine days per 4 weeks from baseline to the last 4 weeks of intervention.

A detailed pen and paper headache diary (similar to www.myheadache.ch) is used to assess the reduction in monthly migraine frequency (i.e. the primary outcome). Reduction of days with migraine (assessed with headache diaries) is the standard primary efficacy outcome measure in RCTs on migraine prevention. The headache diary is available as IOS and android app and is easy to use. Migraine related features such as attack begin, length of attack (in hours), severity of attack (0-10), medication taken (amount and dose), associated symptoms and potential trigger factors are recorded.

2.4.2. Secondary efficacy outcome measures

Secondary objectives are to assess the therapeutic efficacy of externally induced mild ketosis by the verum regarding the following secondary endpoints:

- Rate of treatment responders, defined as >50% reduction in migraine days.
- Change in number of headache days of any severity from baseline (meeting ICHD- 3 criteria) during the last 4 weeks of intervention.
- Change in number of headache days of any severity from baseline (meeting ICHD- 3 criteria) during the last 4 weeks of follow-up.
- Change in consumption of acute migraine medication from baseline (analgesics or triptans) – measured in days with acute headache medication use – during the last 4 weeks of intervention.
- Change in average migraine intensity from baseline – assessed with a VAS from 0 - 10 for each migraine episode– during the last 4 weeks of the intervention period.
- Change in disability from baseline – assessed with the Migraine Disability Assessment (MIDAS), indicating the number of days with migraine-related disability (0–270) – and the Headache Impact Test 6 (HIT-6) to the last 4 weeks of the intervention period.

The secondary outcomes will be measured using the headache diary (myheadache.ch) and the questionnaires, which will be provided as paper copy during the baseline visit, the visit after the 12 weeks interventions and after the wash-out period respectively.

2.4.3. Exploratory *analyses*

Exploratory objectives are to assess the potential mechanisms of action of externally induced mild ketosis by D-βHB supplementation regarding markers of oxidative stress and genetic analyses:

- Serum concentration changes from baseline of oxidative and nitrosative stress markers (malondialdehyde (MDA), carbonylated proteins, nitrate, nitrite, nitrotyrosine) during the last 4 weeks of intervention, examined with ELISA and mass spectroscopy.
- Genetic profile (SNPs) of all patients involved in the study and correlation of the genetic markers with primary and secondary outcomes.
- Gene expression changes before and after intervention using expression microarrays with a special focus on mitochondrial related genes citrate synthase, cytochrome C oxidase subunit 1, succinate dehydrogenase subunit A).
- Correlation of gene expression changes with the genetic profile of the patients (eQTL analysis) in combination with primary and secondary outcomes as possible covariates.
- A further exploratory objective is to examine the change of peak BK levels and glucose levels from baseline to the last 4 weeks of the intervention period. Blood KB and glucose levels will be measured using a portable point-of-care blood ketone meter (Precision Xtra® and matching test stripes) once a week in the morning, 30 min and 60 min after consumption of the study medicament.

2.4.4.Tolerability and safety outcome measures:

Safety and tolerability will be determined by:

- Comparison of treatment-emergent adverse events (any event regardless of potential causality with the drug) between placebo and active treatment.
- Comparison of treatment-related adverse events (such as gastrointestinal upset) as imputed by the principal investigator between placebo and active treatment.
- Any significant changes on routine laboratory and vital signs (see below) compared to baseline and/ or placebo group.
- Routine laboratory (renal and liver function tests, electrolytes, full blood count, C reactive protein, serum cholesterol, triglycerides, serum proteins, albumin, glucose, Hba1c) will be examined at visit 2, 3 and 4 to determine safety of the treatment. The vital parameters (blood pressure, heart rate, weight, height) will be measured at every visit.

2.5. Selection of trial subjects

2.5.1. Recruitment

Recruitment strategies

Patients are informed about the study during the doctor's consultation at the Department of Neurology, University Hospital Basel by their neurologist (e.g. Dr. Bernhard Decard). More flyers are displayed in local pharmacies, local neurologists, the neurological department of the Bruderholzspital (Kantonsspital Baselland) and the Headache Clinic of Bad Zurzach (by Prof. Sandor). In addition, patients previously contacted for a migraine-sport intervention study at the USB (EKNZ-Number 194/13) are contacted again, if they previously agreed and met inclusion criteria for the current study. About 300 research interested patients previously contacted for this study agreed to be contacted for future studies on migraine prevention. Moreover, there are flyers publicly displayed in the waiting room of the neurology and general medicine department, as well as the University Library. An announcement similar to the flyer is posted on the webpages of the University of Basel "Marktplatz" dedicated to research studies (https://markt.unibas.ch/nc/inserate/kategorie/job-angebot-studien/) as well as the USB Website respectively (https://www.unispital-basel.ch/lehre-forschung/studieninserate/).

Feasibility of recruitment

Trial readiness is high in medium- to high- frequency migraine patients as current therapeutic options are very limited and associated with often intolerable side-effects. Migraine is a prevalent disorder and the inventors already have a contingent of 300 patients willing to take part in research on new forms of migraine inventions. Additionally, the co-applicant has access to a big patient pool through his Headache Clinic and together with the neurology department of the USB approximately 100 patients meet inclusion criteria. The inventors are not anticipating any problems with the recruitment of 50 eligible patients.

2.5.2. Inclusion and exclusion criteria

Inclusion Criteria: The subject

1. Is between the ages of 18 and 65 years.
2. Has been previously diagnosed with migraine (with or without aura) in accordance with the ICHD-3 Beta Classification criteria.
3. Experience between 5 and 14 migraine days per month (over the last 3 months) with at least 2 of the migraines lasting more than 4 hours.
4. Has age of onset of migraine less than 50 years old.
5. Agrees to refrain from initiating or changing the type, dosage or frequency of any prophylactic medications (exclusive of medications taken for acute relief of migraine

symptoms) as well as dietary supplements (such as Q10, riboflavin etc.) against migraine and for indications other than migraine that in the opinion of the clinician may interfere with the study objectives (e.g. antidepressant, anticonvulsants, beta blockers, etc.) for the duration of the study.

6. Has not changed type, dosage or frequency of any prophylactic medications (exclusive of medications taken for acute relief of migraine symptoms) as well as dietary supplements (such as Q10, riboflavin etc.) against migraine and for indications other than migraine that in the opinion of the clinician may interfere with the study objectives (e.g. antidepressant, anticonvulsants, beta blockers, etc.) for at least 3 months prior to study onset.
7. Agrees to use the ketogenic powder or placebo as intended, follow all of the requirements of the study including follow-up visit requirements, record required study data in the subject dairy and other self-assessment questionnaires and is okay with drawing blood samples.
8. Is able to provide written Informed Consent.

Exclusion Criteria: The subject

1. Has a concomitant medical condition that will require oral or injectable steroids during the study.
2. Has a history of any significant neurological, psychiatric or other medical condition that in the opinion of the investigator may confound the study assessments
3. Is currently treated for a thyroid disease or has a history thereof.
4. Has a cardiovascular disease (hypertension in particular) or a history thereof.
5. Has a known history of suspected secondary headache.
6. Currently takes simple analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) or triptans greater than 10 days per month for headaches or other body pain.
7. Currently takes prescription opioids.
8. Has previous diagnosis of medication overuse headache (MoH), which has reverted to episodic migraine within the last 6 months.
9. Meets the ICHD-3 Beta Classification criteria for chronic migraine (> 15 headache days per month).
10. Has failed an adequate trial (two months or greater) of at least 3 classes of a drug therapy for the prophylaxis of migraine.
11. Has had surgery for migraine prevention.
12. Has received Botox injections within the last 6 months.
13. Is pregnant or plans to become pregnant during the study period, or of childbearing years and is unwilling to use an accepted form of birth control.

14. Is participating in any other therapeutic clinical investigation or has participated in a clinical trial in the preceding 30 days.
15. Belongs to a vulnerable population or has any condition such that his or her ability to provide informed consent, comply with the follow-up requirements, or provide self-assessments is compromised (e.g. homeless, developmentally disabled and prisoner).
16. Is thinking to start, change or stop a hormone-based contraception.

2.6. Statistics

The ***primary objective*** is to show in moderate- to high-frequency episodic migraineurs the superiority of the ketogenic supplement to placebo with regard to the reduction in migraine days per 4 weeks from baseline to the last 4 weeks of intervention. The ***primary endpoint***, number of migraine days in the last four weeks of treatment, will be measured twice for each patient, once after the placebo treatment period and once after the verum treatment period. The number of migraine days in the four weeks before start of treatment will be assessed for both treatment periods, thus there will be two baseline values than will be used as covariates. This has the aim of correcting for any potential seasonal variation in baseline migraine frequency or carry-over effects

The primary analysis will be performed using a ***linear, mixed effects regression model***. The primary model will include the primary endpoint - number of migraine days in the last four weeks of treatment - as response variable, the respective baseline value as covariate, treatment (verum vs placebo) and period (first vs. second) as main effects, the two interaction terms “treatment x period” and “treatment x baseline value”, and patient as random effect. A significant interaction term between treatment and period would indicate a carry-over effect. Since it is not known how strong the primary end- point correlates with the baseline value, it is not known whether including the base- lines as covariates in the model is sensible. Therefore, the above described primary model will be compared to models without interaction term “treatment x baseline value” and without both interaction term “treatment x baseline value” and baseline value as covariate by means of Akaike’s Information Criterion (AIC).

The primary analysis will be done on the ITT set. Missing values will be imputed as described in section 11.5.

Subgroup analyses: The following a priori defined subgroups will be investigated: sex (male/female), migraine with aura (yes/no), baseline frequency of migraine days (medium = 5–9 days/4 weeks; high = 10–14 days/4 weeks For each subgroup, the main effect of the subgroup and the interaction term “subgroup x treatment” will be added to the above described statistical model. In case of a trend ($p < 0.10$) for an interaction effect – indicating

a difference in the treatment effect between the subgroups–, separate models will be fit for each subgroup.

Sensitivity analysis: The main analysis, without subgroup analyses, will be repeated on the PP set. Potential deviations from the results of the ITT analysis will be described in detail.

Secondary endpoints will be analysed as described for the primary endpoint with the corresponding baseline measure as covariate, if available. A further exploratory objective is to examine the correlation of BK levels with the number of migraine days per 4 weeks from baseline to the last 4 weeks of the follow-up period. The time courses of both variables will be graphically displayed and inspected. Further, the cross-correlation will be calculated. Statistical considerations for the other exploratory objectives (gene expression changes, changes in markers of oxidative stress and potential genetic basis underlying treatment response) are outlined below.

The statistical analysis will be performed using R (http://www.r-project.org/).

Claims

1. A compound for use in a method of treatment or prevention of migraine and/or symptoms thereof, wherein said compound is selected from

   a. beta-hydroxybutyric acid (βHB) or a pharmaceutically acceptable salt thereof,

   b. acetoacetate (AcAc) or a pharmaceutically acceptable salt thereof,

   c. a metabolic precursor of βHB or AcAc selected from

      – 1,3-butanediol (CAS No. 107 88 0) and

      – triacetin (CAS No. 102-76-1),

      or a pharmaceutically acceptable salt thereof, and

   d. a compound comprising an acetoacetyl- or 3-hydroxybutyrate moiety described by any one of the formulae

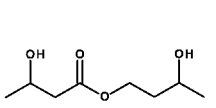
      – Ia, Ib, Ic "D-beta-hydroxybutyrate-D-1,3-butanediol",

      – II "(3R)-hydroxybutyl-(3R)-hydroxybutyrate",

      – IIIa, IIIb, IIIc "acetoacetyl-1,3-butanediol",

      – IV "acetoacetyl-R-3-hydroxybutyrate" and

      – Va, Vb, Vc, Vd, Ve "acetoacetylglycerol",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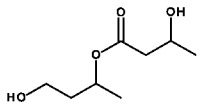
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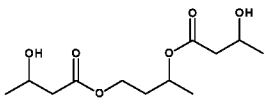
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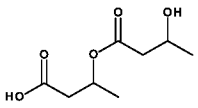
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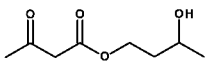
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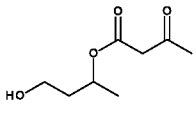
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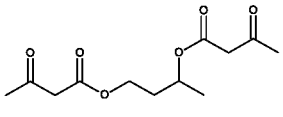
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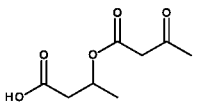
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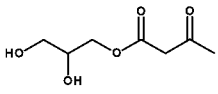
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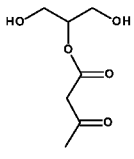
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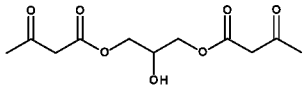
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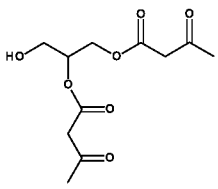
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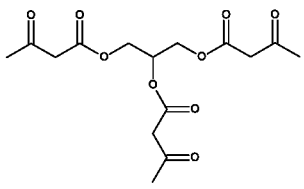

(Ve)

or a pharmaceutically acceptable salt thereof.

2. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to claim 1, wherein said compound is selected from βHB, a metabolic precursor of βHB, a compound comprising a 3-hydroxybutyrate moiety, and a pharmaceutically acceptable salt thereof.

3. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said compound is βHB, or a pharmaceutically acceptable salt thereof.

4. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said compound is D-βHB, or a pharmaceutically acceptable salt thereof.

5. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said pharmaceutically acceptable salt is selected from a potassium salt, a sodium salt, a calcium salt, a magnesium salt, an arginine salt, a lysine salt, a histidine salt, an ornithine salt, a creatine salt, an agmatine salt, a citrulline salt, a methyl glucamine salt and a carnitine salt, or a combination of said salts, in particular a combination of a calcium and a sodium salt.

6. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to claim 5, wherein said combination of salts comprises a combination of a lysine salt and a calcium, potassium, magnesium and/or sodium salt, in particular a combination of a lysine, a calcium, and a sodium salt.

7. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said treatment or prevention includes decreasing migraine attack frequency; decreasing migraine attack severity; decreasing the severity of migraine symptoms; preventing disease progression; preventing disease chronification.

8. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said symptoms of migraine include at least two of the following symptoms: medium to strong predominantly unilateral headache, light, noise and/or smell sensitivity, nausea or sickness, facial pain, sore eyes, balance disturbance, word finding difficulties, other neurological symptoms, such as sensory or motor disturbances, allodynia or any other of the features known to accompany, precede or follow a migraine attack, such as fatigue, nausea, cognitive difficulties, tiredness, ravenous hunger or thirst, reduced libido, depression, mania, mood

swings, as well as changes in brain structure and function, such as white matter lesions or disturbances in functional connectivity.

9. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein said compound is to be administered before occurrence of one or several of the symptoms of a migraine attack recited in claim 8.

10. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein the daily dose to be administered is 0.05 g/kg to 1 g/kg body weight, particularly 0.1 g/kg to 0.7 g/kg body weight, more particularly 0.2 g/kg to 0.4 g/kg body weight.

11. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to claim 10, wherein said daily dose is divided into one to six doses, particularly into two or three doses.

12. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to claim 10 or 11, wherein said daily dose is to be administered over a time period of at least one month, particularly at least 6 months, more particularly at least one year, even more particularly 2 years.

13. The compound for use in a method of treatment or prevention of migraine and/or symptoms thereof according to any one of the above claims, wherein the administration of said pharmaceutical composition to a subject causes elevation of blood ketone body (KB) levels to 0.3 mM to 6 mM, particularly to 0.4 mM to 4 mM, even more particularly to 1 mM to 4 mM.

14. A pharmaceutical composition for use in a method of treatment or prevention of migraine and/or symptoms thereof comprising the compound according to any one of claims 1 to 13.

15. The pharmaceutical composition according to claim 14, wherein said pharmaceutical composition is a combination medicament further comprising an amino acid selected from the group comprising leucine, lysine, isoleucine, tryptophan, tyrosine and phenylalanine.

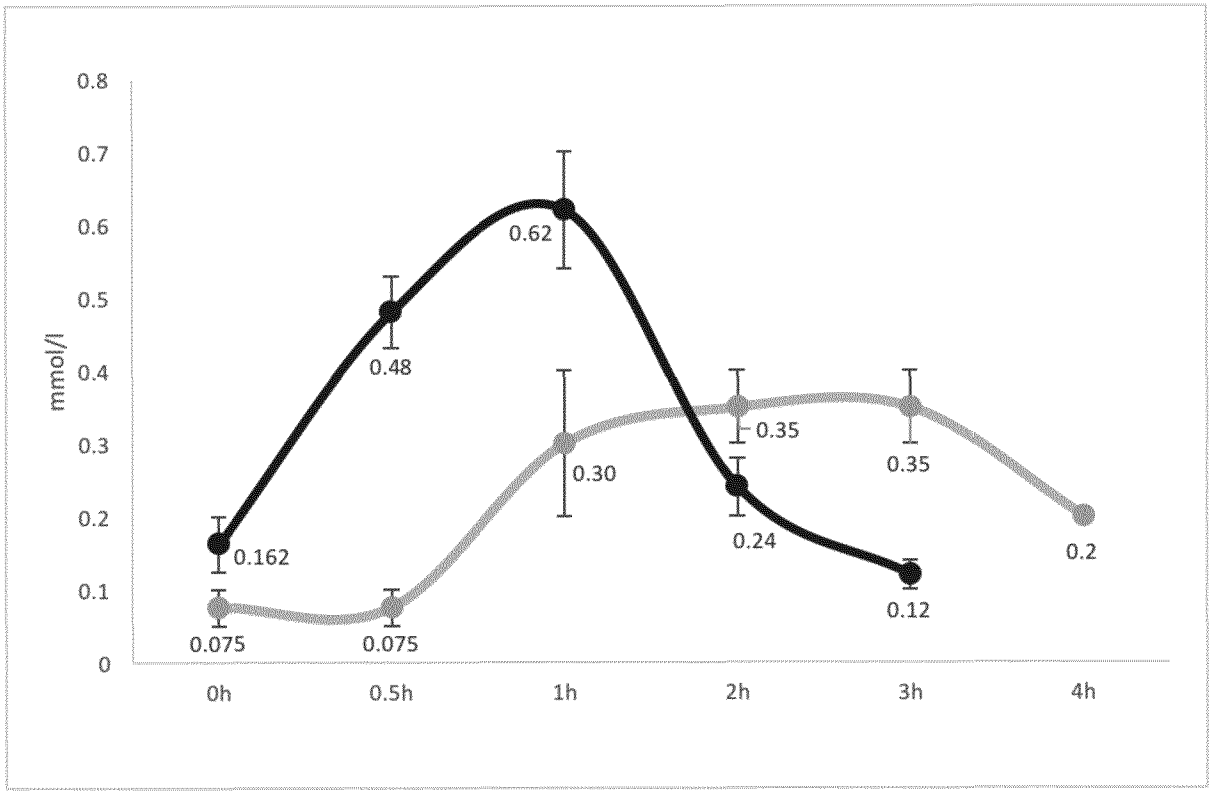
16. The pharmaceutical composition according to any one of claims 14 to 15, wherein the content of the compound according to any one of claims 1 to 13 is at least 25% (w/w), particularly at least 35% (w/w), more particularly 50% to 100% (w/w).

17. The pharmaceutical composition according to any one of claims 14 to 16 formulated for oral administration.

18. The pharmaceutical composition according to claim 17, formulated as a powder for oral administration.

19. The pharmaceutical composition according to any one of claims 14 to 16, wherein said pharmaceutical composition is a drink.

Fig. 1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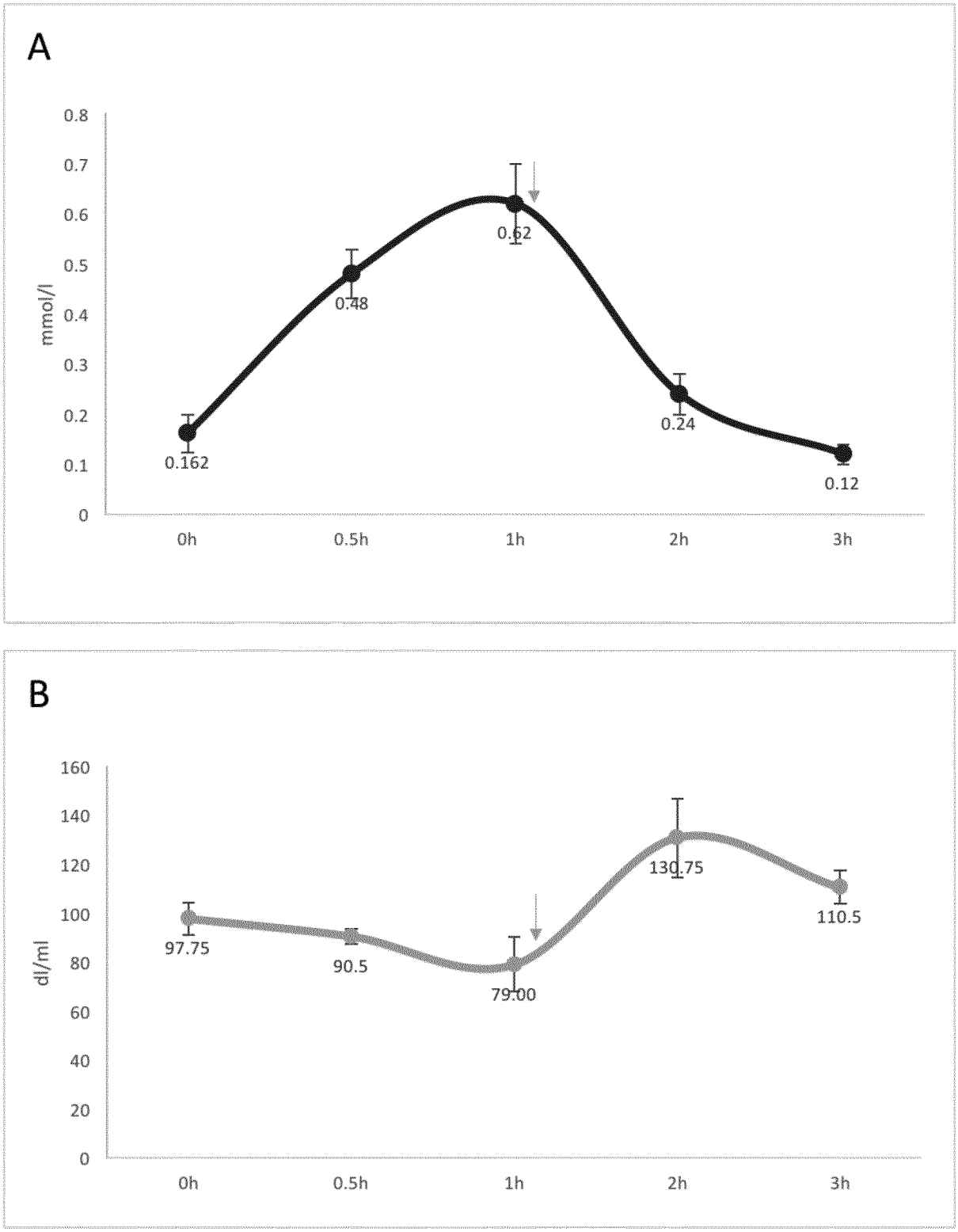
0.6
0.5
0.4
0.3
0.2
0.1
0
mmol/l
0.62
0.48
0.35
0.35
0.30
0.162
0.24
0.2
0.12
0.075
0.075
0h
0.5h
1h
2h
3h
4h

Fig. 2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mmol/l
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.162
0.48
0.62
0.24
0.12
0h
0.5h
1h
2h
3h
B
dl/ml
160
140
120
100
80
60
40
20
0
97.75
90.5
79.00
130.75
110.5
0h
0.5h
1h
2h
3h

Fig. 3

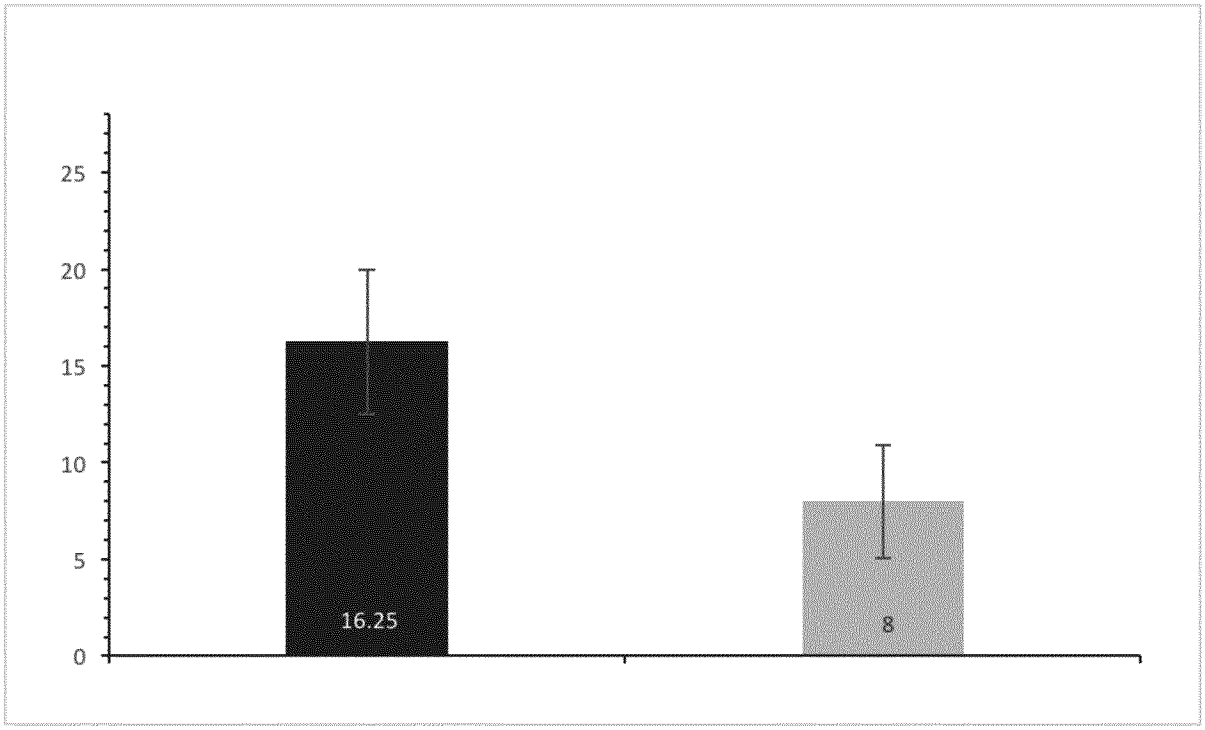
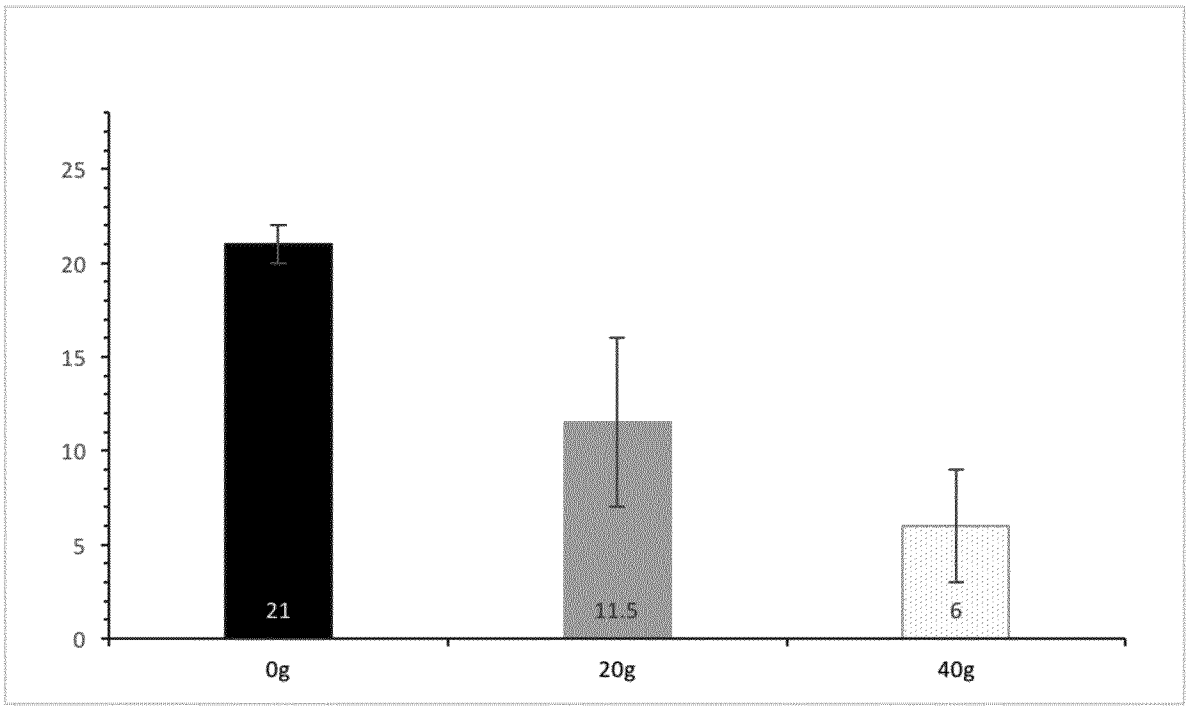


Fig. 4

A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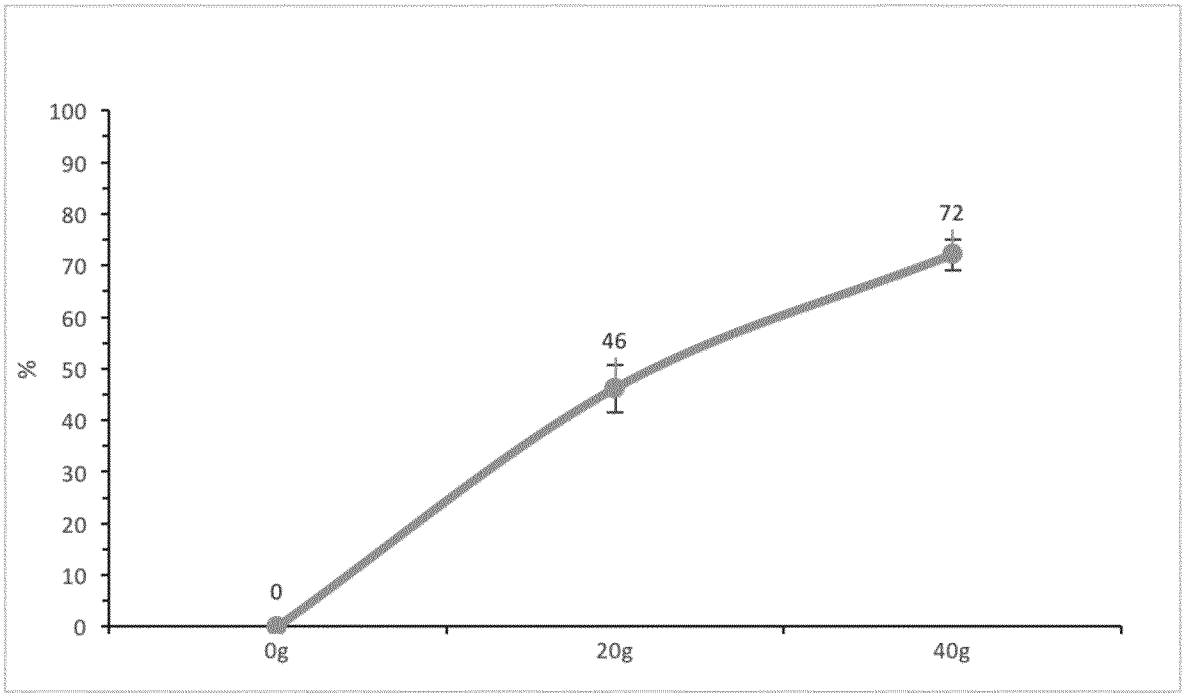
20
15
10
5
0
21
11.5
6
0g
20g
40g

B


100
90
80
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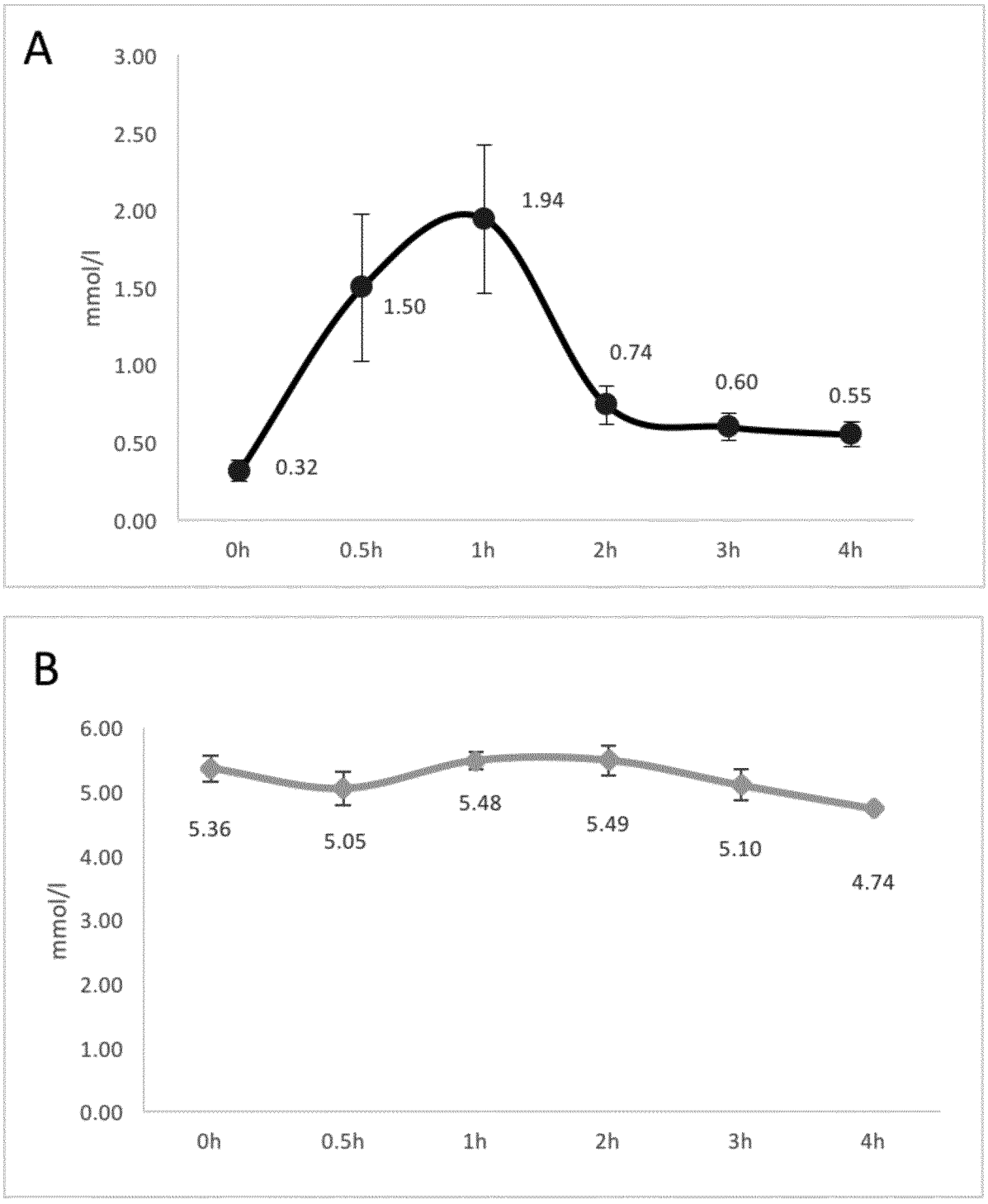
60
50
40
30
20
10
0
%
0
46
72
0g
20g
40g

Fig. 5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1.50
1.00
0.50
0.00
mmol/l
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1.50
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0.60
0.55
0.32
0h
0.5h
1h
2h
3h
4h
B
6.00
5.00
4.00
3.00
2.00
1.00
0.00
mmol/l
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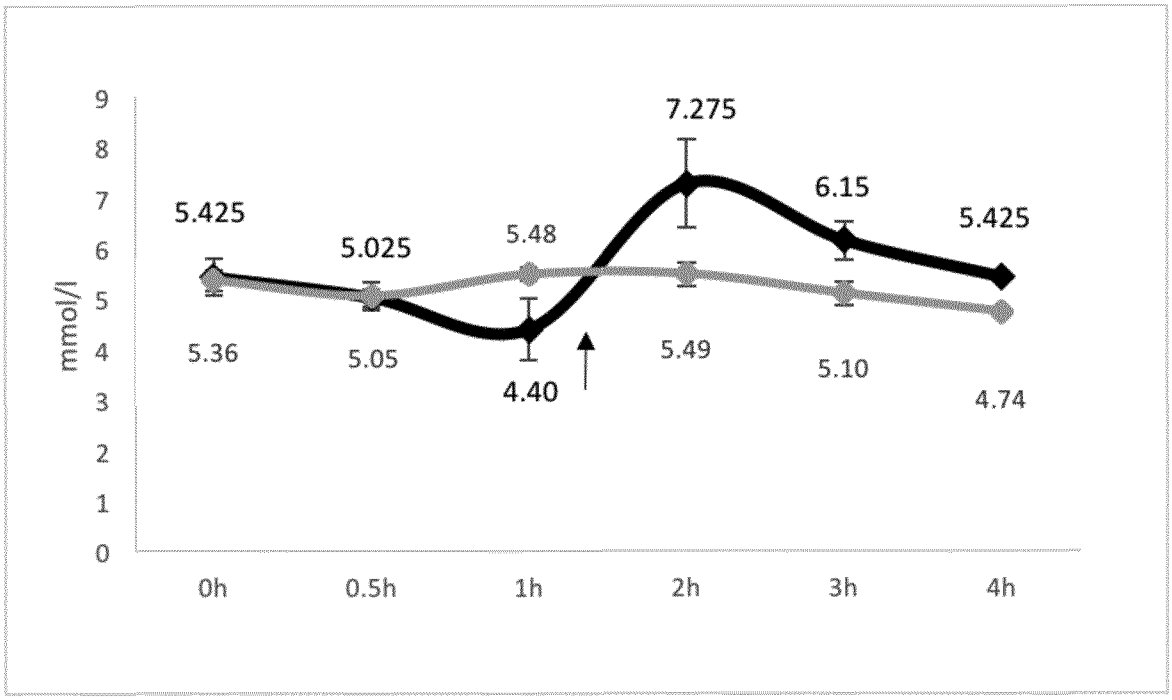
5.49
5.10
4.74
0h
0.5h
1h
2h
3h
4h

Fig. 6


mmol/l
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8
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6
5
4
3
2
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0
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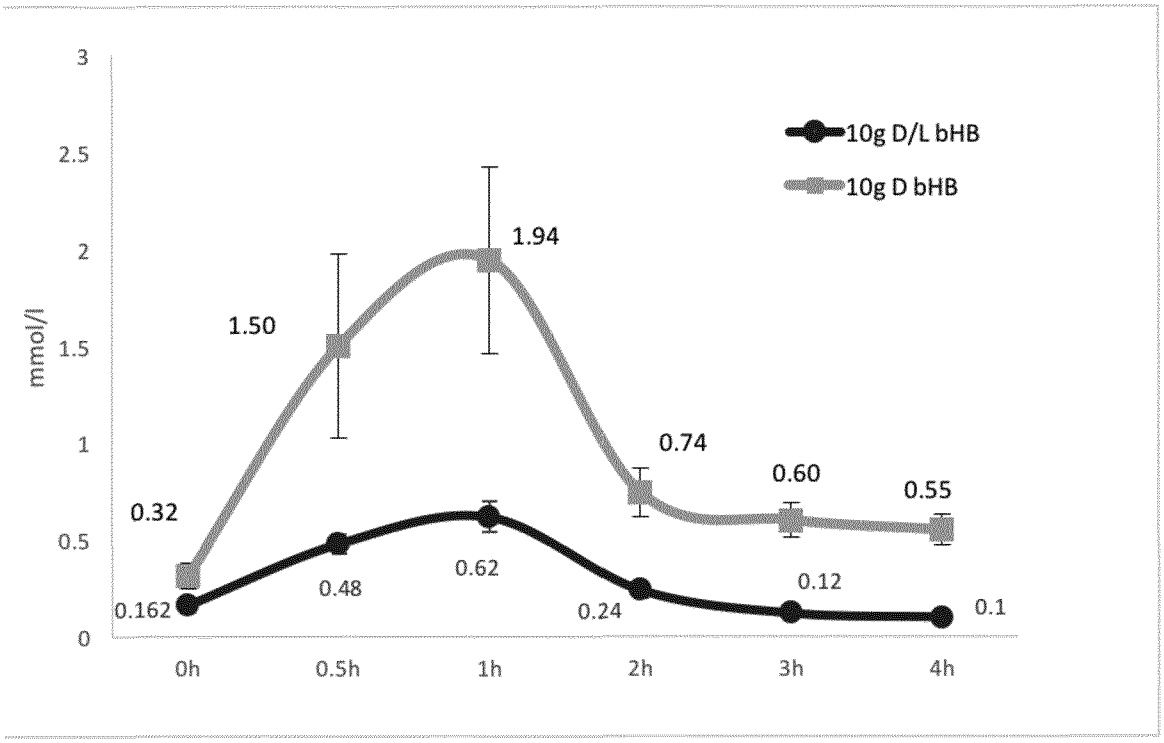
5.025
5.48
7.275
6.15
5.425
5.36
5.05
4.40
5.49
5.10
4.74
0h
0.5h
1h
2h
3h
4h

Fig. 7


mmol/l
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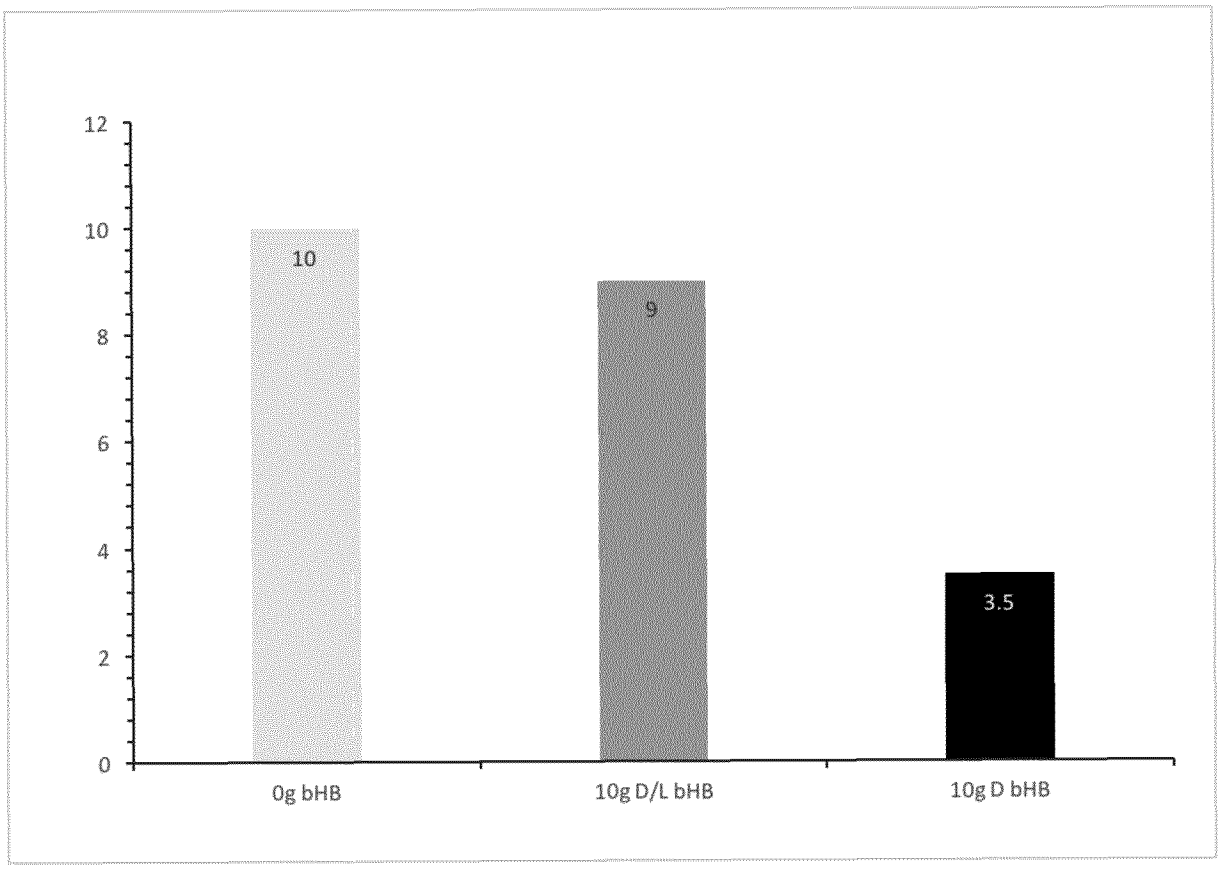
2.5
2
1.5
1
0.5
0
10g D/L bHB
10g D bHB
1.50
1.94
0.74
0.60
0.55
0.32
0.48
0.62
0.24
0.12
0.1
0.162
0h
0.5h
1h
2h
3h
4h

Fig 8



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083880

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/047 A61P25/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	C. DI LORENZO ET AL: "Migraine improvement during short lasting ketogenesis: a proof-of-concept study", EUROPEAN JOURNAL OF NEUROLOGY, vol. 22, no. 1, 25 August 2014 (2014-08-25), pages 170-177, XP55310603, GB ISSN: 1351-5101, DOI: 10.1111/ene.12550 page 175, paragraph 1 ----- -/--	1-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 February 2018	12/03/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cattell, James

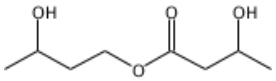
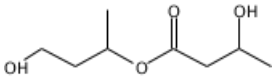
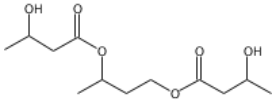
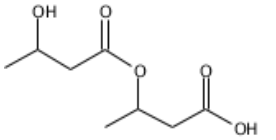
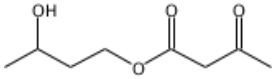
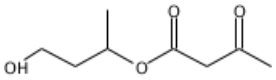
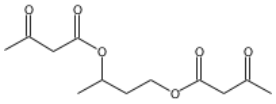
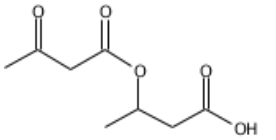
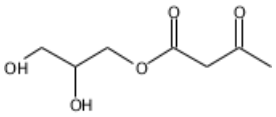
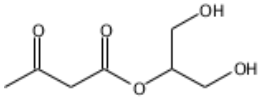
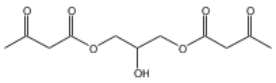
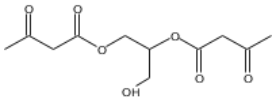
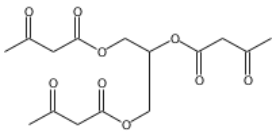
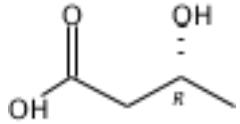
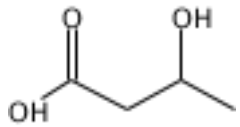
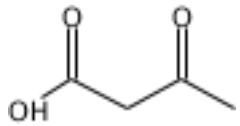
3

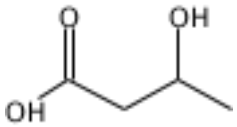
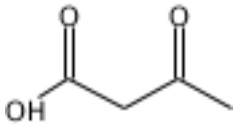
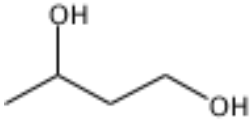
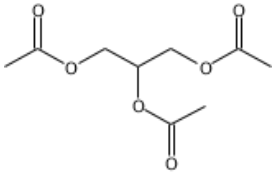
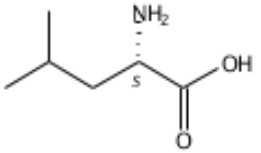
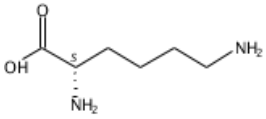
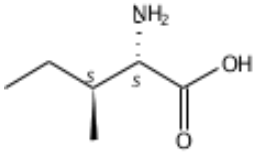
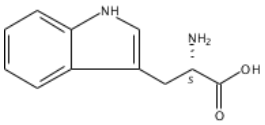
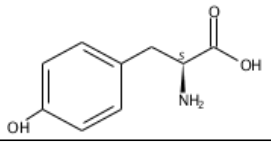
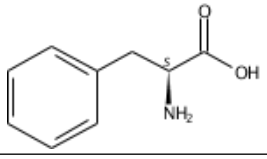
Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOSHIHIRO KASHIWAYA ET AL: "A Ketone Ester Diet Increases Brain Malonyl-CoA and Uncoupling Proteins 4 and 5 while Decreasing Food Intake in the Normal Wistar Rat", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 285, no. 34, 20 August 2010 (2010-08-20), pages 25950-25956, XP55454578, US ISSN: 0021-9258, DOI: 10.1074/jbc.M110.138198 abstract -----	1-19

Key Substances in Patent

Mark	Page #	CAS RN	Name	Structure
7	p.6	125317-37-5	Butanoic acid, 3-hydroxy-, 3-hydroxybutyl ester	
8	p.6	125317-38-6	Butanoic acid, 3-hydroxy-, 3-hydroxy-1-methylpropyl ester	
9	p.6	2230982-34-8		
10	p.6	7565-79-9	Butanoic acid, 3-hydroxy-, 2-carboxy-1-methylethyl ester	
11	p.6	26411-45-0	Butanoic acid, 3-oxo-, 3-hydroxybutyl ester	
12	p.6	124737-11-7	Butanoic acid, 3-oxo-, 3-hydroxy-1-methylpropyl ester	
13	p.6	58213-75-5	Butanoic acid, 3-oxo-, 1,1'-(1-methyl-1,3-propanediyl) ester	
14	p.6	2230982-40-6	Butanoic acid, 3-(1,3-dioxobutoxy)-	
15	p.6	75428-80-7	Butanoic acid, 3-oxo-, 2,3-dihydroxypropyl ester	
16	p.6	104478-74-2	Butanoic acid, 3-oxo-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	
17	p.6	76489-36-6	Butanoic acid, 3-oxo-, 1,1'-(2-hydroxy-1,3-propanediyl) ester	
18	p.6	131134-28-6	Butanoic acid, 3-oxo-, 1-(hydroxymethyl)-1,2-ethanediyl ester	
19	p.6	6079-98-7	Butanoic acid, 3-oxo-, 1,1',1''-(1,2,3-propanetriyl) ester	
26	p.17	625-72-9	Butanoic acid, 3-hydroxy-, (3*R*)-	
1	p.28	300-85-6	Butanoic acid, 3-hydroxy-	
2	p.28	541-50-4	Butanoic acid, 3-oxo-	

3	p.28	300-85-6D	Butanoic acid, 3-hydroxy- metabolic precursors	
4	p.28	541-50-4D	Butanoic acid, 3-oxo- metabolic precursors	
5	p.28	107-88-0	1,3-Butanediol	
6	p.28	102-76-1	1,2,3-Propanetriol, 1,2,3-triacetate	
20	p.30	61-90-5	L-Leucine	
21	p.30	56-87-1	L-Lysine	
22	p.30	73-32-5	L-Isoleucine	
23	p.30	73-22-3	L-Tryptophan	
24	p.30	60-18-4	L-Tyrosine	
25	p.30	63-91-2	L-Phenylalanine	

2.5 Manuscript 5: Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A study protocol for a single-centre, randomised, placebo-controlled, double-blind crossover trial

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Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A study protocol for a single-centre, randomised, placebo-controlled, double-blind crossover trial

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Authors' contributions

EG participated in the design of the study, its organisation, conduct and data acquisition. She drafted the manuscript. NP, ALO, and SS participate in the conduct of the study. DV performs the statistical analysis and calculated the sample size for the study. PS, SC and DF participated in the design of the study, its organisation, data analyses and manuscript creation. All authors read and approved the final manuscript.

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Trials

STUDY PROTOCOL Open Access

Efficacy and safety of exogenous ketone bodies for preventive treatment of migraine: A study protocol for a single-centred, randomised, placebo-controlled, double-blind crossover trial

Elena Gross[1], Niveditha Putananickal[1*], Anna-Lena Orsini[1], Simone Schmidt[1], Deborah R. Vogt[2], Sven Cichon[3], Peter Sandor[4] and Dirk Fischer[1]

Abstract

Background: Currently available prophylactic migraine treatment options are limited and are associated with many, often intolerable, side-effects. Various lines of research suggest that abnormalities in energy metabolism are likely to be part of migraine pathophysiology. Previously, a ketogenic diet (KD) has been reported to lead to a drastic reduction in migraine frequency. An alternative method to a strict KD is inducing a mild nutritional ketosis (0.4–2 mmol/l) with exogenous ketogenic substances. The aim of this randomised, placebo-controlled, double-blind, crossover, single-centre trial is to demonstrate safety and superiority of beta-hydroxybutyrate (βHB) in mineral salt form over placebo in migraine prevention.

Methods/design: Forty-five episodic migraineurs (5–14 migraine days/months), with or without aura, aged between 18 and 65 years, will be recruited at headache clinics in Switzerland, Germany and Austria and via Internet announcements. After a 4-week baseline period, patients will be randomly allocated to one of the two trial arms and receive either the βHB mineral salt or placebo for 12 weeks. This will be followed by a 4-week wash-out period, a subsequent second baseline period and, finally, another 12-week intervention with the alternative treatment. Co-medication with triptans (10 days per months) or analgesics (14 days per months) is permitted. The primary outcome is the mean change from baseline in the number of migraine days (meeting International Classification of Headache Disorders version 3 criteria) during the last 4 weeks of intervention compared to placebo. Secondary endpoints include mean changes in headache days of any severity, acute migraine medication use, migraine intensity and migraine and headache-related disability. Exploratory outcomes are (in addition to routine laboratory analysis) genetic profiling and expression analysis, oxidative and nitrosative stress, as well as serum cytokine analysis, and blood βHB and glucose analysis (pharmacokinetics).

Discussion: A crossover design was chosen as it greatly improves statistical power and participation rates, without increasing costs. To our knowledge this is the first RCT using βHB salts worldwide. If proven effective and safe, βHB might not only offer a new prophylactic treatment option for migraine patients, but might additionally pave the way for clinical trials assessing its use in related diseases.

(Continued on next page)

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(Continued from previous page)
Trial registration: ClinicalTrials.gov, NCT03132233. Registered on 27 April 2017.

Keywords: Migraine, Migraine prevention, Exogenous ketone bodies, Beta-hydroxybutyrate, 3-Hydroxybutyrate, Ketosis, Randomised controlled trial, Placebo-controlled, Crossover, Clinical trial

Background

Migraine is a complex, common and debilitating neurological disorder [1] that affects approximately 17% of women and 8% of men in Europe [2]. With a peak incidence during the most productive years of life, migraine not only causes a huge amount of suffering, but also inflicts a substantial amount of costs on society: approximately €18.5 billion per year in Europe alone [3].

Various lines of research suggest that brain energy metabolism abnormalities are likely to be part of migraine pathophysiology [4–9]. Specifically, there is some evidence for reversible abnormalities in mitochondrial functioning in migraine [7, 8, 10]. For example, treatment with riboflavin and coenzyme Q10 has been shown to have migraine-protective effects [4, 7, 9–12], probably via a positive effect on energy metabolism [7, 10]. Lactic and pyruvic acid, markers of mitochondrial disease, have been found to be increased in migraineurs [13]; ^{31}P-MRS patterns seen in migraine are consistent with what is seen in mitochondrial disorders [5, 14–16]; and COX-negative fibres typical of mitochondrial diseases have also been seen in some patients with migraine [6]. A breakdown of the resting membrane potential due to lack of ATP could explain cortical abnormalities in excitability, which have been reported in migraine [17–21] and would offer a mechanism by which the trigeminal pain pathway, whose afferents densely innervate the meninges and its associated blood vessels, could be activated or sensitised in migraine. The activation and sensitisation of the trigeminal pain pathway is considered the current understanding for the origin of the migraine headache [22–24].

Despite causing a huge amount of suffering and a substantial amount of costs for society [3, 25], current migraine treatment options are limited and their mechanisms of action are also not completely understood [26]. Most of the prophylactic agents licensed to date are not migraine specific and are additionally associated with significant, sometimes intolerable, side-effects. Furthermore, their migraine-preventive properties tend to be moderate at most. Hence, there is a need for developing alternative anti-migraine therapies.

The ketogenic diet (KD) was developed about 100 years ago after the observation that prolonged fasting has anticonvulsive properties [27]. With its high fat, low carbohydrate and medium protein content, the KD simulates the metabolic effects of starvation. With the advent of antiepileptic medication the rather complicated KD fell out of favour. However, within recent years it has received new interest, in particular since ketone bodies (KBs) might be beneficial for a variety of neurological and even psychiatric disorders due to various different mechanisms [28–30], including improved energy metabolism.

Recently, some case studies [31–34] and a first short proof-of-concept study [35] have demonstrated a reduction in migraine attack frequency, severity and use of acute anti-migraine medication during ketosis—with effect sizes ranging from total absence of attacks [31] to a reduction to 1/5th of the run-in period [35]. In addition, preliminary evidence suggests that the migraine-protective effect may outlast the duration of ketosis [31, 32, 35]. This might be a result of longer-lasting gene expression changes [28, 36]. Elevated KB levels in humans have been shown to be well tolerated for extended periods of time (up to several years) [34, 37–48]. However, a strict KD might not provide a feasible long-term solution for all episodic migraine patients, because patient adherence may be limited and it is not easily implemented in an ambulatory setting.

An alternative means to induce a state of mild to medium nutritional ketosis (0.4–2 mmol/l), irrespective of blood glucose levels, is dietary supplementation with ketogenic substances, such as beta-hydroxybutyrate (βHB) salts [45, 49–52]. This approach could be easily implemented with intake of a ketogenic powder dissolved in water (consisting of a calcium–magnesium–βHB salt three times a day). This intervention seems much more feasible than a strict KD in larger patient populations and avoids the complications of a very restricted high-fat diet. These considerations led us to examine the efficacy and safety of KB mineral salts in migraine prevention within the scopes of a double-blind, randomised, placebo-controlled, efficacy and safety trial with a crossover design.

Material and methods

Study design and setting

The study is an investigator-initiated, double-blind, randomised, placebo-controlled, efficacy and safety trial with a crossover design (see Fig. 1) and a treatment period of 36 weeks. It is a single-centre study;

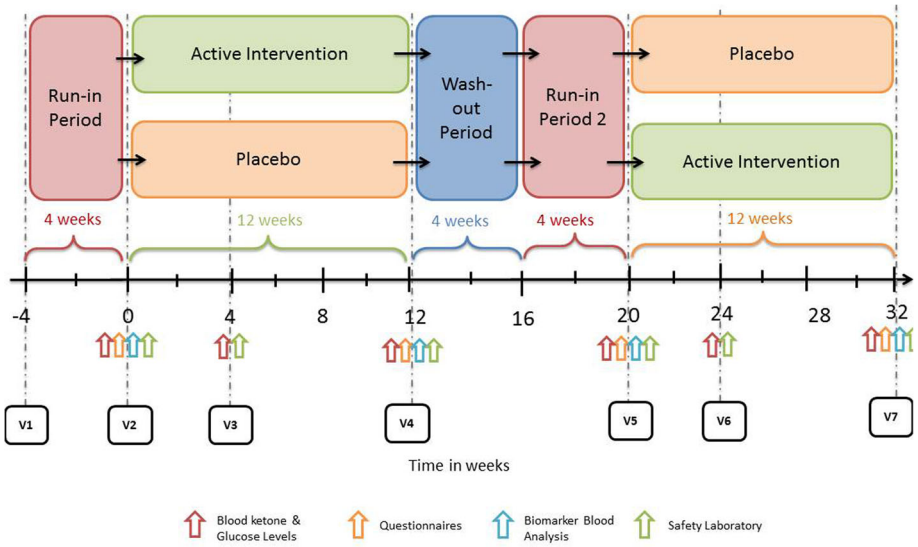

Fig. 1 Flowchart of study design, including timing of measurements and procedures. V = visit

all investigations will take place at the clinical trial unit (CTU) of the University Hospital Basel (USB), Switzerland.

We plan to enrol 45 medium to high-frequency episodic migraineurs (5–14 migraine days/months), with or without aura, aged between 18 and 65 years.

The study period will begin with a 4-week run-in period, during which there is no investigational treatment. The purpose of the run-in period will be observation for baseline comparison. The run-in period will be followed by a 12-week intervention period, when the subjects will receive the investigational medicinal product (IMP) or placebo (orally, three times a day). The intervention period will be followed by a 4-week wash-out and a 4-week second run-in period, during which the subjects will receive no further intervention.

As the study medicament has a half-life of less than 4 h and the outcome measures are based on the last 4 weeks of the 12 weeks intervention only, a 4-week wash-out period was judged to be sufficient. This will be followed by a second 12-week intervention period of the alternative treatment (patients who first received placebo will now receive IMP and vice versa).

Ethics approval has been obtained from the local Ethics Committee (EKNZ 2015-304) and the corresponding competent authority (CA): the National Swiss Drug Agency (2016DR2109). The trial was registered at ClinicalTrials.gov (NCT03132233) prior to starting recruitment. Funding for the study has been received from the Swiss National Science Foundation (SNSF).

Eligibility criteria

Inclusion criteria

Visit 1 (prior to the 4-week run in period) The patient:

1. is between the ages of 18 and 65 years;
2. has been previously diagnosed with migraine (with or without aura) in accordance with the International Classification of Headache Disorders version 3 (ICHD-3) Beta Classification criteria;
3. experiences between 5 and 14 migraine days per month (over the last 4 months) with at least two of the migraines lasting more than 4 h;
4. has an age of onset of migraine younger than 50 years;
5. agrees to refrain from initiating or changing the type, dosage or frequency of any prophylactic medications (exclusive of medications taken for acute relief of migraine symptoms) as well as dietary supplements (such as Q10, riboflavin, etc.) against migraine and for indications other than migraine that in the opinion of the clinician may interfere with the study objectives (e.g. antidepressant, anticonvulsants, beta blockers, etc.) for the duration of the study;
6. has not changed type, dosage or frequency of any prophylactic medications (exclusive of medications taken for acute relief of migraine symptoms) as well as dietary supplements (such as Q10, riboflavin, etc.) against migraine and for indications other than migraine that in the opinion of the clinician may interfere with the study objectives (e.g. antidepressant,

anticonvulsants, beta blockers, etc.) for at least 3 months prior to study onset;
7. refrains from making any drastic changes to their diet for the duration of the study, including periods of fasting;
8. agrees to use the study medication as intended, follow all of the requirements of the study including follow-up visit requirements, record required study data in the subject dairy and other self-assessment questionnaires, and is okay with drawing blood samples; and
9. is able to provide written informed consent.

Visit 2 (baseline visit, just prior to 12-week intervention) Before starting the intervention, the study patient must meet all of the following inclusion criteria.

The patient:

1. continues to meet all baseline (Visit 1) eligibility criteria;
2. has experienced between 5 and 14 migraine days; and
3. has demonstrated compliance with the headache diary during the run-in period.

Exclusion criteria

Visit 1 (prior to the 4-week run-in period) Subjects meeting any of the following criteria cannot be included in this research study.

The patient:

1. has a concomitant medical condition that will require oral or injectable steroids during the study;
2. has a history of any significant neurological, psychiatric or other medical condition that in the opinion of the investigator may confound the study assessments (no liver or kidney diseases in particular);
3. has a cardiovascular disease (hypertension in particular) or a history thereof;
4. has a known history of suspected secondary headache;
5. currently takes simple analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) for more than 14 days per 4 weeks or triptans for more than 10 days per 4 weeks for headaches or other body pain;
6. currently takes prescription opioids;
7. has previous diagnosis of medication overuse headache (MoH), which has reverted to episodic migraine within the last 6 months;
8. meets the ICHD-3 Beta Classification criteria for chronic migraine (> 15 headache days per month);
9. has failed an adequate trial (2 months or longer) of at least three classes of a drug therapy for the prophylaxis of migraine;
10. has had surgery for migraine prevention;
11. has received Botox injections within the last 6 months;
12. is pregnant or thinking of becoming pregnant during the study period, or is of childbearing years and unwilling to use an accepted form of birth control;
13. is participating in any other therapeutic clinical investigation or has participated in a clinical trial in the preceding 30 days;
14. belongs to a vulnerable population or has any condition such that his or her ability to provide informed consent, comply with the follow-up requirements or provide self-assessments is compromised (e.g. homeless, developmentally disabled or prisoner); and
15. is thinking to start, change or stop a hormone-based contraception.

Visit 2 (baseline visit, just prior to 12-week intervention) Before starting the intervention, the study patient must meet none of the following exclusion criteria.

The patient:

1. has initiated or changed the type, dose or frequency of any prophylactic medication for indications other than migraine that in the opinion of the clinician may interfere with the study objectives during the 4-week run-in period

Interventions

Experimental intervention

The investigational medicinal product (IMP) used in this clinical trial is D-L-beta-hydroxybutyrate (βHB) in powdered calcium (Ca^{2+})–magnesium (Mg^{2+})–salt form (Ca-Mg-βHB). D-L-Beta-hydroxybutyrate calcium salt (Ca-βHB) dissolves in water (i.e. in the body) into Ca^{2+} and D-L-beta-hydroxybutyrate (βHB), the compound of interest. D-L-Beta-hydroxybutyrate magnesium salt trihydrate (Mg-βHB) dissolves in water (i.e. in the body) into Mg^{2+} and βHB. Also known as beta-hydroxybutyric acid, 3-hydroxybutyric acid or 3-hydroxybutyrate, βHB is an endogenous metabolite with the formula $CH_3CH(OH)CH_2CO_2H$. It is a beta-hydroxy acid and a keto acid. The IMP was purchased from Ergomax (https://www.ergomaxsupplements.com) in bulk powder of GMP quality and packaged at Hänseler AG (Herisau, Switzerland). It does not contain anything else other than the βHB mineral salts. The flavour is masked using a sucralose-based sugar-free syrup. The daily dose of 9 g Ca-βHB contains 7.54 g of βHB and 1.47 g Ca^{2+}, and will be divided into three servings

supplied in individual sachets containing 2.51 g βHB and 0.49 g Ca^{2+}, respectively. The daily dose of 9 g Mg-βHB contains 6.6 g βHB and 0.77 g Mg^{2+}, and will also be divided into three servings supplied in individual sachets containing 2.2 g βHB and 0.26 g Mg^{2+}. Both IMPs are provided as a water-soluble powder. During the 12-week intervention period participants will consume the IMPs in three oral doses, to be taken with or after breakfast, lunch and dinner, respectively. This adds up to less than 100 kcal per day. Each serving will raise KB levels for approximately 3 h. To minimise possible gastrointestinal symptoms such as bloating or diarrhoea, patients are instructed to increase the dosage over time, starting with half the dose during the first week before reaching the maximum dosage by day 7.

Elevated ketone body (KB) levels have been shown to be well tolerated for extended periods of time (up to several years) [31–35, 37–49]. During fasting, the healthy adult is capable of producing up to 185 g of KBs [53]. Previously, orally administered sodium βHB salts with higher doses ranging between 0.5 and 1 g per kg have been shown to be tolerated in both the short term [39, 50, 54, 55] and the long term [45, 49, 52, 56, 57] with no significant side-effects. The rather conservative dose of 18 g βHB mineral salt per day (as compared to endogenous production during starvation) was determined largely by the mineral load of Mg^{2+} and Ca^{2+}, which we wanted to keep within acceptable ranges. Not going over the suggested maximum supplemental guidelines meant 9 g of Mg-βHB and 9 g of Ca-βHB, respectively. A similar dose of 5 g βHB/day was shown to lead to a modest elevation in blood KB (up to 0.4 mmol/l) [52], supporting the safety of our chosen dose. A Ca-βHB and Mg-βHB salt was chosen to avoid the potentially negative long-term consequences of high sodium intake.

To the best of our knowledge, no human controlled trials using βHB mineral salts have been done, either for migraine or for any other indication, and there seems to not yet be other human published data on specifically Ca-βHB and Mg-βHB. However, recently, βHB supplements, mostly in mineral salt form similar to our IMP, are being produced and sold in the USA, marketed as a sport/life-style supplement. A couple of million servings with a similar dosing to our IMP have been consumed without any incidents reported.

Control intervention

The placebo powder consists of mannitol, a sugar alcohol, which has the same texture, colour and packaging. Taste and smell are masked in the applied form (both are diluted in sugar-free syrup) and therefore similar. It is used by the USB Pharmacy as the standard placebo substance. In higher doses it can also lead to gastrointestinal symptoms [58], which means it has similar potential side-effects to the IMP.

Packaging, labelling and supply

The IMP and placebo are provided in sachets containing either one dose of Ca-βHB (3 g) or one dose of Mg-βHB (3 g), respectively, in powder form (see earlier). The whole supply for the study (ca. 3 kg per patient, > approximately 70 kg IMP and 70 kg placebo in total) will be delivered to and stored at the pharmacy of the University Hospital Basel. Patients will be provided with sufficient quantity to last from each visit to the next. The IMP will be labelled in accordance with regulatory requirements.

Storage conditions

The IMP is stored at room temperature. After delivery it will be stored at the USB Pharmacy until the end of the study.

Concomitant interventions (treatments)

The use of analgesics and triptans is allowed for less than either 14 days (analgesics) or 10 days (triptans), respectively, per month. They are not predicated to have an effect on the study outcomes. Steroids (oral or injectable) as well as prescription opioids are not permitted for the duration of the study period, including run-in and follow-up. Migraine-related surgery and Botox injections within the last 6 months are also not permitted. Prophylactic medications (exclusive of medications taken for acute relief of migraine symptoms) as well as dietary supplements (such as CoenzymeQ10, riboflavin, etc.) against migraine and for indications other than migraine that in the opinion of the clinician may interfere with the study objectives (e.g. antidepressant, anticonvulsants, beta blockers, etc.) are permitted as long as the type, dosage or frequency is not changed for the duration of the study and has not been changed at least 3 months prior to study onset. Hormone-based contraception is permitted as long as the patient does not intend to start, stop or change it for the duration of the study and at least 3 months prior to the intervention. Other hormonal treatment is not permitted.

Outcome measures

Primary outcome measure

Mean change from baseline in number of migraine days (meeting ICHD-3 criteria) during the last 4 weeks of intervention compared to placebo In order to assess the therapeutic efficacy of externally induced mild ketosis over placebo in migraine prevention, a detailed headache diary in pen and paper form is used to record the change in monthly migraine frequency. The headache diary includes: month, days 1–31, distinction migraine/headache, pain intensity (Likert scale 0–10), medication, dosage, treatment effectiveness of acute

medication used (Likert scale 0–10), migraine-associated symptoms, days with menstruation and potential trigger factors (if known).

A day with head pain will only be classified as a migraine day if it meets ICHD-3 classification criteria. According to the International Headache Association and the European Medical Association Guidelines, the recommended measure to assess migraine frequency reduction is the change in migraine days per 4 weeks compared to baseline. This approach has one major advantage over the other frequently used method of recording the number migraine attacks: attack duration is also taken into consideration.

Secondary outcome measures

Mean change from baseline in number of headache days of any severity (meeting ICHD-3 criteria) during the last 4 weeks of intervention compared to placebo The same headache diary in pen and paper form is used to record the change in 4-week headache frequency. A day with headache will only be classified as a headache day if it does not meet ICHD-3 migraine classification criteria. According to the International Headache Association and the European Medical Association Guidelines, the change in migraine days versus headache days per 4 weeks compared to baseline should be recorded separately in migraine patients who experience both headache types.

Mean change from baseline in consumption of acute migraine medication (analgesics or triptans) measured in days with acute headache medication use during the last 4 weeks of the intervention The same headache diary in pen and paper form is used to record the change in days with acute headache medication use (analgesics or triptans). With this approach the number of tablets is not of primary interest, but rather the number of days on which one or more analgesics or triptans were consumed. A clinically meaningful migraine preventative is predicted to lower the days during which migraine acute medication is necessary.

Mean change from baseline in migraine intensity (measured with a numerical rating scale from 1 to 10) during the last of 4 weeks of the intervention period The same headache diary in pen and paper form is used to record a potential change in migraine intensity, as measured with a numerical rating scale from 1 to 10. Each migraine or headache day, respectively, is given an intensity score, with 0 being not painful at all and 10 being an operation without anaesthesia.

Change in disability from baseline during any treatment period, as assessed with the Migraine Disability Assessment and the Headache Impact Test (comparison baseline and post-intervention score) In order to assess a change in migraine and headache-related disability, two commonly used validated and reliable questionnaires are used: the Migraine Disability Assessment (MIDAS) and the Headache Impact Test (HIT-6) [59–63]. The German translations were also shown to have adequate reliability and validity [60, 63]. Both questionnaires will be provided as pen and paper versions and will be filled out at the baseline visits (V2 and V5) and the end of innervation visits (V4 and V7), respectively.

Exploratory outcome measures

The demographic characteristics and neurological examination will be assessed at one time point (Visit 1). To determine the potential mechanisms of action of successful migraine treatment, we are going to examine single nucleotide polymorphism (SNP) markers in order to assess the genetic background of migraine patients involved in this study. In addition to this, we also plan to conduct gene expression analysis. SNP and gene expression analysis will be conducted using microarrays. In our analysis strategy we especially focus on, but not limit to, genes coding for mitochondrial-related enzymes (citrate synthase, cytochrome C oxidase subunit 1, succinate dehydrogenase subunit A).

We will also examine the serum concentration of oxidative and nitrosative stress markers (malondialdehyde (MDA), carbonylated proteins, nitrate, nitrite, nitrotyrosine) using enzyme-linked immunosorbent assay (ELISA) and mass spectroscopy. In addition to Hba1c, insulin, cortisol, lactate and markers of functioning, cytokines will be analysed using the MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel—Premixed 41 Plex—Immunology Multiplex Assay.

Optionally, patients will also receive an Abbott FreeStyle Libre Blood Glucose Monitoring System for 2 weeks at visits V2, V3, V5 and V6, respectively, which will allow permanent tissue glucose monitoring without finger pricking. This allows us to examine a potential association between blood glucose levels (hyperglycaemia or hypoglycaemia) and migraine, and the potential effect of the study medication on glucose levels.

Safety outcomes measures

Safety and tolerability will be determined by:

1. comparison of treatment-emergent adverse events (any event regardless of potential causality with the drug) and treatment-related adverse events (such as gastrointestinal upset) as imputed by the principal

investigator between active treatment and placebo; and
2. examination for potential effects of the intervention on routine laboratory parameters (renal and liver function tests, electrolytes, full blood count, lipids, glucose, CRP, Hba1c, insulin, cortisol, lactate, TSH, FT4 and FT3) in the treatment group compared to the control group.

Study procedure

At screening (Visit 1 (V1), week − 4), patients are informed about preclinical data, alternative treatments, potential risks and benefits of the study (see Fig. 2). Further, written informed consent, including consent for the collection of blood for genetic analysis, from the patients is obtained by the trial physician. After signing the informed consent form, the inclusion and exclusion criteria are verified. If the criteria are fulfilled, the patient will be enrolled in the study under reserve. During V1 the following additional procedures are performed: a detailed first clinical interview/examination, vital signs, migraine diary explanation, where necessary a pregnancy test and a neurological examination. After screening, visits will be scheduled for baseline (V2, week 0). V1 will last approximately 30 min.

At the start of the intervention (baseline/V2, week 0) the following procedures are performed: check inclusion/exclusion criteria and, if met, confirmation of enrolment, migraine diary check, diet check, consumption of first dose of IMP/placebo, adverse events, vital signs, physical examination if necessary, blood draw (for safety, biomarkers and genetic analysis), standardised migraine questionnaires, KB and glucose concentration measurements using a portable point-of-care blood ketone meter (precision xtra from Abbot) and/or the Abbott FreeStyle Libre Blood Glucose Monitoring System. Patients will be randomly assigned to the treatment or control group and receive the according study medication, which will be consumed three times daily for the following 12 weeks. V2 takes approximately 60 min.

After 4 weeks of intervention, there will be another visit (V3, week 4), during which KB and glucose levels will be measured, adverse events will be recorded, vital signs, diet and migraine diary will be checked, a dose of IMP/placebo will be consumed, blood for safety will be drawn, physical examination will be performed if necessary, participants will be provided with the rest of the study medication for the first intervention period and sachets of used study medication will be collected for compliance control. V3 takes about 30 min.

During the visit after the first intervention period (V4, week 12), the following procedures are performed: migraine questionnaires, migraine diary and diet check, consumption of IMP/placebo, KB and glucose measurements, vital signs, blood draw for biomarker and safety analysis, physical examination if necessary and sachets of used study medication will be collected for compliance control. V4 takes about 60 min.

After 8 weeks without intervention, V5 (week 20) takes place, which is identical to the baseline visit (V2). V5 includes the following procedures: standardised migraine questionnaires, migraine diary and diet check, consumption of IMP/placebo, blood draw for biomarker and safety analysis, physical examination if necessary, KB and glucose concentration and vital signs. At this visit the patients will receive the alternative treatment to the first intervention.

After 4 weeks of the second intervention, V6 (week 24, analogous to V3) takes place. The following procedures are performed: migraine diary and diet check, consumption of IMP/placebo, KB and glucose measurements, adverse effects, vital signs, blood draw for safety and physical examination, if necessary. Participants will be provided with the rest of the study medicament and sachets of used study medication will be collected for compliance control. V5 takes about 30 min.

After completion of the second intervention, the last visit (V7, week 32, analogous to V4) takes place. The following procedures are performed: migraine questionnaires, migraine diary and diet check, consumption of IMP/placebo, KB and glucose measurements, vital signs and blood draw for biomarker and safety analysis, physical examination if necessary and collection of sachets of used study medication for compliance control.

All investigations will take place at the clinical trial unit of the University Hospital Basel, Switzerland. Participants are required to keep a detailed headache diary for the entire duration of the study.

Sample size estimation

Determination of sample size

Sample size is estimated to be able to show the superiority of IMP over placebo. A crossover design with 1:1 IMP/placebo:placebo/IMP randomisation is planned.

Fixed sample size estimation

Assumptions Sample size estimation is based on the following assumptions:

- We expect the baseline number of migraine days per 4 weeks to be 10 days in our patient population.
- Placebo effect: based on recent findings [64], we assume a rather strong placebo effect of 32% reduction in the primary endpoint. This corresponds to an absolute reduction of 3 migraine days per 4 weeks.

STUDY PERIOD	Screening	Baseline	Intervention 1		Wash-out	Baseline 2	Intervention 2	
VISIT	V1	V2	V3	V4		V5	V6	V7
TIMEPOINT (in weeks)	-4 (+/-2)	0 (+/-2)	4 (+/-1)	12 (+/-1)		20 (+/-2)	24 (+/-1)	32 (+/-1)
ENROLLMENT:								
Demographics	X							
Medical History	X							
Pregnancy test	X							
Informed consent	X	X						
Inclusion/Exclusion	X	X						
Randomisation		X						
INTERVENTIONS:								
Observational run-in	◆—	—◆				◆—		
Treatment/ Placebo 1		◆—	—	—◆				
Wash-out					◆—	—◆		
Treatment/ Placebo 2							◆—	—
Dispensing of study medication		X	X	X		X	X	X
Collection of study medication			X	X			X	X
ASSESSMENTS:								
Adverse Events			X	X		X	X	X
Vital Signs[1]	X	X	X	X		X	X	X
Physical examination	X							
Migraine Diary[2]	X	X	X	X		X	X	X
MIDAS & HIT-6 questionnaire[3]		X		X		X		X
Blood ketone & glucose level[4]		X	X	X		X	X	X
Blood draw for safety analyis[5]		X	X	X		X	X	X
Blood draw for genetic analysis[6]		X		X		X		X
Blood draw for markers of oxidative / nitrosative stress and cytokines[7]		X	X	X		X	X	X

Fig. 2 Detailed study schedule. [1]Blood pressure, heart rate, weight and height. [2]Pen and paper headache dairy. [3]Migraine Disability Questionnaire (Migraine Disability Assessment (MIDAS)) and Headache Impact Test (HIT), German versions, standard questionnaires for assessing the extent of migraine-related disability. [4]Blood beta-hydroxybutyrate and glucose levels, measured with a portable ketone meter (precision xtra by Abbot). [5]Routine laboratory (renal and liver function tests, electrolytes, full blood count, C-reactive protein, serum cholesterol, triglycerides, serum proteins, albumin, glucose, Hba1c, insulin, cortisol, lactate, TSH, FT4 and FT4). [6]Blood draw (1 × EDTA, 1 × PAXgene) at each time point for genetic profiling and gene expression analysis using microarrays. [7]Blood draw at each time point for oxidative and nitrosative stress markers (malondialdehyde (MDA), carbonylated proteins, nitrite, nitrotyrosine) and serum cytokine measurements (including, but not limited to, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, MCP-1, TNF-α, TNF-β, TGF-β1). V = visit

- IMP effect: synthesising previous findings [4, 64] and our pilot data [65], we aim to detect a difference of 2 days between placebo and IMP.
- We assumed the absolute reduction in migraine days to be normally distributed with a standard deviation of 3 days.
- We assume a conservative intra-patient correlation between IMP and placebo of 0.4.
- Drop out: a high drop-out rate of 30% is assumed.

Re-sampling The sample size was estimated using a re-sampling method. Each sample size ($n_i = 1,...,49 = 12$,

..., 60) was evaluated by sampling $R = 999$ times the reduction in migraine days from a bivariate normal distribution as already described. For each sample, whether superiority of the IMP over placebo could be shown (i.e. whether a two-sided paired t test resulted in significant $p < 0.05$) was tested.

In order to show the superiority of the IMP over placebo with a statistical power of 90%, 45 patients should be recruited in total to ensure 31 evaluable patients, assuming a drop-out rate of 30%. Figure 3 shows how the sample size depends on the expected reduction in number of migraine days in the IMP arm.

Recruitment

Patients will be informed about the study at the Department of Neurology, University Hospital Basel (USB). Moreover, there will by flyers publicly displayed in the waiting room of the neurology and general medicine department of the University Hospitals in Basel, Bern, Zurich and St Gallen, as well as the University Library. An announcement similar to the flyer will be posted on the webpages of the University of Basel "Marktplatz" dedicated to research studies (https://markt.unibas.ch/nc/inserate/kategorie/job-angebot-studien/) as well as the USB website (https://www.unispital-basel.ch/lehre-forschung/studieninserate/) and the University Children's Hospital Basel (UKBB) website (http://www.ukbb.ch/en/research/research-groups/neuromuscular-research.php), respectively. More flyers will be displayed in local pharmacies and pharmacies in Germany (with a radius of approximately 100 km around Basel), local neurologists, the neurological department of the Bruderholzspital (Kantonsspital Baselland) and the Headache Clinic of RehaClinic, located in Baden as well as Bad Zurzach, and also in the neurological outpatient clinic in Brugg (team of Prof. Sandor). Flyers will also be displayed in local busses and trains. The Swiss Headache Society (SKG) and the German migraine and headache society (DMKG) will advertise the trial on their website. All websites may include the link to a short recruitment video (https://www.youtube.com/watch?v=2YzNjIX-k_eY&t=19s) explaining the clinical trial with similar wording to the flyer. The video and an advertisement with similar wording to the flyer will also be advertised on Facebook (for users in a radius of 200 km of Basel). Patients previously contacted for a migraine–sport intervention study at the USB (EKNZ-Number 194/13) will be contacted again, if they previously agreed and met the inclusion criteria for the current study.

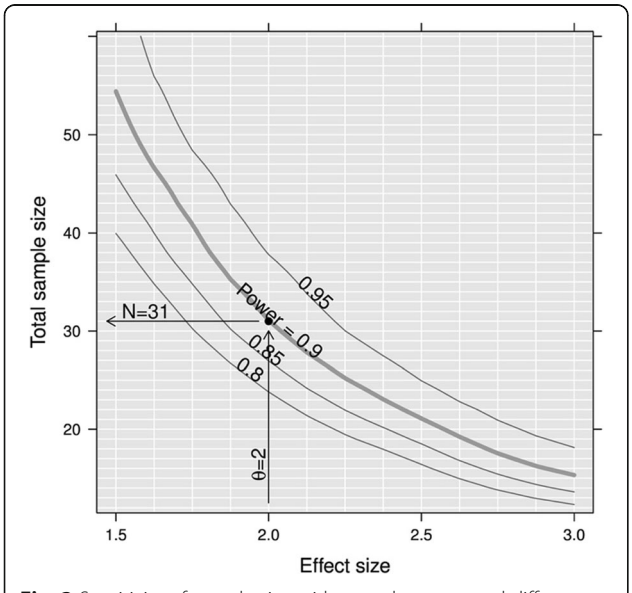

Fig. 3 Sensitivity of sample size with regard to expected difference in reduction in number of migraine days per 4 weeks of IMP compared to placebo. Example given, based on an effect size of 2 and a statistical power of 90%. The curves are smoothed and are for illustrative purposes only

Randomisation and blinding

Methods of minimising bias

Bias will be minimised by randomisation in 1:1 allocation and blinding of patients and investigators to the intervention. Randomisation will be done using an electronic data capture (EDC) system (SecuTrial) through an independent individual. The medication will be numerically labelled at the Pharmacy of the University Hospital of Basel and will then be provided to the ward and applied to the patient. This will allow a double-blinded randomisation (patient and treating physician will be blinded to the treatment).

The placebo powder has the same texture, colour and packaging as the IMP, so they cannot be distinguished in their appearance. The placebo also has a similar side-effect profile to the IMP. Data will be checked for protocol violation by the independent monitoring institution (see Quality assurance and control).

Randomisation

A crossover design with 1:1 AB/BA (IMP/placebo:placebo/IMP) randomisation is planned. The randomisation list will be computer generated and uploaded into the electronic data capture software SecuTrial by the responsible Data Manager at the Clinical Trial Unit (CTU) of the University Hospital Basel. Only unblinded personnel at the Pharmacy of the University Hospital Basel and at the CTU Basel will have access to the randomisation list. Just before the baseline visit, a clinical investigator will use SecuTrial to automatically assign a randomisation number from the randomisation list to the patient.

An additional list with medication numbers complementing the treatment arm of the first intervention

period will be provided by the CTU, in order to allow opposite treatment allocation during the second intervention period without unblinding the trial staff.

Blinding procedures

The study medication (IMP or placebo) will be provided as similar-looking medication in sachets. The medication will be packed by the Pharmacy of the University Hospital of Basel and will be numerically labelled using the randomisation list provided by the responsible Data Manager at CTU Basel. All investigators and patients will remain blinded until the trial is completed and the database has been locked.

Unblinding procedures (code break)

In the case of problems and safety concerns that cannot be solved with ongoing randomisation, the participant's allocated intervention will be revealed. Unblinding can be performed by authorised investigators using the EDC software SecuTrial. Each unblinding is documented in the EDC's integrated audit trail system and automatically reported to the principal investigator.

Data management

The study data recorded in the CRF will be transferred to a corresponding electronic CRF (e-CRF) by the clinical investigators. The principal investigator and co-investigator at the study site will be responsible for assuring that the data entered into the e-CRF is complete and accurate, and that the entry and updates are performed in timely manner. All information recorded in the e-CRFs will be traceable to the source documents in the patient's file and in the date source files.

Data management system

Data management will be conducted fulfilling all ethical and legal requirements according to Good Clinical Practice (GCP) and the Swiss Laws as "Bundesgesetz über die Forschung am Menschen" (Humanforschungsgesetz (HFG)).

The e-CRF will be implemented by the data management group at the Clinical Trial Unit (CTU) of the University Hospital Basel using the electronic data capture (EDC) software SecuTrial. The EDC system runs on a server maintained by the IT department of the University Hospital Basel.

Data entry will be performed by trained clinical investigators at the UKBB.

Data security, access and back-up

The EDC system is accessible via a standard browser on a www-connected device. Password protection and user-right management ensures that only authorised UKBB or CTU staff can enter the system to view, add or edit data according to their permissions. User administration and user training is performed by the CTU Basel according to predefined processes.

Back-up of SecuTrial study data is performed regularly according to the processes of the IT department of the University Hospital Basel. An integrated audit trail system will maintain a record of initial entries and changes made, reasons for change, time and date of entry, and user name of the person authorising entry or change.

Source data will be available at the site to document the existence of the study participants and will include the original documents relating to the study (patient demographics, medical history, medication, neurological examination, informed consent forms).

Analysis and archiving

The EDC system will be locked after e-CRF data entry is completed, all data have been monitored and raised queries have been resolved. The complete study dataset is exported from the database and transferred to the study statistician as well as the principal investigator through a secure channel. The exported data will be archived for 10 years by the principal investigator.

Electronic and central data validation

Data entered into the e-CRF will be validated for completeness and discrepancies automatically. The data will be reviewed by the responsible investigator as well as an independent monitor. The monitor will raise queries using the query management system implemented in SecuTrial. Designated investigators have to respond to the query and confirm or correct the corresponding data. Thereafter, the monitor can close the query.

Data monitoring

To ensure the quality of the study conduct and of the data, monitoring of the study is performed by organisations independent of the study (CTU, USB and Kammermann Monitoring Services GmbH). All inclusion and exclusion criteria are checked, and the monitor controls whether the data have been recorded correctly in the CRF, whether the drug accountability is correct and whether serious adverse events (SAEs) have occurred during the study.

Statistical analyses

Detailed methodology for summaries and statistical analyses of the data collected in this study will be documented in a statistical analysis plan. The statistical analysis plan will be finalised before database closure and will be under version control at the CTU, University Hospital Basel.

The primary endpoint, the number of migraine days in the last 4 weeks of treatment, will be measured twice for

each patient, once after the placebo treatment period and once after the IMP treatment period. The number of migraine days in the 4 weeks before the start of treatment will be assessed for both treatment periods, thus there will be two baseline values that will be used as covariates. This process has the aim of correcting for any potential seasonal variation in baseline migraine frequency or carry-over effects.

Hypothesis

The *null hypothesis* is that there is no difference in the difference in number of migraine days per 4 weeks from baseline to the last 4 weeks of intervention between the IMP and the placebo treatment.

The corresponding *alternative hypothesis* is that the difference in the number of migraine days per 4 weeks from baseline to the last 4 weeks of intervention differs between the IMP and the placebo treatment.

Statistical criteria for termination of trial

No early stopping is planned, either for efficacy or for futility.

Planned analyses

Datasets to be analysed, analysis populations The full analysis set (FAS) consists of all patients who are randomised and for whom the number of migraine days per 4 weeks at baseline is available.

The intention to treat (ITT) will include all randomised patients for whom the number of migraine days of at least the first 4 weeks of the first treatment period is available.

The per protocol (PP) will include all patients from the ITT set for whom the primary endpoint is available for both treatment periods, who are compliant as per the protocol (see later) and who have no protocol violations (to be defined in detail in the statistical analysis plan).

Primary analysis The primary endpoint, the number of migraine days in the last 4 weeks of treatment, will be measured twice for each patient, once after the placebo treatment period and once after the IMP treatment period. The number of migraine days in the 4 weeks before start of treatment will be assessed for both treatment periods, thus there will be two baseline values that will be used as covariates. This process has the aim of correcting for any potential seasonal variation in baseline migraine frequency or carry-over effects.

The primary analysis will be performed using a linear, mixed-effects regression model. The primary model will include the primary endpoint (the number of migraine days in the last 4 weeks of treatment) as the response variable, the respective baseline value as a covariate, treatment (IMP vs placebo) and period (first vs second) as main effects, the two interaction terms "treatment × period" and "treatment × baseline value", and patient as random effects. A significant interaction term between treatment and period would indicate a carry-over effect. Since it is not known how strongly the primary endpoint correlates with the baseline value, it is not known whether including the baselines as covariates in the model is sensible. Therefore, the already described primary model will be compared to models without the interaction term "treatment × baseline value" and without both the interaction term "treatment × baseline value" and the baseline value as a covariate by means of Akaike's Information Criterion (AIC).

The primary analysis will be done on the ITT set.

Subgroup analyses The following a priori defined subgroups will be investigated: sex (male/female), migraine with aura (yes/no) and baseline frequency of migraine days (medium = 5–9 days/4 weeks; high = 10–14 days/4 weeks). For each subgroup, the main effect of the subgroup and the interaction term "subgroup × treatment" will be added to the already described statistical model. In the case of a trend ($p < 0.10$) for an interaction effect—indicating a difference in the treatment effect between the subgroups—separate models will be fit for each subgroup.

Sensitivity analysis The main analysis, without subgroup analyses, will be repeated on the PP set. Potential deviations from the results of the ITT analysis will be described in detail.

Secondary analysis The secondary (exploratory) objectives are to assess the therapeutic efficacy of externally induced mild ketosis by the IMP regarding the following secondary endpoints:

- change in number of headache days of any severity from baseline (meeting ICHD-3 criteria) during the last 4 weeks of intervention;
- change in number of headache days of any severity from baseline (meeting ICHD-3 criteria) during the last 4 weeks of follow-up;
- change in consumption of acute migraine medication from baseline (analgesics or triptans)—measured in days with acute headache medication use—during the last 4 weeks of intervention;
- change in average migraine intensity from baseline—assessed with a VAS from 0 to 10 for each migraine episode—during the last of 4 weeks of the intervention period; and

- change in disability from baseline—assessed with the Migraine Disability Assessment (MIDAS) and the Headache Impact Test (HIT-6)—to the last of 4 weeks of the intervention period.

All of these secondary endpoints will be analysed as described for the primary endpoint with the corresponding baseline measure as covariate, if available.

All secondary analyses are done on the ITT set.

Exploratory analyses The exploratory objectives are to assess the potential mechanisms of action of externally induced mild ketosis by the IMP regarding markers of oxidative stress, markers of inflammation, glucose, fat, protein metabolism and genetic analyses:

- Serum concentration changes from baseline of oxidative and nitrosative stress markers (malondialdehyde (MDA), carbonylated proteins, nitrate, nitrite, nitrotyrosine) using ELISA and mass spectroscopy. This exploratory endpoint will be analysed as described for the primary endpoint with the corresponding baseline measure as a covariate.
- Serum concentration changes from baseline in markers of fat (triglycerides, cholesterol, HDL, LDL) or glucose metabolism (insulin, glucose, cortisol, Hba1c and lactate) during the last 4 weeks of intervention. This exploratory endpoint will be analysed as described for the primary endpoint with the corresponding baseline measure as a covariate.
- Serum concentration changes from baseline in serum inflammatory markers (cytokines including, but not limited to, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, MCP-1, TNF-α, TNF-β, TGF-β1) during the last 4 weeks of intervention, using a multiplex immunoassay analysed with a BioPlex 200. This exploratory endpoint will be analysed as described for the primary endpoint with the corresponding baseline measure as a covariate.

The following exploratory endpoints will be analysed with standard methods for gene and/or gene expression variation analysis:

- genetic profile (single nucleotide polymorphisms (SNPs)) of all patients involved in the study and correlation of the genetic markers with other outcome measures;
- gene expression changes before and after diet using expression microarrays with a special focus on mitochondrial-related genes (citrate synthase, cytochrome C oxidase subunit 1, succinate dehydrogenase subunit A); and
- correlation of gene expression changes with the genetic profile of the patients (eQTL analysis in combination.

All exploratory analysis is done on the ITT set.

Safety analysis Safety and tolerability will be determined by:

- comparison of treatment-emergent adverse events (any event regardless of potential causality with the drug) and treatment-related adverse events as defined by the principal investigator between active treatment and placebo; and
- examination for potential effects of the intervention on routine laboratory parameters (renal and liver function tests, electrolytes, full blood count, CRP, lipids, Hba1c, insulin, cortisol, lactate, TSH, FT4, FT3) in the treatment group compared to the control group.

Deviation(s) from the original statistical plan If substantial deviations of the analysis as outlined in these sections are needed for whatever reason, the protocol will be amended. All deviations of the analysis from the protocol or from the detailed analysis plan will be listed and justified in a separate section of the final statistical report.

Handling of missing data and drop-outs

The frequency of, timing of and reasons for, as well as all side-effects of, drop-outs will be reported for each treatment. Patients who drop out during the first run-in period or during the first 4 weeks of the first treatment period will be excluded. All patients who drop out later will be included in the ITT set.

For patients who drop out after the first 4 weeks and before the end of the first treatment period, the primary endpoint for the first treatment period will be imputed using multiple imputations. If appropriate, imputations will be accounted for baseline value and number of migraine days during the first treatment period, as far as available. The primary endpoint for the second treatment period will not be imputed for these patients.

For patients who drop out after the end of the first treatment period and before the first 4 weeks of the second treatment period are finished, the primary endpoint for the second treatment period will not be imputed. The primary endpoint for the first treatment period will be available.

For patients who drop out after the first 4 weeks and before the end of the second treatment period, the primary endpoint for the second treatment period will be imputed as already described.

Thus, for each patient included in the ITT set, the primary endpoint will be available (whether measured or imputed) for at least the first treatment period and will be taken into account with the proposed mixed effects models.

In case there are indications for missing data not at random, the inverse probability of censoring weights (IPCW) will be considered.

Statistical criteria for termination of trial No early stopping is planned, either for efficacy or for futility.

Quality assurance and control

The principle investigator (PI) is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions. The PI is responsible for proper training of all involved study personnel. To assess high-quality conduct of the trial in accordance with the protocol, all medical staff involved in this study are certified in good clinical practice (GCP).

Data handling and record-keeping/archiving

Paper documents including the results of the blood analysis, the headache diaries, questionnaires and all study-related documents will be filed in the study files and stored in the hardcopy archive of UKBB on a dedicated shelf.

Case report forms

For each subject included in this study, a case report form (CRF) will be completed, dated and signed by a study investigator. Data will be recorded in the CRF from the source documents, which may include medical notes and results obtained from laboratory reporting systems.

All participants receive a unique identification number (patient ID) and no identifying data such as name, initials or birth date will be collected in the CRF.

Specification of source documents

Source data will be available at the site to document the existence of the study participants. Source data will include the original documents relating to the study (patient demographics, medical history, medication, neurological examination, informed consent forms) as well as the MIDAS and the HIT-6 questionnaire.

Record-keeping/archiving

All study data, including CRFs and informed consent forms, will be archived for a minimum of 10 years after termination (or premature termination) of the clinical research project. Paper documents including the results of the blood analysis and gene expression changes as well as questionnaires will be stored in the hardcopy archive of the UKBB.

Monitoring

To ensure the quality of the study conduct and of the data, monitoring of the study will be performed by a person independent of the study (Kammermann Monitoring Services GmbH, Zug, Switzerland). All inclusion and exclusion criteria will be checked and whether the data have been recorded correctly in the CRF, whether the drug accountability is correct and whether SAEs have occurred during the study.

Audits and inspections

All study documentation and the source data/documents will be accessible to auditors/inspectors (also EKNZ and CA) and questions will be answered during inspections. All involved parties must keep the participant data strictly confidential.

Confidentiality and data protection

Direct access to source documents will be permitted for purposes of audits and inspections (ICHE6, 6.10). The investigators of the study will have access to the protocol, dataset (including questionnaires, demographical/clinical data) and statistical code during and after the study. The patients' identities will never be published in any abstracts or publications. A transfer of data will only take place for study purposes and only in encoded form. Third persons will not gain any insight into original data. For inspection purposes, insight into the original data will be permitted to the members of the appropriate authorities and also for the members of the local ethics committee, EKNZ. During the study, confidentiality will be guaranteed. The principal investigator will guarantee compliance with national and international data security.

Storage of biological material, related health data and returned study medication

Blood samples will be sent immediately to the earlier specified research laboratories. DNA and RNA extraction will be conducted immediately after arrival at the research laboratory. The extracted DNA/RNA will be sent for microarrays analysis on dry ice to Life&Brain, Bonn, Germany.

Biological material and related health data will be stored in an encrypted format for follow-up analyses.

In order to assess compliance of study medication intake, empty and full sachets are returned by the patients at visits 3, 4, 6 and 7. A member of the study team will count and balance the returned containers and can check the correct intake. This will be captured in an appropriate form. A qualified person from the study team will check the number of dispensed/taken

medications and complete a study-specific drug accountability form. After completion of the clinical trial, leftover study medication will be destroyed.

Safety assessments

Adverse events are monitored throughout the study. At every study visit, patients are asked about adverse events and their vital parameters are measured. If an AE is reported, a clinical examination is performed. The following safety parameters amongst other parameters are checked at visits 2, 3, 4, 5, 6 and 7 to determine safety of the treatment: routine laboratory parameters (renal and liver function tests, electrolytes, full blood count, C-reactive protein, serum cholesterol, triglycerides, serum proteins, albumin, glucose, Hba1c, insulin, cortisol, lactate, TSH, FT4 and FT3), blood pressure, heart rate, weight and height, assessed after 5 min of resting in a supine position.

As βHB is an endogenous substance we are not expecting any treatment-related serious adverse events on routine laboratory measures. Nevertheless, the intake of the IMPs will be stopped in the case of clinically significant changes in any of the parameters measured. In the event of any serious adverse events (treatment related or unrelated) occurring during intake of the IMPs, treatment will also be stopped immediately. If pathologic changes should be detected, whether related to or independent of migraine, the affected patients will be informed immediately and the possibilities of further investigation, respectively treatment of these abnormalities according to current medical knowledge, will be discussed.

Reporting of serious adverse events and other safety-related events

Treatment-emergent serious adverse events (any event regardless of potential causality with the drug) and treatment-related adverse events as imputed by the principal investigator (such as gastrointestinal upset) will be recorded. Reporting to the EKNZ will take place according to the clinical trials of medicinal products guidelines for notification and reporting of Swissethics. In brief:

- Serious adverse events (SAEs) with fatal consequences or where a connection is suspected with the intervention will be reported within 7 days.
- Suspected unexpected serious adverse reactions (SUSARs) with fatal consequences will be reported within 7 days, other SUSARs within 15 days.
- SAEs that may be related to the intervention under investigation in other clinical trials will be reported within 15 days.

AEs of this trial are graded in the most recent Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, which was published in November 2017 and became effective in April 2018 [66], published by the National Cancer Institute (NCI) of the National Institutes of Health (NIH).

Follow-up of (serious) adverse events

Patients with adverse reactions which have occurred in the context of the study will be followed up by the investigator up to 30 days after the last visit.

Discussion

We propose a single-centre, randomised, double-blind, placebo-controlled, crossover trial to determine whether treatment with βHB in mineral salt form has a positive effect on migraine frequency and associated symptoms. To our knowledge this is the first RCT using exogenous KB salts worldwide. If proven effective, βHB might offer a new prophylactic treatment option for moderately to strongly affected migraine patients, or at least a subgroup thereof. A demonstration of its safety might additionally pave the way for clinical trials assessing its use in related diseases.

Planning clinical trials in migraine is challenging for the following reasons: migraine is an episodic disease with a fluctuating nature (i.e. in some patients, migraine frequency can vary substantially from one month to the next or one season to the other, which makes it harder to demonstrate a treatment-related effect); the placebo effect is quite large, between 20 and 40% [67], which further adds to this problem; individual migraine attacks are of different length, and in more severely affected patients are sometimes hard to identify [68]; some patients suffer from headache of a different quality in addition to migraine and this distinction must be made by the patient subjectively [68]; and there is no objective biomarker for migraine or disease severity [68].

In order to address these problems, we have: incorporated two baseline periods to account for seasonal changes, and chosen a conservative effect size as well as a study population of moderate to high-frequency episodic migraineurs (5–14 headache days per month), in order to make it easier to demonstrate a sufficiently large effect size within a short timeframe, without introducing any confounds associated with chronic migraine, such as frequent co-morbidities [69]; calculated with a quite large placebo effect of 30%; chosen migraine days versus migraine attack frequency as the primary outcome; included a thorough briefing of each patient on the characteristic features of a migraine versus a headache attack; and included a detailed medical history and

diagnostic consultation by a neurologist, as well as a carefully constructed headache diary.

We have decided in favour of a crossover design in this single-centre RCT for the following reasons. Despite all efforts, recruitment has been slow and screening failures were a little higher than expected; in addition, we found that patients tended to be discouraged when they learned that they had solely a 50% chance of trying the IMP and would only find out which treatment arm they belonged to upon trial completion (in over 2 years time).

A crossover design in migraine is typically not recommended [68] because of the following limitations [67]: the possibility of a carry-over effect; the need for a long total period of treatment (extended by a wash-out period) with concomitant increases in drop-outs over time and in turn loss of statistical power [70]; and the increased likelihood of adverse events, which can unmask the blinding when a subject is exposed to both treatments.

In our case, a crossover design has three key advantages:

(1) A crossover design greatly improves statistical power, as each patient can be his/her own control (within-subject analysis versus between-subject analysis), which can be especially useful in a heterogeneous disease such as migraine, and hence fewer patients would be necessary to demonstrate a given effect. The sample size is effectively halved, even when more conservative a priori assumptions are employed, which is advantageous in single-centre studies. To compensate for some of the aforementioned weaknesses of crossover designs in migraine, we decided to make our a priori assumptions to determine the sample size more conservative than we would have with a parallel group design: a statistical power of 90% and a drop-out rate of 30% were chosen (in addition to a 30% placebo effect).
(2) A crossover design gives each patient the chance to try the IMP, which—from our experience—increases compliance, motivation and participation rates. We asked 25 prospective subjects for their preference and all of them favoured a crossover over a parallel group design. Instead of 6 months including a follow-up period, patients are now participating for a total of 9 months. The longer duration might lead to a slight increase in drop-out rates; however, on the other hand, it also leads to much improved participation rates, while only needing half of the patients. Additionally, it is known that vigilant patient education, monitoring and follow-up may reduce drop-out rates in longer trails [70]. From our experience, the moderate increase in trial duration has nowhere near negatively outweighed the positive impact of being guaranteed exposure to the IMP. A subsequent open-label period at the termination of the parallel group design would have a similar effect, but would also increase the costs substantially, as it does not have any impact on statistical power. This can be problematic, particularly for investigator-initiated trials.
(3) In addition to adding a wash-out period, the crossover design also allowed us to incorporate a second baseline period. This might help control for any potential seasonal effects on migraine frequency.

The possibility of a carry-over effect is always there; however, with a very short half-life of approximately 3–4 h, a 4-week wash-out period was judged to be sufficient.

Finally, we addressed the possibility of unblinding due to exposure to both substances. While there is no way to completely avoid this issue, we chose a placebo with a similar gastrointestinal side-effect profile to the IMP: mannitol, a sugar alcohol, can cause gastrointestinal disturbances, without having any systemic effect as it does not leave the gastrointestinal tract [58].

Various explorative outcomes have been included in order to be able to identify some of the potential protective mechanisms of exogenously induced ketosis in migraine. In addition, we are hoping this might help us distinguish responders and non-responders on both a phenotypical as well as physiological level.

Trial status

The trial started enrolment in May 2017 and is expected to be completed by the end of January 2020.

The newest protocol version is V6 of 5 September 2018. All protocol modifications have been and will be reported to the local ethic committee (Swissethics) and other relevant parties (such as Swissmedics, investigators and trial participants).

Additional file

Additional file 1: SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents. (DOC 122 kb)

Abbreviations

βHB: Beta-hydroxybutyrate; CA: Competent authority; Ca^{2+}: Calcium ion; CRF: Case report form; CTU: Clinical Trial Unit, University Hospital Basel, University of Basel; EDC: Electronic data capture; EKNZ: Local ethic committee of northern central Switzerland; ELISA: Enzyme-linked immunosorbent assay; FAG: Freie Akademische Gesellschaft; FAS: Full analysis set; GCP: Good clinical practice; HFG: Humanforschungsgesetz; HIT-6: Headache Impact Test; ICHD-3: International Classification of Headache Disorders version 3; IMP: Investigational medicinal product; ITT: Intention to treat; KB: Ketone body; KD: Ketogenic diet; MDA: Malondialdehyde; Mg^{2+}: Magnesium ion; MIDAS: Migraine Disability Assessment; MoH: Medication overuse headache; NSAID: Non-steroidal anti-inflammatory drug; PP: Per protocol; RCT: Randomised controlled trial; SAE: Serious adverse event;

SKG: Swiss Headache Society; SNF: Swiss National Foundation; SNP: Single nucleotide polymorphism; SOP: Standard operating procedure; SUSAR: Suspected unexpected serious adverse reactions; UKBB: University Children's Hospital Basel; USB: University Hospital Basel; V1: Visit 1; VAS: Visual analogue scale

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Availability of data and materials
For inspection purposes, insight into the original data will be permitted for the members of the appropriate authorities and also for the members of the local ethics committee, EKNZ. This study adhered to the SPIRIT checklist, available via Additional file 1.

Publication and dissemination policy
The results of the study will be published independent of the results in a medical journal.

Authors' contributions
EG participated in the design of the study and its organisation, conduct and data acquisition, and drafted the manuscript. NP, A-LO and SS participated in the conduct of the study. DRV performed the statistical analysis and calculated the sample size for the study. PS, SC and DF participated in the design of the study and its organisation, data analyses and manuscript creation. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethics approval has been obtained from the local Ethics Committee (EKNZ 2015-304) and the National Swiss Drug Agency (2016DR2109).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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3. Further Publications

3.1 Auf dem Weg zu neuen Applikationswegen und neuen Therapieprinzipien

Sprenger, T. & **Gross, E.** (2015). Migränetherapie- Update. Auf dem Weg zu neuen Applikationswegen und neuen Therapieprinzipien, *Hausarzt Praxis,* 10(1).

Migränetherapie – Update
Traitement de la migraine – Mise à jour

Auf dem Weg zu neuen Applikationswegen und neuen Therapieprinzipien

Vers de nouvelles voies d'application et de nouveaux principes thérapeutiques

Till Sprenger, Elena Gross, Basel

Seit der Einführung der Triptane in den 1990er Jahren gab es bei der Entwicklung der Migränetherapie nur wenige Neuerungen. Momentan befindet sich aber eine Reihe vielversprechender Substanzen bzw. Neurostimulationsmethoden in fortgeschrittenen Phasen der klinischen Prüfung – sowohl zur Akuttherapie der Migräne als auch zur Prophylaxe.

Le développement de la thérapie de la migraine a connu peu de nouveautés depuis l'introduction des triptans dans les années 1990. Il existe cependant actuellement une série de substances très prometteuses ou de méthodes de neurostimulation en phases avancées d'essais cliniques – aussi bien pour le traitement aigu de la migraine que pour la prophylaxie.

Die Migräne ist eine neurologische Erkrankung mit stereotyp rezidivierenden Attacken von mittelstarken bis starken, häufig pulsierenden Kopfschmerzen, Licht-, Lärm- und Geruchsempfindlichkeit sowie Übelkeit. Der Schmerz verstärkt sich meist bei körperlicher Aktivität [1]. Im Global Burden of Disease Survey 2010 der WHO wird die Migräne als dritthäufigste humane Erkrankung gelistet. Es wurde festgestellt, dass die Migräne mehr als 50% der durch neurologische Erkrankungen verursachten «years lived with disability» verursacht.

In den 1990er Jahren wurden mit den Triptanen wichtige neue Behandlungsoptionen zur Akuttherapie der Migräne verfügbar, die inzwischen einen festen Platz in Therapiealgorithmen haben. Seither waren sowohl im Bereich der Akuttherapie als auch der Prophylaxe trotz erheblicher Forschungsanstrengungen nur wenige Neuerungen zu verzeichnen. Erfreulicherweise scheint sich das Blatt vor allem in Bezug auf die Prophylaxe nun wieder zu wenden: Eine Reihe vielversprechender Substanzen bzw. Neurostimulationsmethoden wurde entweder bereits erfolgreich getestet oder befindet sich aktuell in fortgeschrittenen Phasen der klinischen Prüfung. Eine Auswahl aktueller Studienergebnisse zu pharmakologischen und interventionellen Therapieoptionen soll im Folgenden getrennt für die Akuttherapie und Prophylaxe der Migräne besprochen werden. Nicht-medikamentöse (z.B. psychologische) Verfahren werden in diesem Artikel nicht erläutert, haben aber ebenfalls einen hohen Stellenwert.

Akuttherapie der Migräne

Bei schweren Migräneattacken sind meist Triptane wie Sumatriptan 50 mg in Tablettenform Mittel der Wahl. Einer oralen Applikation stehen jedoch teilweise die Migräne-assoziierte Übelkeit und Erbrechen entgegen. In diesen Fällen kommen z.B. Sumatriptan oder Zolmitriptan Nasenspray, Sumatriptan Zäpfchen oder Rizatriptan bzw. Zolmitriptan Schmelztabletten in Frage, bei besonders heftigen Attacken allenfalls auch Sumatriptan 6 mg subkutan.

Die FDA hat 2013 mit einem iontophoretischen transdermalen System eine weitere Therapieoption

für solche Fälle zugelassen, dieses hat bislang jedoch keine Zulassung durch die Swissmedic. Bei diesem System wird Sumatriptan durch einen schwachen elektrischen Strom unter Umgehung des Magen-Darm-Trakts transdermal resorbiert. Es liess sich gegenüber Placebo eine eindeutige Wirksamkeit nachweisen, die jedoch etwas schwächer zu sein scheint als bei manchen oralen Triptanen.

Patienten, die sich mit schweren Migräneattacken notfallmässig vorstellen, sind eine besondere Herausforderung, da heftige Migräneattacken schlechter auf Medikamente ansprechen und diese Patienten in der Regel bereits orale nicht-steroidale Antirheumatika bzw. Triptane eingenommen haben. Daher kommen für solche Patienten insbesondere parenterale Substanzen in Frage. In einer kürzlich erschienenen doppelblinden Studie mit 330 Patienten wurden die intravenösen Gaben von Valproat (1000 mg), Metoclopramid (10 mg) bzw. Ketorolac (30 mg) verglichen. Hierbei schnitt Valproat schlechter ab als Metoclopramid und Ketorolac bezüglich der primären und sekundären Effektivitätsparameter. Erstaunlicherweise war Metoclopramid effektiver als das nicht-steroidale Analgetikum Ketorolac [2].

Eine Metaanalyse (fünf randomisiert-kontrollierte Studien) widmete sich der Studienlage von intravenösem Magnesium zur Migräneakuttherapie [3]. Es wurde kein positiver Effekt von Magnesium in Bezug auf Schmerzreduktion und die Einnahme von Rescue-Medikamenten festgestellt, und die Magnesium-behandelten Patienten klagten häufiger über Nebenwirkungen. Magnesium zur Akuttherapie kann insofern nicht als sinnvolle Therapieoption betrachtet werden, im Gegensatz zu Magnesium zur Migräneprophylaxe.

Interessant ist auch eine Fallserie zur Nutzung von Betablockern in Form von Augentropfen zur Migräne-Akuttherapie. Die beschriebenen sieben Patienten sprachen dabei auf die Gabe von entsprechenden Augentropfen (meist Timolol 0,5%) mindestens partiell an. Die Autoren argumentierten, dass durch die topisch okuläre Administration relativ schnell wirksame Plasmaspiegel erreicht werden können und daher Betablocker auch zur Akuttherapie geeignet sein könnten. Allerdings muss diese interessante Beobachtung erst noch in prospektiven klinischen Studien validiert werden.

Ein anderer interessanter Therapieansatz nutzt die Stimulation peripherer Nerven zur akuten, teilweise auch prophylaktischen Migränetherapie. Verschiedene Geräte und Stimulationsorte wurden dabei bislang untersucht, allerdings jeweils in überwiegend eher kleinen und teilweise nur unkontrollierten Studien. Die nicht-invasive Vagusnervstimulation (Gamma-Core® Gerät) ergab in einer offenen Studie eine Rate an schmerzfreien Patienten von 21% nach zwei Stunden [4]. Diese Rate entspricht etwa der Ansprechrate bei der erwähnten transdermalen Sumatriptangabe. Weitere Sham-kontrollierte Studien sind geplant. Ein Vorteil von nicht-invasiven Neurostimulationsverfahren wäre sicher, dass diese ohne Probleme mit einer medikamentösen Akuttherapie kombiniert werden könnten.

Abbildung: Elena Gross

Selbstbildnis einer Migränepatientin, das den hohen Leidensdruck veranschaulicht

Migräneprophylaxe

Zu den klassischen Migräneprophylaktika gehören Antiepileptika (vor allem Topiramat und Valproat), Kalziumantagonisten (Flunarizin), Betablocker (z.B. Propranolol) und als zweite Wahl auch Antidepressiva (z.B. Trizyklika). Durch die Einnahme von prophylaktischen Medikamenten kann bei rund jedem zweiten Patienten eine Reduktion der Attackenfrequenz um etwa 50% erwartet werden. Nebenwirkungen der genannten Substanzen (u.a. Gewichtszunahme, Müdigkeit, kognitive Nebenwirkungen, Übelkeit etc.) stellen ein grosses Problem der Migräneprophylaxe dar, so dass weitere Optionen wünschenwert sind.

Bereits seit mehreren Jahrzehnten wird angenommen, dass das Neuropeptid «Calcitonin Gene-related Peptide» (CGRP) im Rahmen der Migräne-Pathophysiologie eine wichtige Rolle spielt. So konnten erhöhte CGRP-Spiegel bei Migräneattacken nachgewiesen werden [5], und es wurde gezeigt, dass CGRP-Infusionen Migräneattacken auslösen können. Ferner waren CGRP-Rezeptorantagonisten wirksam in der Mi-gräne-Akuttherapie und -Prophylaxe [6,7]. Allerdings traten insbesondere in der letztgenannten Prophlaxestudie Transaminasenerhöhungen auf, die vermutlich einen Klasseneffekt darstellen, der auch auf andere CGRP-Rezeptorantagonisten (sog. Gepants) zutrifft. Die weitere klinische Entwicklung dieser Substanzen wurde daher vorerst gestoppt.

Das grundsätzliche Wirkprinzip wurde nun jedoch mit CGRP-Antikörpern wieder aufgegriffen. Hierbei handelt es sich um monoklonale Antikörper direkt gegen CGRP. Auch Antikörper gegen den CGRP-

Rezeptor werden aktuell getestet. Kürzlich wurden die Ergebnisse der ersten Phase II-Studien zur Prophylaxe der episodischen Migräne (<15 Kopfwehtage im Monat) mit CGRP-Antikörpern veröffentlicht [8,9]. Die beiden humanisierten CGRP-Antikörper ALD403 (einmalige i.v. Gabe von 1000 mg) und LY2951742 (alle zwei Wochen subkutan über 12 Wochen) zeigten dabei eine gegenüber Placebo bessere Wirksamkeit hinsichtlich Reduktion der Anzahl an Migränetagen bei guter Verträglichkeit. Es wird erwartet, dass diese und weitere Substanzen in Kürze in die Phase III der klinischen Prüfung eintreten. Auch Schweizer Zentren werden vermutlich an diesen Studien teilnehmen. Inwieweit die parenterale Applikationsform der Antikörper von Migränepatienten angenommen wird, muss abgewartet werden. Glücklicherweise sind die Intervalle zwischen den Applikationen recht lang (mindestens zwei Wochen). Vorteil dieser Substanzen könnte die Tatsache sein, dass sie nach derzeitigem Kenntnisstand nicht wie viele etablierte Migräneprophylaktika zu Gewichtszunahme (z.B. Valproat und Flunarizin) und Müdigkeit (z.B. Betablocker, Flunarizin, Trizyklika) führen.

Bei bereits zugelassenen Substanzen gibt es Evidenz, dass der Angiotensin II-Rezeptorblocker Candesartan wirksam in der Migräneprophylaxe ist. Dies hatte bereits eine frühere kleinere, doppelblinde, Placebo-kontrollierte Cross-over-Studie mit Candesartan (16 mg oral) und 60 Patienten nahegelegt [10]. Die gleichen Autoren haben nun Candesartan 16 mg in einer erneuten Cross-over-Studie gegen das etablierte Migräneprophylaktikum Propranolol (160 mg täglich) sowie gegen Placebo getestet. Candesartan zeigte eine ähnliche Effektstärke bei der Reduktion von Migräne- und Kopfwehtagen wie Propranolol. Beide Wirkstoffe waren Placebo überlegen. Eine frühere Studie (n=95) zur episodischen Migräneprophylaxe mit Telmisartan 80 mg war zumindest hinsichtlich des primären Endpunkts negativ geblieben [11], so dass Candesartan möglicherweise spezifische Effekte aufweist und sich als relativ nebenwirkungsarme (off-label) Alternative zu den gängigen Migräneprophylaktika anbietet.

Hormonelle Faktoren spielen bei der Migräne eine wichtige Rolle. Dies wird unterstrichen durch die häufige Triggerung von Migräneattacken durch die Menstruation und die Tatsache, dass Frauen nur zwischen Menarche und Menopause häufiger als Männer von Migräne betroffen sind – in der Kindheit und im Alter kommt Migräne bei beiden Geschlechtern etwa gleich häufig vor. Ferner besteht bei Patientinnen, die unter Migräne mit Aura leiden und die östrogenhaltige Kontrazeptiva anwenden ein erhöhtes Risiko für zerebrovaskuläre Ereignisse. Eine erste kleinere, offene Studie (n=37) aus Zürich hat Hinweise auf positive Effekte einer reinen Gestagenkontrazeption (Desogestrel) auf Migränefrequenz und Lebensqualität gezeigt [12]. Prospektive Untersuchungen zu diesem Thema sind geplant.

Zur Behandlung der chronischen Migräne (≥15 Tage pro Monat mit Kopfweh, davon mindestens sieben migränetypisch) wurde Botulinumtoxin A in der EU zugelassen, von Swissmedic jedoch nicht. Prinzipiell ist eine Botulinumtoxintherapie bei Migräne daher in der Schweiz nur off-label möglich. In der Vergangenheit waren diverse Studien mit Botulinumtoxin zur Prophylaxe der episodischen Migräne und von Spannungskopfweh negativ geblieben, zwei grosse Placebo-kontrollierte Studien zur chronischen Migräne hatten jedoch einen Effekt von Botulinumtoxin A gezeigt. Interessant sind neue Befunde von Burstein et al., die tierexperimentell zeigen konnten, dass durch extrakranielle Botulinumtoxininjektionen bestimmte Äste meningealer Nozizeptoren inhibiert werden können [13]. Somit besteht zumindest eine gewisse pathophysiologische Plausibilität von Botulinumtoxinjektionen bei chronischer Migräne.

Frühere Beobachtungsstudien zeigten einen positiven Effekt von invasiven Vagusnervstimulatoren, die Patienten mit therapierefraktärer Epilepsie implantiert wurden, auf deren komorbide Migränefrequenz. Der bereits erwähnte, nicht-invasive Vagusnervstimulator wird daher auch zur Migräneprophylaxe untersucht. Erste Ergebnisse bei chronischer Migräne zeigen eine gute Tolerabilität bei täglicher Applikation, allerdings eine nur mässige Reduktion der Attackenfrequenz.

Bereits mittels einer multizentrischen, randomisierten, Sham-kontrollierten Studie untersucht wurde die nicht-invasive supraorbitale Neurostimulation (Cefaly®-Gerät) [14]. 67 Patienten mit episodischer Migräne wurden täglich für 20 Minuten über drei Monate stimuliert. Die 50%-Responderrate war in der Verum-behandelten Gruppe signifkant höher (38,1%) als bei den Sham-stimulierten Patienten (12,1%).

Fazit für die Praxis

- Die Migräne ist eine häufige und behandelbare Erkrankung, die zu einem hohen Leidensdruck führen kann.
- Im Bereich der Akuttherapie werden in Zukunft neue Applikationswege bekannter Substanzen (z.B. Sumatriptan Patch) und möglicherweise auch Neurostimulationsverfahren (z.B. Gamma-Core® Gerät) zur Verfügung stehen.
- Auch im Rahmen der Migräneprophylaxe könnten nicht-invasive Nervenstimulationsverfahren in Zukunft eine Rolle spielen.
- Candesartan kommt als medikamentöse Alternative zu etablierten Migräneprophylaktika in Frage, ist jedoch für diese Indikation nicht zugelassen.
- CGRP-Antikörper greifen direkt in den pathophysiologischen Prozess bei Migräne ein, befinden sich derzeit in der klinischen Prüfung und könnten ein völlig neues Therapieprinzip zur Migräneprophylaxe ermöglichen.

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3.2 Headache in acute ischaemic stroke: a lesion mapping study

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Headache in acute ischaemic stroke: a lesion mapping study

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Headache is a common symptom in acute ischaemic stroke, but the underlying mechanisms are incompletely understood. The aim of this lesion mapping study was to identify brain regions, which are related to the development of headache in acute ischaemic stroke. Patients with acute ischaemic stroke ($n = 100$) were assessed by brain MRI at 3 T including diffusion weighted imaging. We included 50 patients with stroke and headache as well as 50 patients with stroke but no headache symptoms. Infarcts were manually outlined and images were transformed into standard stereotaxic space using non-linear warping. Voxel-wise overlap and subtraction analyses of lesions as well as non-parametric statistics were conducted. The same analyses were carried out by flipping of left-sided lesions, so that all strokes were transformed to the same hemisphere. Between the headache group as well as the non-headache there was no difference in infarct volumes, in the distribution of affected vascular beds or in the clinical severity of strokes. The headache phenotype was tension-type like in most cases. Subtraction analysis revealed that in headache sufferers infarctions were more often distributed in two well-known areas of the central pain matrix: the insula and the somatosensory cortex. This result was confirmed in the flipped analysis and by non-parametric statistical testing (whole brain corrected P-value < 0.01). To the best of our knowledge, this is the first lesion mapping study investigating potential lesional patterns associated with headache in acute ischaemic stroke. Insular strokes turned out to be strongly associated with headache. As the insular cortex is a well-established region in pain processing, our results suggest that, at least in a subgroup of patients, acute stroke-related headache might be centrally driven.

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Abbreviations: PWH = patients with ischaemic stroke and headache; PWOH = patients with ischaemic stroke without headache

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Introduction

Headache is frequently reported by patients with cerebrovascular events, even though severe neurological symptoms such as paresis, ataxia and loss of speech are usually the predominant clinical features. In previous studies, the prevalence of headache in acute ischaemic stroke has been reported with a range of 8% to 34% in different studies (Mohr *et al.*, 1978; Portenoy *et al.*, 1984; Vestergaard *et al.*, 1993; Jorgensen *et al.*, 1994; Ferro *et al.*, 1995; Kumral *et al.*, 1995; Evans and Mitsias, 2009). Prior investigations showed a lower prevalence of headache at the onset of stroke symptoms in patients with ischaemic stroke compared with patients with intraparenchymal haemorrhage (Mohr *et al.*, 1978; Portenoy *et al.*, 1984; Gorelick *et al.*, 1986; Vestergaard *et al.*, 1993; Arboix *et al.*, 1994; Kumral *et al.*, 1995; Evans and Mitsias, 2009). Some authors have suggested that the occurrence of headache in stroke may be related to infarct location. Headache during acute ischaemic stroke was reported to be more common with ischaemic events in the posterior than in the anterior circulation (Mohr *et al.*, 1978; Edmeads, 1984; Portenoy *et al.*, 1984; Gorelick *et al.*, 1986; Kumral *et al.*, 1995; Libman *et al.*, 2001; Mitsias *et al.*, 2006). Moreover, headache at stroke onset seems to be less frequent in lacunar stroke, compared with non-lacunar stroke (Mohr *et al.*, 1978; Gorelick *et al.*, 1986; Vestergaard *et al.*, 1993; Arboix *et al.*, 1994; Ferro *et al.*, 1995; Evans and Mitsias, 2009). Headache in stroke is frequently regarded as non-specific (Arboix *et al.*, 1994; Jorgensen *et al.*, 1994) and the characteristics of headache in stroke considered by the International Headache Classification (IHC) are more similar to the criteria of tension-type headache than migraine (Vestergaard *et al.*, 1993; Verdelho *et al.*, 2007). However, attendant symptoms such as hypersensitivity to light or noise (25%), nausea or vomiting (23–40%) can occur (Vestergaard *et al.*, 1993; Verdelho *et al.*, 2007). It should be mentioned that most of the studies referenced above did not differentiate between patients with and without primary headaches prior to stroke. The mean duration of headache in stroke has been reported to be 3.8 days (Verdelho *et al.*, 2007). Former studies on the relation between infarct size and the probability of headache have not shown a correlation (Vestergaard *et al.*, 1993; Jorgensen *et al.*, 1994; Verdelho *et al.*, 2007). However, no detailed lesion-mapping studies have been performed thus far.

Pain receptors are absent in brain tissue and mechanisms producing pain in ischaemic stroke must be different compared to other ischaemic disorders such as for example, in peripheral artery disease. Lesions of pain processing structures and descending pain pathways may play a role in the development of acute stroke-associated headache similar to other central post-stroke pain syndromes (Sprenger *et al.*, 2012; Seifert *et al.*, 2013). To date, no study has used modern neuroimaging techniques to assess the correlation of headache with lesions in specific locations or patterns of the ischaemic lesions (Evans and Mitsias, 2009). The aim of this lesion-mapping study was to phenotype patients with headache during acute ischaemic stroke and to relate the occurrence of head pain to lesion location. Patients with ischaemic stroke and headache (PWH) were compared to patients with ischaemic stroke but without headache (PWOH) in terms of their infarct patterns and in correlation with clinical data. We hypothesized that there are specific lesion patterns, which are associated with the development of acute headache in ischaemic stroke.

Materials and methods

Patients and recruitment

We investigated 100 patients with acute ischaemic stroke, who were treated in the stroke unit at the Klinikum rechts der Isar in Munich, Germany. Patients who had been transferred to our stroke unit with the diagnosis of an acute ischaemic stroke were consecutively included in case they met our inclusion criteria and either assigned to the PWH group or the PWOH group depending on whether they answered our question on headache 'yes' or 'no'. To ensure equal group sizes, inclusion was stopped when 50 cases were met in the respective group. All patients were interviewed within the first 10 days after the onset of symptoms of stroke. Only patients aged >18 years with ischaemic stroke as indicated by the MRI scan were included in this study. We excluded patients with strokes other than of ischaemic origin, with persistent aphasia or other reasons interfering with the assessment of the clinical phenotype (e.g. dementia or unconsciousness) and patients with contraindications for MRI (e.g. heart pacemakers) were excluded from this study.

After a detailed explanation of the study procedures, every patient provided written informed consent. The study was approved by the local ethics committee of the Technische Universität München, Klinikum rechts der Isar and was performed in accordance with the Declaration of Helsinki. The workflow of the study from screening to final analysis with inclusion and exclusion criteria is demonstrated in Supplementary Fig. 1.

Headache phenotyping and classification

All patients were interviewed by a special trained investigator (E.M.S.) using a semi-standardized questionnaire. The past medical history was obtained and patients were asked about specific features and qualities of headache in accordance with the second edition of the International Classification of Headache Disorders (Headache Classification Subcommittee of the International Headache, 2004). Pre-existing headaches (i.e. before the stroke) and their phenotype were also determined. Patients were asked about headache lateralization, the localization (frontal, temporal, occipital, nuchal, cervical), quality (pulsating, tensioning, stabbing) and intensity indicated on a visual analogue scale from 0–10 corresponding to minimal, medium and maximal intensity of pain. Additional symptoms such as nausea, vomiting, hypersensitivity to light and/or

noise, as well as cranial autonomic symptoms (facial sweating, conjunctional injection, rhinorrhoea and tearing) were assessed. We used the Pain DETECT questionnaire (Freynhagen *et al.*, 2006) for the purpose of investigating potential neuropathic components of stroke-related headache. The Pain DETECT questionnaire includes questions evaluating pain intensity (three numeric rating scales, pain course pattern, a pain drawing reflecting pain radiation, and seven questions addressing somatosensory phenomena, which the patient rates on a six-category Likert scale ranging from 'never' to 'very strongly'). Finally, a score ranging between 0 and 38 is calculated. A Pain DETECT score $\geqslant 19$ indicates that a neuropathic pain component is likely, a score of 13–18 is considered equivocal, and a score $\leqslant 12$ indicates that a neuropathic pain component is unlikely. Its specificity and sensitivity were shown to be 85% and 80%, respectively, with a predictive value of 83% (Freynhagen *et al.*, 2006).

All patients underwent the routine stroke MRI protocol applied at Klinikum rechts der Isar. MRI scans were routinely performed 2–4 days after stroke onset (computed tomography is usually applied initially when patients are admitted to our institution). All treatment decisions were made independently of this study.

Magnetic resonance imaging

All patients underwent brain MRI including FLAIR (fluid attenuated inverse recovery), diffusion-weighted imaging and a high-resolution T_1 sequence. All MRI measurements were performed on the same 3 T MRI system using a standard 16-channel head coil (Philips Achieva, Philips Medical Systems). High-resolution isotropic diffusion-weighted images were acquired including 73 sequential axial slices, echo time = 55 ms, repetition time = 3388 ms, voxel size $2 \times 2 \times 2$ mm, reconstructed voxel size 0.88 mm, max b-value = 1000. The neuroradiologists who performed the MRI scans were unaware of the existence of headache.

Lesion mapping

In a first step, axial diffusion-weighted images (73 slices) were imported with the freely available software MRIcro (Version 6 June 2013; Chris Rorden, Columbia, SC, USA; www.mricro.com). Lesions were drawn manually on the 3D diffusion-weighted images as regions of interest by a trained investigator (E.M.S.). The individually drawn lesions were checked (and corrected when necessary) by an experienced neurologist (C.L.S.) in a blinded manner.

As the proportion of right to left hemispheric lesions was not significantly different between PWH and PWOH, and to improve the data power of the lesion overlay analysis, region of interest images were transformed so that all lesions could be mapped to one hemisphere in a second analysis (referred to as 'Flipped Analysis' below). Thereby, all left-sided lesions were flipped to the right side. This seems justifiable since experimental studies on pain processing usually showed bilateral without a dominance of one hemisphere (Bingel *et al.*, 2002).

The data were then spatially normalized using the Statistical Parametric Mapping package (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) (Ashburner and Friston, 2005). Thereby, the brain MRI scans were normalized after masking of the lesions (Brett *et al.*, 2001). The normalization parameter file of every single patient was used to normalize the lesions [region of interest (ROI) files].

After non-linear normalization, the lesions were further analysed in terms of lesion overlaps with MRIcro and MRIcron software (http://www.mccauslandcenter.sc.edu/mricro/mricron/; Chris Rorden, Columbia, South Carolina).

Table 1 Baseline characteristics of PWH and PWOH

	PWH, *n* = 49	PWOH, *n* = 50	*P*
Age, mean (SD)	61.5 (14.7)	65.3 (13.4)	0.095
Male *n* (%)	29 (58%)	34 (68%)	0.362
Risk factors			
Hypertension	32 (63%)	32 (64%)	0.939
Smoking			
ever	28 (57%)	15 (30%)	0.006
former	5 (10%)	8 (16%)	0.012
Current smokers	23 (47%)	7 (14%)	0.012
Hyperlipidaemia	10 (20%)	22 (44%)	0.012
Obesity	13 (27%)	10 (20%)	0.442
Diabetes mellitus	9 (18%)	10 (20%)	0.837
NIDDM	8 (16%)	6 (12%)	0.303
IDDM	1 (2%)	4 (8%)	0.303
Atrial fibrillation	5 (10%)	12 (24%)	0.069
Prior stroke	1 (2%)	4 (8%)	0.362
Prior TIA	6 (12%)	2 (4%)	0.160
Cause of stroke			0.321
Cryptogenic	20 (41%)	20 (40%)	
Embolic	19 (39%)	19 (38%)	
Microangiopathic	5 (10%)	9 (18%)	
Dissection	2 (4%)	0 (0%)	
ACI stenosis	2 (4%)	0 (0%)	
VA stenosis	0 (0%)	1 (2%)	
Fabry's disease	1 (2%)	0 (0%)	
Vascular territory			
MCA	21 (43%)	20 (40%)	0.773
ACA	0 (0%)	2 (4%)	0.495
PCA	7 (14%)	9 (18%)	0.616
Lateralization			0.287
Bilateral	9 (18%)	10 (20%)	
Right	24 (49%)	17 (34%)	
Left	16 (33%)	23 (46%)	
NIHSS at onset, mean SD (*n* = 48)	4.2 (SD 4.5)	3.8 (3.8)	0.721
NIHSS at discharge, mean SD (*n* = 47)	2.2 (3.7)	2.4 (2.8)	0.553
Infarct volume, median voxels (SD)	7340 (55110)	4796 (23372)	0.300
Prior primary headache			0.308
Migraine	3 (6%)	8 (16%)	
Tension-type headache	27 (55%)	14 (28%)	
No headache	17 (35)	27 (54%)	
Unclassified headache	2 (4)	1 (2%)	

TIA = transient ischaemic attack; NIDDM = non-insulin-dependent diabetes mellitus; IDDM = insulin-dependent diabetes mellitus; MCA = middle cerebral artery; ACA = anterior cerebral artery; PCA = posterior cerebral artery; ACI = internal carotid artery; VA = vertebral artery; NIHSS = National Institutes of Health Stroke Scale.

Statistical analysis

The comparison of lesions between PWH and PWOH was analysed with the voxel-based lesion–symptom mapping (VLSM) method implemented in the non-parametric mapping (NPM) software, which is part of MRIcron. We considered headache as a binary variable (absent/present) according to a binary images/binary behaviour design. Non-parametric mapping was conducted using the Brunner and Munzel test with 4000 permutations (Medina *et al.*, 2010). We used the NPM software module of the MRIcron software to generate non-parametric tests. $P < 0.01$ corrected for multiple comparisons with the false discovery rate (FDR) approach was used. Coloured VLSM maps were generated and overlaid onto the automated anatomical labelling white matter templates (Johns Hopkins University) provided with MRIcron software. The same procedures were applied in the flipped analysis. In this analysis, the lesions with the centre of gravity in the left hemisphere were mirrored at the *x*-axis.

Comparisons of the clinical characteristics of PWH and PWOH were performed with the χ^2 test. We further conducted a correlation analysis between infarct volumes and pain intensity ratings using Spearmen correlation.

Results

One patient of the headache group with a medullary infarct had to be excluded from the final analysis as the infarct was not within the Montreal Neurological Institute and Hospital (MNI) space. Interestingly, this patient reported a very strong headache. The lesions of 21 patients with headache were flipped and lesions of 27 patients without headache were flipped to the right hemisphere.

All patients were interviewed within the first 10 days after stroke. The median time duration between stroke onset and the interview was 3 days [mean 3.4 days; standard deviation (SD) 2.1 days].

Baseline characteristics

The mean age of the patients in the headache group was 61.5 years (SD 14.7) with no significant difference compared to the non-headache group (mean 65.3 years, SD 14.9). Both groups were well matched in terms of risk factors for stroke; the only differences among them were their smoking status and the presence of hyperlipidaemia (Supplementary Table 1).

Of patients with transient or mild aphasia, five patients in the headache group and three patients in the non-headache group were included in our study. There were patients with a previous history of headache (before stroke onset) in both groups, patients who developed headache in acute stroke reported more often previous history of tension-type headache (55%), which was, however, not significantly different between both groups. Even though the infarct volumes were numerically larger in PWH, the statistical comparison between PWH and PWOH did not show a significant difference ($P = 0.300$).

Table 2 Characteristics of headache in PWH

Lateralization	
Bilateral	39 (80%)
Right	9 (18%)
Left	1 (2%)
Localization	
Frontal	33 (67%)
Temporal	31 (63%)
Occipital	26 (53%)
Nuchal	15 (31%)
Diffuse	34 (69%)
Quality	
Pulsating	8 (16%)
Tension-like	39 (80%)
Stabbing	5 (10%)
Intensity on VAS	
Mean of minimum pain, (SD)	3.8 (2.5)
Mean of mean pain, (SD)	4.9 (2.6)
Mean of maximum pain (SD)	5.9 (2.7)
Other symptoms	
Nausea/vomiting	14 (28%)
Photophobia	15 (30%)
PhonophobiaCranial autonomic symptoms	12 (24%)
Sweating	7 (14%)
Conjuntival injection	4 (8%)
Rhinorrhoea	2 (4%)
Lacrimation	3 (6%)
	2 (4%)
Pain DETECT score, mean, (SD)	2.76 (5.9)

VAS = Visual Analogue Scale.

Headache characteristics

The characteristics of headache in PWH are summarized in Table 2. Most of the patients reported bilateral pain (80%). The location of the pain was frontal in 67%, temporal in 63%, occipital in 53% and in the neck area in 31%. Most patients (69%) reported more than one head pain location. Patients with unilateral ischaemic infarcts reported headache on the same side in 60% of cases. Results of the Pain DETECT questionnaire suggest that the observed headache shows less signs of neuropathic pain. Only one patient with a right-sided insular infarct indicated a Pain DETECT score > 19. Only one other patient with a border zone infarct of the left middle cerebral artery indicated a score of 13. All other patients indicated ⩽ 12.

Imaging results

Figure 1 shows lesion probability maps of PWOH and PWH. In PWOH, the regions of highest lesion probability were seen in the left putamen, the left insula, the brainstem, the cerebellum, and the somatosensory cortex. The regions of highest lesion probability in PWH were detected in the right anterior insula and with lower probability in the posterior insula, the caudate nucleus, periventricular white matter, the cerebellum, and the somatosensory cortex

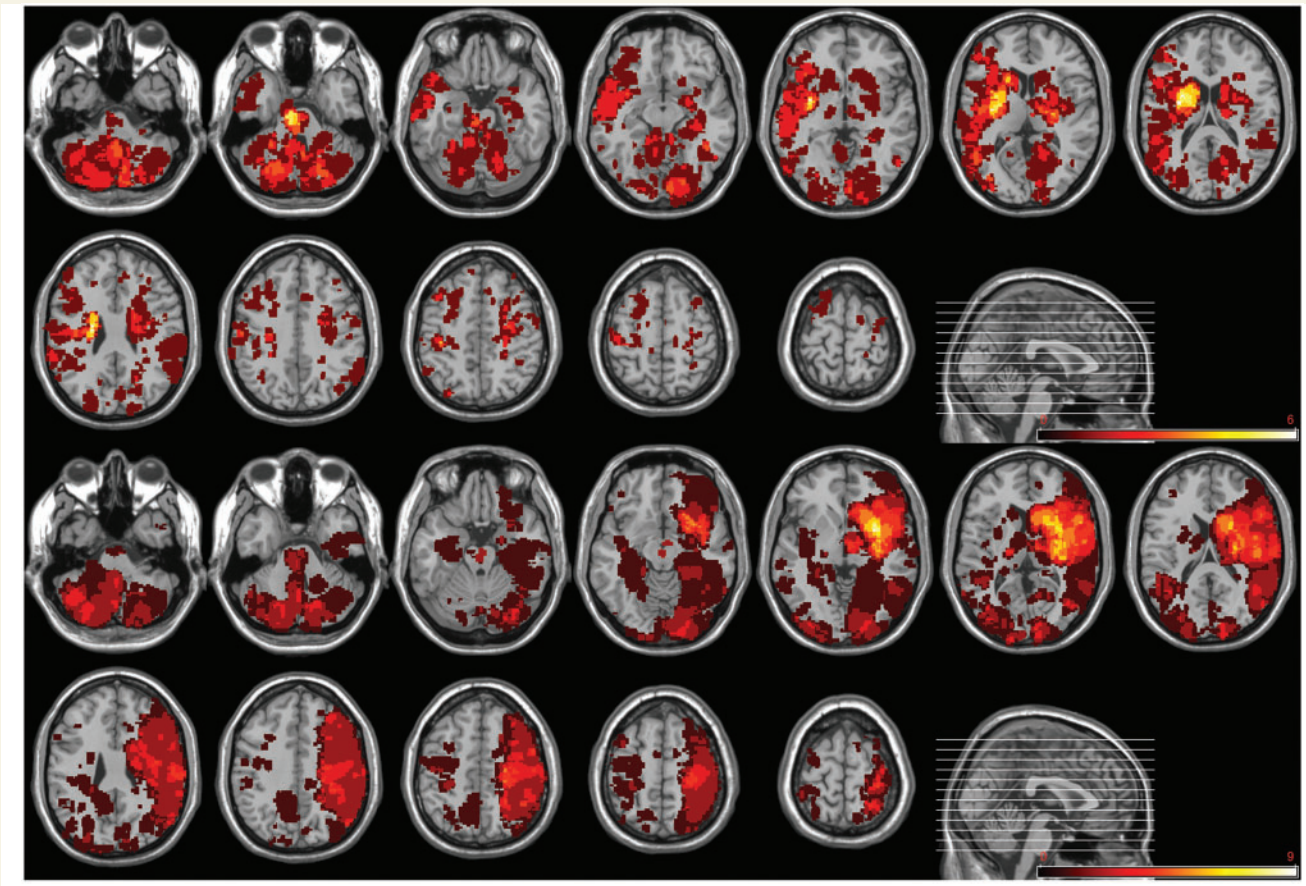

Figure 1 Lesion probability maps in PWOH (*top two rows*) and in PWH (*bottom two rows*), unflipped analysis. The colour bar indicates the number of patients with lesion overlaps.

(MNI-coordinates are given in Supplementary Tables 1 and 2).

Figure 2 shows the distribution of probability values for flipped lesions in PWOH and PWH. The regions of highest lesion probability in PWOH were also seen in the putamen/nucleus lentiformis, the caudate nucleus, the posterior cingulate, and the amygdala. The maximal overlap by summation of flipped lesions in the PWH group was seen in the insula, and with lower probability in the putamen, the cerebellum, and the somatosensory cortex (MNI-coordinates are given in Supplementary Tables 1 and 2).

In Fig. 3, summed lesions in the PWOH group were subtracted from the summed lesions in the PWH group. The corresponding Table 3 describes where the difference (PWH > PWOH) was maximal. The right anterior insula shows the maximal overlap (MNI coordinates 34, 7, −3). In the flipped analysis, the subtraction analysis of PWH minus PWOH showed that PWH lesions more often occurred in the anterior insula (MNI coordinates 33, 9, −6) as well as in the posterior insula (34, −15, 6).

Figure 4 demonstrates the results of the non-parametric statistics using the Brunner Munzel test with 4000 permutations for unflipped and flipped data. Anterior, posterior, and middle parts of the insula were found to be significantly associated with headache in the unflipped analysis. The flipped analysis confirmed our results (anterior and posterior insula) and moreover pointed out an association of lesions in the primary somatosensory cortex and the cerebellum with headache. The exact *z*-values and coordinates are summarized in Table 4.

An additional analysis with exclusion of the two patients with an underlying carotid dissection was performed because pain caused by vessel dissection might be due to a different pathophysiology. The results after exclusion were not significantly different.

Correlation analysis

The correlation analysis of infarct volumes with pain ratings (visual analogue scale) showed a correlation coefficient of 0.311 (Spearman).

Discussion

To the best of our knowledge, this is the first lesion mapping study investigating headache in acute ischaemic stroke. As a novel finding, this study was able to identify specific

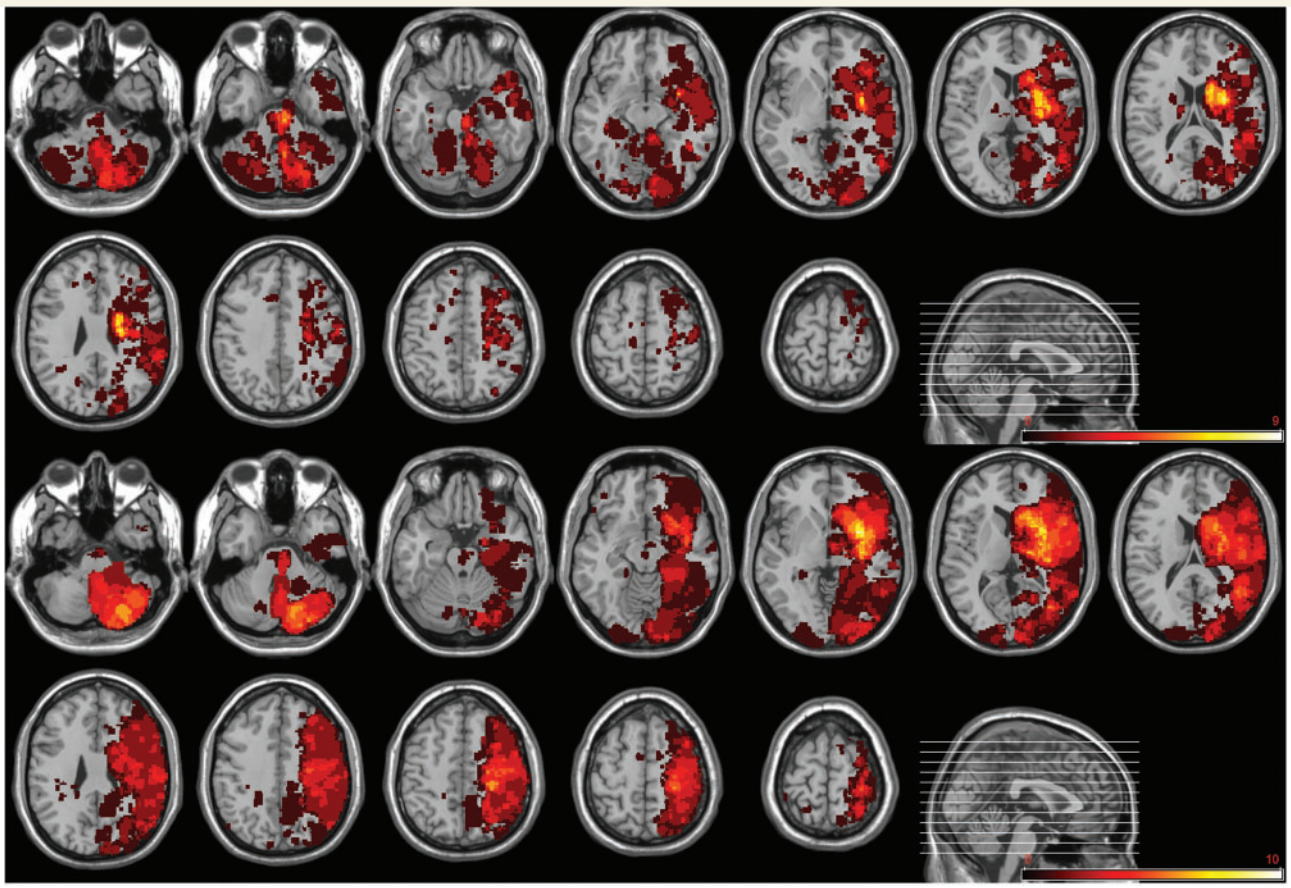

Figure 2 Lesion probability maps in PWOH (*top two rows*) and in PWH (*bottom two rows*), flipped analysis. The colour bar indicates the number of patients with lesion overlaps.

lesion patterns associated with the development of headache in acute ischaemic stroke. The insular cortex was identified as a key region in our conventional lesion mapping approach (i.e. subtraction analysis), which was confirmed by non-parametric voxel-wise statistics. In the flipped analysis, we were able to confirm that the insular cortex, and in particular the anterior part, is associated with headache in acute ischaemic stroke. Furthermore, with lower probability, lesions in the somatosensory cortex and the cerebellum seem to predispose for headache in stroke.

The insular cortex has been shown to be involved in the processing of painful as well as non-painful somatosensory inputs (Craig *et al.*, 2000; Mazzola *et al.*, 2006). It is part of the so-called 'pain matrix' (Apkarian *et al.*, 2005; Tracey and Mantyh, 2007) and its activation is one of the most consistent findings in pain stimulation paradigms in functional neuroimaging studies (functional MRI and PET) (Apkarian *et al.*, 2005; Tracey and Mantyh, 2007). The anterior part of the insula seems to be involved in the emotional and attentional processing of pain (Cauda *et al.*, 2011; Mazzola *et al.*, 2012), whereas the posterior insula is a key area for encoding pain intensity (Craig *et al.*, 2000; Bornhovd *et al.*, 2002; Moayedi and Weissman-Fogel, 2009). Further, a dissociation of latency coding and intensity coding of painful stimuli in the anterior and posterior parts of the insula has been proposed (Monteith *et al.*, 2014). In line with this finding a recent functional MRI study (Pomares *et al.*, 2013) revealed an association of painful sensations and increased amplitude of the blood oxygen level-dependent response in the middle and posterior parts of the insular cortex to painful laser stimuli.

Apart from general pain processing, the insular cortex is also thought to play a role in the pathophysiology of primary headache syndromes. It has been shown by voxel-based morphometry that the volume of the grey matter in the bilateral insula is reduced in migraine patients compared to controls (Kim *et al.*, 2008; Valfre *et al.*, 2008). In addition to structural changes, increased interictal resting state connectivity of the insula in patients suffering from migraine provides further support for the notion of the insular playing a role in migraine pathophysiological mechanism (Schwedt *et al.*, 2013; Tso *et al.*, 2015). One could hypothesize that ischaemic lesions of the insula lead to changes in functional connectivity networks and could cause pain by this mechanisms.

Similar to migraine, significant grey matter decreases have also been demonstrated in the insula bilaterally in

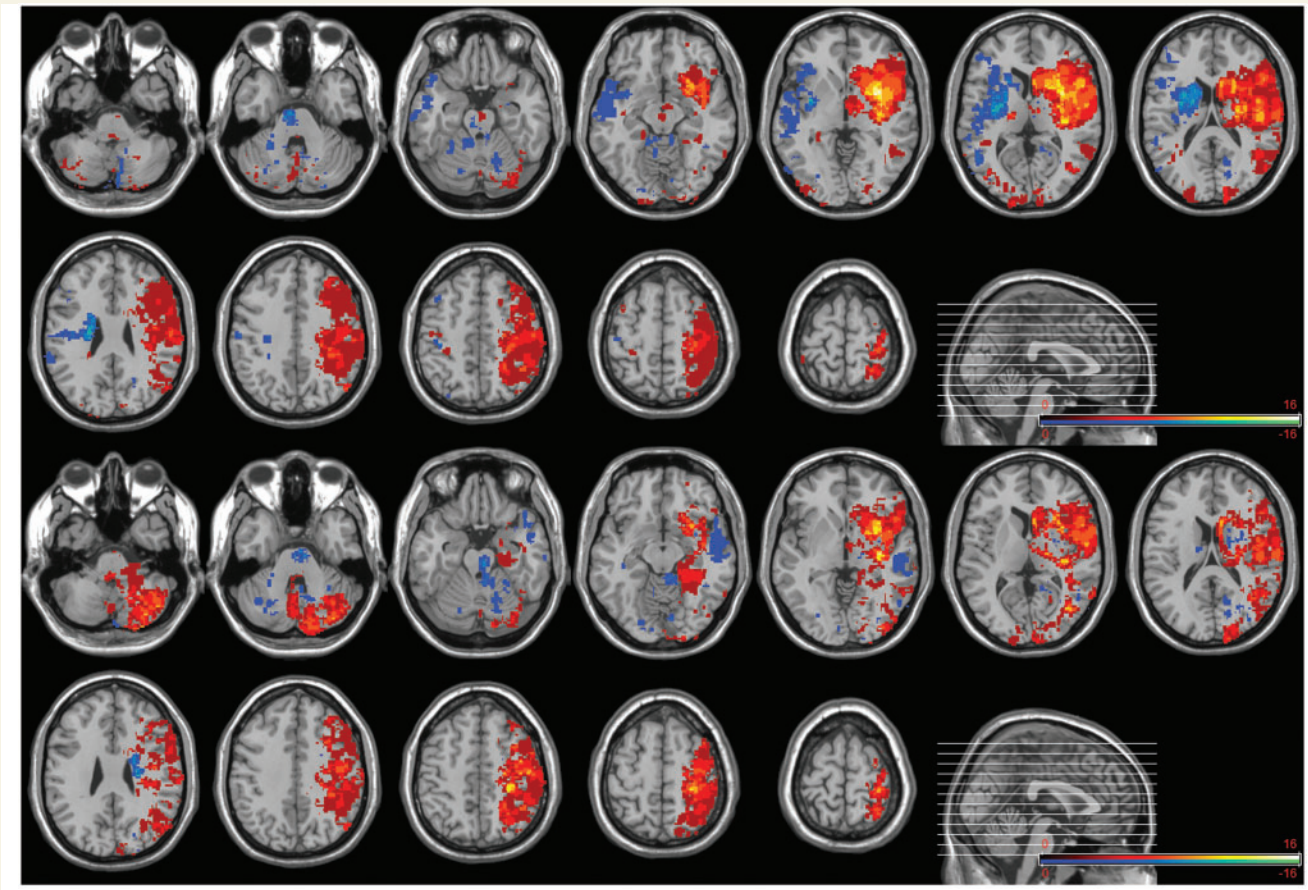

Figure 3 Subtraction map of lesions in PWH minus PWOH, unflipped analysis (*top two rows*) and flipped analysis (*bottom two rows*). The colour bar indicates the number of patients with lesion overlaps: red to yellow means positive and blue to green means negative.

Table 3 Subtraction analysis of summated lesions of PWH minus PWOH with the count of patients with overlaps, the coordinates in the MNI and the Talairach space and their lateralization

Region	MNI coordinates (*x*, *y*, *z*)	Overlaps after subtraction
Unflipped analysis		
Anterior insula right	34, 7, −3	+16
Caudate nucleus right	24, −14, 22	+12
Posterior insula right	34, −18, 4	+12
Somatosensory cortex right	33, −33, 66	+10
Lentiform nucleus, putamen right	30, 10, −16	+10
Putamen left	−20, 3, 15	−12
Periventricular area left	−23, −15, 26	−12
Insula left	−34, −5, 4	−12
Brainstem left	−4, −14, −32	−10
Flipped analysis		
Anterior insula	33, 9, −6	+14
Posterior insula	34, −15, 6	+14
Cerebellum	22, −89, −31	+12
	14, −83, −28	+12
Internal capsule, thalamus	19, −15, 6	+12
Somatosensory cortex	31, −29, 50	+12
Putamen	28, 10, −16	+10
Caudate nucleus	23, 0, 24	+10

chronic tension-type headache (Schmidt-Wilcke *et al.*, 2005). From visual inspection, reductions in grey matter in this voxel-based morphometry study included both the anterior as well as the posterior part of the insula.

The insular cortex shows anatomical and functional connectivity (Wiech *et al.*, 2014) to the secondary somatosensory cortex (SII), which is the second region we identified. Similar to the insula, the somatosensory cortex has also been demonstrated to be also involved in pain processing (Tracey and Mantyh, 2007). Additionally, neuroimaging studies support the role of the somatosensory cortex in headache pathophysiology. In migraine, thickening of the somatosensory cortex was demonstrated in two independent MRI studies (DaSilva *et al.*, 2007; Kim *et al.*, 2014). The character of the headache found in the PWH in this study was most similar to characteristics of tension type headache consistent with the criteria suggested by the second edition of the international classification of headache disorders (Headache Classification Subcommittee of the International Headache, 2004). This is in line with previous studies, in which a predominance of tension-type like headache in ischaemic strokes was found (Vestergaard *et al.*, 1993; Verdelho *et al.*, 2007). At present there seems to be no data supporting the involvement of the somatosensory cortex in tension-type headache specifically,

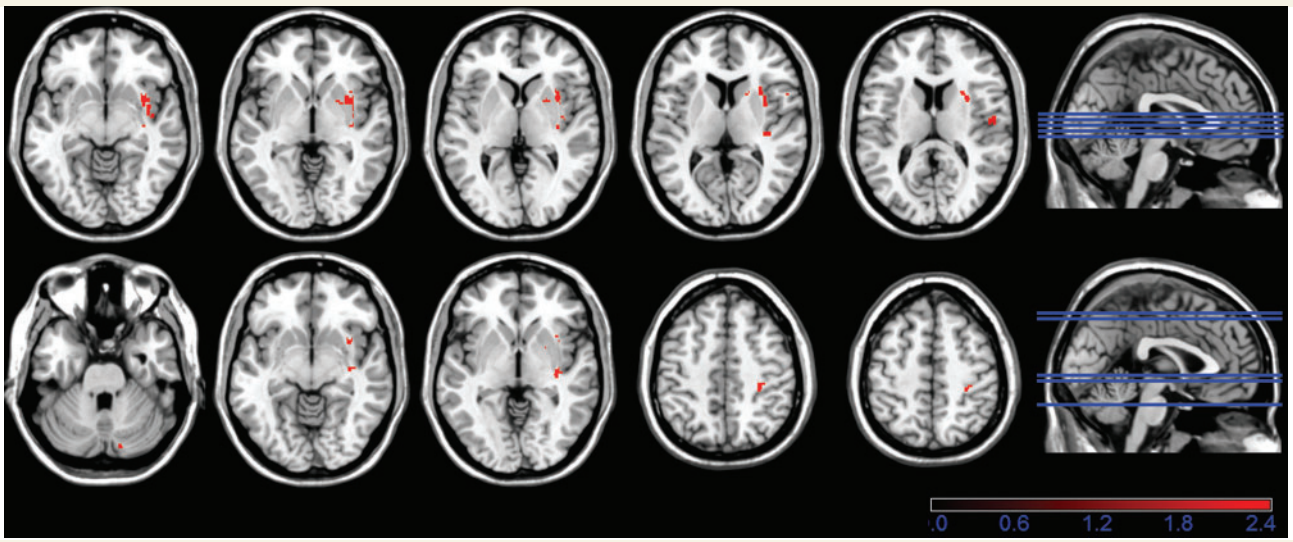

Figure 4 Non-parametric statistical map of the unflipped data (*top row*) and the flipped data (*bottom row*) using the Brunner Munzel test with 4000 permutations. The red scale indicates the z-score.

Table 4 Results of the non-parametric statistical test (Brunner Munzel with 4000 permutations); unflipped analysis and flipped analysis

Region	MNI coordinates (x, y, z)	Talairach coordinates (x, y, z)	Voxels, *n*	*Z*-Score
Unflipped analysis				
Anterior insula right	33, 7, 3	29, 4, 8	1632	3.04
Middle insula right	52, −11, 14	47, −13, 16	192	2.52
Putamen right	24, 6, 1	21, 4, 6	126	2.52
Posterior insula right	35, −19, 5	31, −20, 7	114	2.52
Flipped analysis				
Somatosensory cortex	31, −30, 51	27, −34, 47	378	2.52
Posterior insula	35, −17, −3	31, −18, 0	196	2.79
Anterior insula	34, 11, −4	31, 9, 2	164	3.04
Cerebellum	34, 11, −4	31, 9, 2	164	3.04

but a role of SII in the pathophysiology of primary headache syndromes seems to be well established.

Another region we found to be associated with headache in the flipped analysis is the cerebellum. This is in line with previous studies reporting a higher incidence of stroke-related headache with posterior circulation strokes (Mitsias *et al.*, 2006). The posterior cerebral artery has meningeal branches, which could partly explain why headache is more frequent in ischaemic lesions in the posterior circulation (Weinstein *et al.*, 1974, 1977; Ono *et al.*, 1984). Nevertheless, the role of the cerebellum in pain processing is less clear. A recent study supports the notion that the cerebellum might play a role in pain processing: it was shown that after cerebellar infarction, patients perceived heat and repeated mechanical stimuli as more painful than healthy controls (Ruscheweyh *et al.*, 2014). Cerebellar infarcts can lead to oedema (Wijdicks *et al.*, 2014), mass effects and consecutive distension of the pain sensitive dura mater, it is conceivable that cerebellar infarcts cause acute headache via local compressive effects rather than interaction with central pain processing.

Study limitations

In our study, stroke patients with aphasia or dementia as well as other disabling conditions were excluded as the study relied on patient self-report and headache history. This might bias our results, especially with regard to lateralization. As aphasia is mostly associated with left hemispheric infarcts, the lateralization in our study must be interpreted with caution. A recent meta-analysis (Duerden and Albanese, 2013) showed that pain-related brain activation tends to be bilateral, at least when it comes to the anterior insula. One could therefore argue that lateralization might play a minor role at most, as both damage to the left and the right insula can lead to headache in ischaemic stroke.

Conclusion

We found that headache in acute ischaemic stroke is often associated with infarcts in the anterior and posterior parts of the insular cortex, a brain area that has been repetitively shown to play a key role in both physiological pain processing as well as the generation of clinical pain conditions. Whether pain arises from disrupted processing of painful and non-painful somatosensory inputs or even altered interoception (Craig, 2002) remains to be established. The

present study provides evidence for the notion that at least in a subgroup headache in stroke might be centrally driven.

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The study was presented as a poster at the European Stroke Conference 2015 in Glasgow.

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Conflict of interest

The University Hospital Basel, as the employer of T.S., received compensation for him serving on scientific advisory boards and speaking from Actelion, ATI, Electrocore, Biogen Idec, Genzyme, Mitsubishi Pharma and Novartis. TS received funding from the Swiss National Science Foundation (SNF), Novartis Pharma, and the Swiss MS Society. H.P. received compensation for serving on scientific advisory boards and speaking from Bayer Healthcare, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo. M.M. has received research support from Merck Serono and Novartis; he has received travel expenses for attending meetings from Bayer, and Merck Serono. T.R.T received compensation for serving on scientific advisory boards and lectures from Astellas, Abbot, Grünenthal, Janssen-Cilag, Lilly, Mundipharma and Pfizer.

Supplementary material

Supplementary material is available at *Brain* online.

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3.3 Der Keto Kompass – Exogene Ketonkörper & Ketone, Ketose und Low Carb gegen Migräne

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1986 wurde es ins Englische übersetzt *Dismantling a Myth: The Role of Fat and Carbohydrates in Our Diet*. Lutz hoffte, damit die Diskussion und den wissenschaftlichen Austausch anzuregen. Leider scheiterte er damit wie schon der amerikanische Kardiologe Dr. Robert Atkins vor ihm. Dennoch haben diese beiden Ärzte wesentliche Akzente gesetzt. Wolfgang Lutz starb 2010 im Alter von 92 Jahren in Salzburg.

Dies sind nur einige der Pioniere kohlenhydratreduzierter, fettbetonter und ketogener Ernährungsweisen. Was vor 150 Jahren klein begann, entwickelte sich in den letzten Jahren zu einer regelrechten Bewegung! Und je mehr wir über die ketogene Ernährung lernen, desto spannender werden auch die Ketonkörper selbst. Denn auch mit der Zufuhr exogener Ketone lässt sich eine Ketose erzeugen.

Exogene Ketonkörper
(mit Elena Gross)

Wenn der Körper selbst Ketonkörper herstellt, spricht man von einer endogenen Ketose und endogenen Ketonkörpern (endo = innen/innerhalb). Hier soll es jedoch um »vorgefertigte« Ketonkörper, um die Moleküle selbst, gehen. Mit der Entdeckung der Ketonkörper und ihrer besonderen Wirkungen auf den Organismus und die zellulären Signalwege interessierte sich die Forschung zunehmend für diese Substanzen. Letztlich gelang es, die Ketonkörper zu isolieren und in Form von Supplementen einzusetzen. Wird der Körper auf diese Weise mit Ketonen versorgt, spricht man von exogener Ketose beziehungsweise von exogenen Ketonkörpern (exo = außen).

Versuche mit ß-Hydroxybutyrat (BHB) und Acetoacetat reichen bis in die 1940er-Jahre zurück. Die Arbeiten aus dieser Zeit zeigten, dass extern zugeführte Ketone von den Zellen sogar dann bevorzugt aufgenommen werden, wenn keine Unterzuckerung vorliegt, also auch, wenn genug Glukose vorhanden ist.[96] Die Ketonkörper wurden damals zu »normaler«, das heißt kohlenhydratreicher Kost verabreicht. Damit war eine wichtige Grundvoraussetzung für die Nutzung von Ketonkörpern als Nahrungsergänzungsmittel erfüllt: Sie können auch begleitend zu einer kohlenhydratreicheren Ernährung im Körper transportiert und verstoffwechselt werden. Die zweite wichtige Erkenntnis aus dieser Zeit ist, dass auch die Nutzung der exogenen Ketonkörper in direktem Zusammenhang mit der im Blut vorhandenen Menge steht. Das heißt: Je mehr Ketone zur Verfügung stehen, umso höher ist ihre Aufnahme in die zur Ketolyse fähigen Zellen und zwar unabhängig vom aktuellen Blutzuckerspiegel.[97 98]

Daraus erwächst ein großes Potenzial für exogene Ketonkörper. Denn überall da, wo eine ketogene Ernährung nicht erwünscht, nicht möglich oder nicht streng genug umsetzbar ist, könnten sie – ihre Sicherheit und Anwendbarkeit vorausgesetzt – die Lücke schließen: für

* Es steht gratis online zur Verfügung: http://79.170.40.49/watercar.ch/gesundheit/Lutz,Wolfgang-Leben_ohne_Brot(1985,271S.).pdf

Menschen, denen eine strenge ketogene Diät auf Dauer zu radikal ist, bei hochbetagten oder demenzkranken Patienten, die keine Ernährungsumstellung mehr wollen oder bewerkstelligen können oder in Situationen, wo sehr hohe Ketonkörperwerte für einen idealen therapeutischen Nutzen erreicht werden müssten, die jedoch alleine durch eine Ernährungsumstellung nur schwer zu erreichen wären.

BHB und Acetoacetat findet man in der Natur bestenfalls im Fleisch von hungernden Tieren. Frei kommen die beiden Ketonkörper nicht vor, und als starke Säuren können sie auch nicht in freier Form verabreicht werden. Verträglich sind sie in Form ihrer Salze, das heißt in Verbindung mit Mineralstoffen oder mit verschiedenen Aminosäuren sowie in veresterter Form*. Insofern müssten wir hier eigentlich von exogenen Ketonkörperverbindungen sprechen. Der Einfachheit halber bleiben wir jedoch bei exogenen Ketonkörpern beziehungsweise ketogenen Supplementen. Lange war ihre Anwendung ausschließlich Forschungseinrichtungen vorbehalten. Erst in den letzten Jahren haben es einige ketogene Supplemente auf den Markt geschafft.

Keton-Salze

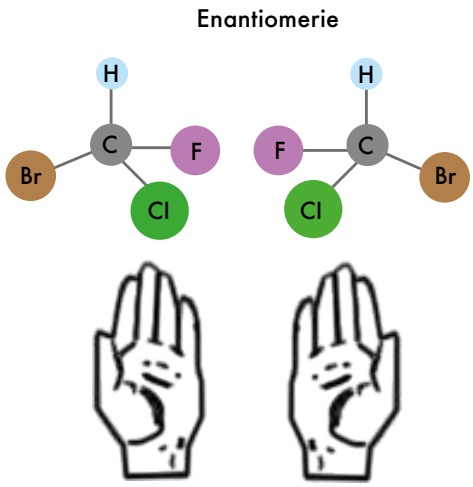
Keton-Salze sind weiße, pulverförmige Feststoffe. Sie bestehen hauptsächlich aus einem BHB-Molekül, das an einen Mineralstoff gebunden ist. Anfänglich war das BHB meist an Natrium gebunden, was jedoch zu unerwünscht hohen Natriumzufuhrmengen führte. Das veranlasste die Entwicklung weiterer Keton-Salze, bei denen das BHB an Kalzium, Magnesium oder Kalium gebunden ist. Um Mineralstoff-Dysbalancen zu vermeiden, enthalten viele der heute verfügbaren Produkte eine Mischung dieser Salze. Allerdings müssen Personen mit bestimmten Erkrankungen, beispielsweise mit Herzkrankheiten, vorsichtig sein und die durch Keton-Salze aufgenommenen Mineralstoffe in ihren täglichen Konsum mit einrechnen.

Eine Weiterentwicklung stellt die Bindung von BHB an Aminosäuren dar, also an Eiweißbausteine. Damit könnte man zwei Fliegen mit einer Klappe schlagen: So ließe sich nicht nur die Salzproblematik lösen, eine Verwendung der beiden ketogenen Aminosäuren Leucin und Lysin würde die Ketonbildung zusätzlich unterstützen. Ketonkörper-Salze mit Aminosäuren sollen ab Winter 2019 auf dem europäischen Markt verfügbar sein.

Wie rechte und linke Hand

Um weitere Unterschiede zwischen den verschiedenen Salzen zu verstehen, müssen wir uns ein wenig mit ihrer Isomerie beschäftigen. Sie beschreibt, dass Moleküle zwar aus den gleichen Elementen aufgebaut sein können, die gleiche Formel und die gleiche Masse besitzen, sich aber dennoch in wesentlichen Punkten unterscheiden können. Ein solcher Punkt ist ihre räumliche Anordnung. Verhalten sie sich wie Bild und Spiegelbild oder wie die rechte zur linken Hand, spricht man von Enantiomeren. Wie rechte und linke Hände sehen sie zwar sehr ähnlich aus, haben aber meist unterschiedliche Eigenschaften und Fähigkeiten. So gibt es häufig eine biologisch aktive Form, die unser Körper produziert und auch nutzt, während die andere Form weniger effizient oder überhaupt nicht genutzt werden kann. Vom Ketonkörper BHB gibt es zwei wichtige Enantiomere: Die beiden Hauptformen werden als D- und L-BHB bezeichnet und verhalten sich wie Bild und Spiegelbild zueinander. Die natürliche, vom Körper selbst produzierte Form ist das D-BHB.

* Ester = chemische Verbindung, die durch eine Kondensationsreaktion zwischen einer Säure und einem Alkohol unter Abspaltung von Wasser entsteht



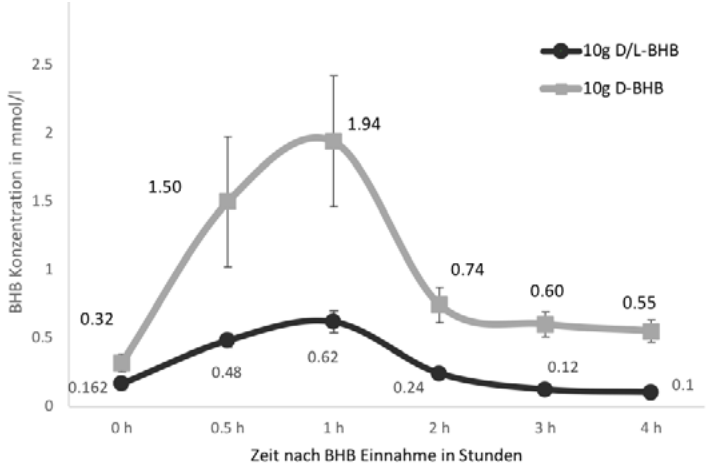
Steht die Energiegewinnung aus Ketonkörpern oder die Signalwirkung Die meisten Keton-Supplemente bestehen allerdings aus einer Mischform, dem DL-BHB. Solche Mischungen werden auch als Razemate bezeichnet. Sie sind in der Herstellung wesentlich günstiger als reines D-BHB, denn bei der chemischen Synthese entsteht etwa zur Hälfte D- und L-BHB, ihre Trennung ist aufwändig und teuer.

Was macht der Körper mit der L-Form? Direkt nutzen lässt sich das L-BHB nicht, doch ein Teil kann in die D-Form umgewandelt werden[99]. Zudem verfügt L-BHB über ein paar günstige Eigenschaften, es ist zum Beispiel ein starkes Antioxidans und es kann den Blutzuckerspiegel senken (siehe Grafik). Wir wissen noch nicht, ob L-BHB alleine oder in der Mischung mit der D-Form (Razemat) ungefährlich ist. Aber wir wissen, dass es natürlicherweise im Körper eines Erwachsenen nur in Spuren vorkommt, denn es macht nur einen winzigen Bruchteil des endogen gebildeten BHB aus. Wer also auf der sicheren Seite sein möchte, sollte zur Supplementation die D-Form wählen, bis es ausreichende Daten über die Eigenschaften des Razemats beim Menschen gibt.

Die Keton-Salze müssen im Körper nicht weiter verarbeitet werden. Da sie einfach durch den Darm ins Blut gelangen, können schon 15 Minuten nach der Einnahme erhöhte Ketonkörperspiegel gemessen werden. Kurz ist jedoch auch ihre Halbwertzeit: Nach etwa einer Stunde ist bereits der Peak erreicht, und nach durchschnittlich drei Stunden liegen die Blutspiegel wieder beim Ausgangswert.

Welche Blutwerte messbar sind, hängt unter anderem davon ab, ob ein Razemat verwendet wurde oder nicht, denn die Blutmessgeräte können nur D-BHB erkennen. Das L-BHB ist für sie und uns unsichtbar, zumindest so lange, bis es in die D-Form umgewandelt wurde. In der folgenden Abbildung sieht man es: Aufgezeichnet sind die Ergebnisse von fünf Probanden, die auf nüchternen Magen entweder 10 Gramm D- oder DL-BHB zu sich genommen hatten.[100] Erstaunlicherweise lag der höchste Wert des D-BHB im Schnitt nicht doppelt, sondern dreimal so hoch wie beim Razemat, das ja zur Hälfte aus der D-Form besteht. Eine andere Arbeitsgruppe kam zu einem ähnlichen Ergebnis.[101] Noch gibt es keine plausible Erklärung für diesen Befund; möglicherweise spielt die Aufnahme im Darm eine Rolle. Mit einem BHB-Wert von durchschnittlich knapp 2 mmol/l kann sich das Keton-Salz jedoch sehen lassen.

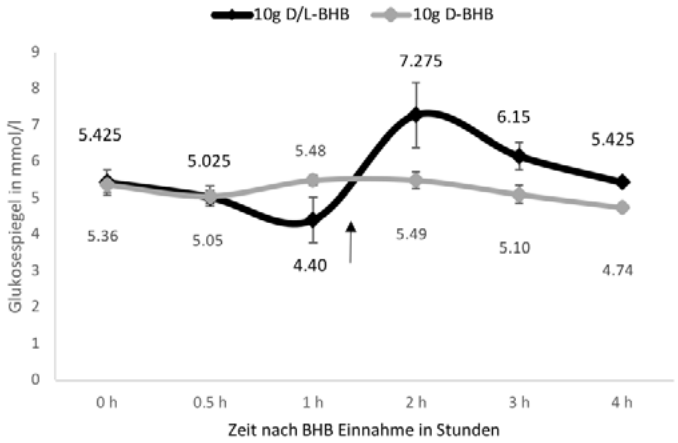
Vergleich der Pharmakokinetik von D/L- und D-BHB[72]



Steht die Energiegewinnung aus Ketonkörpern oder die Signalwirkung des BHB im Vordergrund, sind Präparate der D-Form gegenüber Razematen klar im Vorteil. Andererseits scheint das Razemat besser als D-BHB darin zu sein, den Blutzuckerspiegel zu senken, wie die nächste Abbildung zeigt. Während die Blutzuckerspiegel nach einer Gabe von 10 Gramm D-BHB praktisch unverändert blieben, sanken sie eine Stunde nach 10 Gramm des Razemats um 1 mmol/l. Das ist enorm. Dass der Blutzucker danach wieder anstieg, liegt daran, dass die Probanden etwas zu essen bekamen, um eine Unterzuckerung zu vermeiden.

Hier zeigt sich deutlich, dass die Wahl eines Supplements davon abhängt, was bezweckt werden soll. Bei Migränepatienten (siehe Seite 205) könnte ein blutzuckersenkender Effekt kontraproduktiv sein, bei einem Typ-2-Diabetes möglicherweise therapeutisch nutzbar. Wie kommt der Effekt zustande? Man weiß es nicht sicher, vermutet aber, dass die L-Form des BHB die Glukoneogenese in der Leber stärker hemmt. Dass die exogenen Ketonkörper bei Blutwerten um die 2 mmol/l die Insulinausschüttung beeinflussen, ist dagegen eher unwahrscheinlich. Auch wenn die Studienlage dazu nicht ganz einheitlich ist, so deutet doch viel darauf hin, dass erst hohe Ketonkörperspiegel ab etwa 5 mmol/l eine Insulinantwort auslösen.[102]

Vergleich der Effekte von D/L-BHB und D-BHB auf den Blutzuckerspiegel[72]



Nicht nur Geschmackssache
Ein Nachteil der Keton-Salze war bislang, dass sie nicht gut schmeckten. Mittlerweile gibt es sie zumindest in den USA schon in vielen Geschmacksrichtungen. Die neue Generation der Aminosäuren- und D-BHB-Salze ist geschmacklich sogar recht angenehm. Die Qualität der derzeit angebotenen Supplemente unterscheidet sich allerdings sehr. Oft ist nicht transparent, wie viel BHB sie enthalten. Die Gehalte sind teilweise so niedrig, dass kaum eine Auswirkung auf den Ketonkörperspiegel möglich ist. Die Supplemente unterscheiden sich in ihrer Herstellung (chemisch oder natürlich durch Fermentation), darin, ob sie aus einem Razemat bestehen oder reines D-BHB enthalten, im Anteil der Mineralsalze, die teilweise in bedenklich hohen Mengen darin vorkommen und bei der Verwendung von Zusatzstoffen, die nicht nur gesundheitlich bedenklich sein, sondern auch einen Effekt auf den Blutzucker haben können. Wer also mit Keton-Salzen experimentieren möchte, sollte auf die Qualität der Produkte achten. Stichworte sind: pharmazeutische Qualität (Good Manufacturing Practice, GMP), möglichst D-BHB, wenige Zusatzstoffe und eine ausgewogene Menge an Mineralien oder noch besser Salze mit Aminosäuren.

Zusammenfassend lässt sich sagen, dass Keton-Salze zu einer »Instant-Ketose« führen, mit der die Leber keine Arbeit hat. Neuere Präparate sind mit etwa 120 Euro für 30 Portionen nicht mehr allzu teuer und tolerabel im Geschmack. Sie führen zu respektablen Ketonkörperspiegeln, allerdings mit kurzer Halbwertszeit. Wer länger anhaltend hohe Ketonpegel wünscht, muss zusätzliche Maßnahmen ergreifen.

Keton-Ester
Keton-Ester sind ölige Flüssigkeiten, bei denen BHB- oder Acetoacetat-Moleküle über eine sogenannte Esterbindung an ein Trägermolekül gekoppelt sind. Idealerweise kommt dafür eine Verbindung zum Einsatz, die selbst ketogene Eigenschaften besitzt wie beispielsweise 1,3-Butandiol (BD), das im Körper in BHB umgewandelt werden kann[103]. Es handelt sich um einen zweiwertigen Alkohol, der als Lösungsmittel für Aromastoffe in der Lebensmittelherstellung breite Anwendung findet. BD wurde von der amerikanischen Lebensmittel- und Arzneimittelbehörde FDA als sicher eingestuft. Für die Herstellung von Keton-Estern wird ein BD an ein oder zwei Moleküle BHB oder Acetoacetat gebunden. Anstelle von BD kann auch Glyzerin für die Veresterung verwendet werden. Wie bei den Keton-Salzen gibt es auch bei den Estern Varianten: Es gibt Ester, die aus Razematen bestehen und Ester aus der D-Form beider Substanzen. Auch die Vor- und Nachteile sind hier ähnlich wie bei den Keton-Salzen.

Da die Ester aber erst noch enzymatisch gespalten werden müssen, ist ihre Halbwertszeit etwas länger als bei den Keton-Salzen. Anders als bei den Salzen sind bei den Estern höhere Blutketonspiegel ohne die Gefahr einer Mineralstoffüberladung möglich. Auch lässt sich mit einigen Keton-Estern gezielt der Acetoacetat-Spiegel erhöhen. Das könnte für einige Erkrankungen wie etwa die Epilepsie relevant sein.

Keton-Ester sind derzeit noch sehr teuer in der Herstellung, eine Dosis kostet etwa 100 US-Dollar. Erst seit kurzem sind sie als kommerzielles Produkt erhältlich, allerdings ist der Geschmack noch ein großes Problem. Keton-Ester wecken im Mund Assoziationen von Diesel oder Flugzeugbenzin. Wer sie einmal probiert hat, sagt oft, es sei die scheußlichste geschmackliche Erfahrung überhaupt gewesen. Diesen grässlichen Geschmack und Geruch zu maskieren, ist wohl die größte Herausforderung auf dem Weg zu einer breiteren Anwendung von Keton-Estern.

Haben Ketone Kalorien?

Da Ketone eine Energiequelle darstellen, haben sie auch Kilokalorien. Dieser Aspekt wird gerade im Hinblick auf Keton-Supplemente gerne vergessen. Je nachdem, in welcher chemischen Verbindung das Beta-Hydroxybutyrat in einem Supplement verwendet wird, kann der Kaloriengehalt leicht schwanken. Er liegt zwischen 4,7 und 5,4 Kilokalorien pro Gramm[104]. Eine Tagesdosis von 10 bis 20 Gramm BHB hätte dann circa 50 bis 100 Kilokalorien.

Brennstoffe des Stoffwechsels

Substrat	Kilokalorien / Gramm
freie Fettsäuren (i. D.)	9,31
ß-Hydroxybutyrat (BHB)	4,69
Glukose (Traubenzucker)	3,72
Essigsäure (Acetat)	3,48
Pyruvat	3,17

modif. n. Cahill, F Jr., Ann Rev Nutr 2006;26: 1–22

Wird unter einer Supplementierung mit Ketonkörpern Gewicht verloren, dann liegt das an der Sättigungswirkung der Ketonkörper, die dazu führen kann, dass man insgesamt weniger isst. Wird am Essverhalten nichts geändert, nimmt man durch exogene Ketonkörper wie mit jedem energiehaltigen Getränk oder Lebensmittel zusätzliche Kilokalorien zu sich und kann damit auch zunehmen.

Exogene versus endogene Ketose

Exogene Ketonkörper versorgen den Körper direkt mit BHB oder – im Falle einiger Keton-Ester – mit Acetoacetat, ohne dass eine endogene Ketogenese stattgefunden hat.[105] Sie können die endogene Ketogenese sogar hemmen, wenn ausreichend hohe Blutspiegel um 5 mmol/l erreicht werden.[106] Mit exogenen Ketonen ist es zudem leichter, die Ketonspiegel im Blut deutlich zu erhöhen, auch über die Werte hinaus, die durch endogene Ketose erreichbar sind.[107]

Entscheidend ist jedoch ein weiterer Unterschied zur endogenen Ketose: Werden die exogenen Ketonkörper zu einer »normalen« kohlenhydratreichen Ernährung eingenommen, ändert sich die Glukosezufuhr nicht. Auch wenn sie die Glukoneogenese hemmen und die Glykogeneinlagerung fördern können[108], entfallen so die positiven Effekte einer Kohlenhydratreduktion: Blutzuckerschwankungen, reaktive Hyperinsulinämie, erhöhter oxidativer Stress und vermehrte Entzündungsmarker und deren Folgen werden mit exogenen Ketonen also nicht angegangen. Positiver formuliert: Exogene Ketone induzieren keine Nahrungsketose, sind aber in der Lage, einige ihrer positiven Effekte zu imitieren.

Bislang gibt es noch keine klinische Studie am Menschen, in der eine ketogene Ernährung direkt mit einer Supplementierung exogener Ketone verglichen wurde. Die Unbedenklichkeit der Präparate wurde zunächst an Mäusen untersucht.[109] Zur kurzfristigen Sicherheit und Wirkweise der Ester und Salze gibt es bislang erst wenige Humanstudien. In einer dieser Studien führten beide Substanzen zu niedrigeren Blutzucker- und Triglyzeridspiegeln und zu weniger freien Fettsäuren im Blut. Die Elektrolytspiegel waren nicht beeinträchtigt.[110]

Wie so oft, wenn es um neue Substanzen geht, sind Sportler unter den ersten, die sie zu testen bereit sind. 2013 wurde an kleinen Gruppen von Athleten die Auswirkungen eines D-BHB-Keton-Esters getestet.[111] Es zeigte sich, dass unter diesen Umständen Glukose und Ketonkörper gleichzeitig oxidiert werden können. Das Insulin blieb unbeeinflusst, die Lakatspiegel fielen geringer aus, der Muskelabbau war gehemmt und die Leistungsfähigkeit gesteigert: So fuhren Rennradfahrer in einer halben Stunde durchschnittlich 411 Meter weiter als ohne Supplemente. Aus Berichten über die Tour de France 2018 wurde deutlich, dass mittlerweile mehr und mehr Teams die Strategie *Dual Fuel* für ihre enormen Leistungen nutzen: zweierlei Brennstoffe, Ketonkörper und Glukose.[112] Wie die Verkaufszahlen zeigen, wurden in den USA mittlerweile mehrere Millionen Portionen exogener Ketone konsumiert, jedoch sicher nicht nur von Leistungssportlern.

Könnten exogene Ketonkörper auch bei der Behandlung von Erkrankungen von Nutzen sein? Im Tiermodell erwies sich die exogen induzierte Ketose tatsächlich bereits als recht vielversprechend, unter anderem auf die körperliche und kognitive Leistungsfähigkeit.[113] In einer Studie im Maus-Alzheimer-Modell verdoppelte D-BHB-Salz die neuronale Überlebensrate und schützte die Zellen vor den schädlichen Konsequenzen der Amyloid-Plaques.[114] Exogene Ketonkörper könnten auch bei Parkinson und Schädel-Hirn-Traumata relevant sein.[115] [116] [117] Neuere Mäusestudien bestätigten einen antiepileptischen Effekt exogener Ketonkörper[118] sowie reduzierte Ängstlichkeit, was auf einen antidepressiven Effekt hindeutet.[119]

Dr. Angela Poff aus dem Labor von Dr. Dominique d`Agostino, dem weltweit führenden Forschungszentrum für exogene Ketone, untersucht unter anderem die Auswirkungen exogener Ketone auf das Krebsgeschehen. Sie fand bei Mäusen mit metastasierendem Krebs ein stark verlangsamtes Tumorwachstum und eine um 60 Prozent längere Lebensdauer.[120] Es scheint zunächst verwunderlich, dass die Ketone ohne begleitende Verringerung der Glukosezufuhr helfen können. Man weiß auch noch nicht, warum das so ist. Es zeichnet sich jedoch ab, dass exogene Ketone möglicherweise auch einen Platz in der unterstützenden Therapie bei Krebs verdienen könnten. Dazu sind jedoch kontrollierte Studien am Menschen nötig.

Ein erster Fallbericht von einem an fortgeschrittenem Alzheimer erkrankten Patienten liegt bereits vor (siehe auch Seite 214): Hier führte die zusätzliche Gabe von 28,7 Gramm D-BHB-Keton-Ester pro Tag zu einer deutlichen Besserung des Befindens und vieler Alltagshandlungen.[121] Die Verbesserungen zeigten sich dosisabhängig. Hoffnung machen auch vereinzelte Berichte über den Einsatz exogener Ketone bei Kindern mit schweren Glukosestoffwechselerkrankungen wie der Glykogenspeicherkrankheit[122] [123] [124] [125] sowie bei Migräne (siehe Seite 205). Definitive Aussagen über die Wirksamkeit und Sicherheit exogener Ketone unter verschiedenen Stoffwechselbedingungen lassen sich allerdings erst treffen, wenn Daten aus Placebo-kontrollierten Humanstudien vorliegen. Insofern »dopt« man seinen Stoffwechsel damit derzeit noch auf eigene Gefahr.

Ganz aktuell: Ketone, Ketose und Low Carb gegen Migräne

von und mit Elena Gross

Was Migräneattacken bedeuten, ist für die davon Verschonten schwer nachzuvollziehen. Migräne bedeutet viel mehr als nur Kopfschmerz. Betroffene leiden unter anderem zusätzlich unter starker Licht-, Geräusch- und/oder Geruchsempfindlichkeit, Übelkeit bis hin zum Erbrechen und in einem Drittel der Fälle unter einem Phänomen, das sich Aura nennt – ein kurzzeitiger sensorischer Ausfall, meist visuell, also das Gesichtsfeld betreffend, aber teilweise auch bis zur halbseitigen Lähmung.

Frauen sind zwei- bis dreimal so häufig von Migräne betroffen wie Männer. Insgesamt leidet jeder siebte Mensch auf der Welt unter Migräne.[41] Laut WHO handelt es sich um die dritthäufigste Krankheit und die neurologische Erkrankung, die weltweit die meisten Menschen beeinträchtigt.[42] Neben großem Leid verursacht Migräne auch unglaubliche Kosten, allein 18,5 Milliarden Euro im Jahr in Europa.[43]

Trotz allem sind die Ursachen der Migräne noch immer weitgehend ungeklärt. Wir wissen zwar mittlerweile, dass dem Migräneschmerz höchstwahrscheinlich eine Aktivierung des trigeminovaskulären Systems zugrunde liegt, das die Interaktion zwischen den Blutgefäßen des Gehirns und der Hirnhaut und dem sie innervierenden Trigeminus-Nerv bezeichnet.[44 45 46] Aber warum es zu dieser Aktivierung kommt, ist ungeklärt. Deswegen sind auch die Möglichkeiten der Migränetherapie noch immer sehr eingeschränkt und häufig mit signifikanten, oft auch intolerablen Nebenwirkungen verbunden.

Aufgrund der großen Einschränkungen der Lebensqualität, der Schmerzen und anderer Symptome, die viele Migräniker erfahren und erleiden, sind sie auf Akutmedikamente zur Behandlung der einzelnen Attacken angewiesen. Die unterdrücken allerdings nur die Symptome, nicht die Ursache. Zudem dürfen sie nur an maximal zehn Tagen pro Monat eingenommen werden, damit keine Abhängigkeit und kein Medikamentenübergebrauchskopfschmerz entsteht. Schon deswegen sollte die Migräneprävention ein wichtiges Ziel sein. Doch um die Attackenhäufigkeit reduzieren zu können, muss man wissen, wie Migräne entsteht und was sie auslöst.

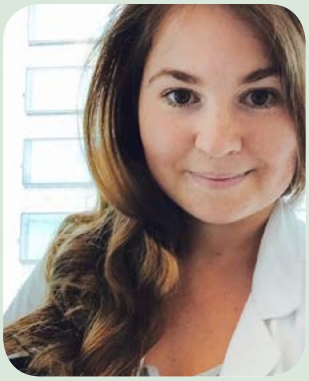
Derzeit mehren sich die Hinweise, dass es sich bei Migräne, zumindest zum Teil, um ein Energiedefizit des Gehirns handeln und dass Veränderungen des Energiestoffwechsels zur Entstehung beitragen könnten.[47 48 49 50 51 52] Dafür sprechen beispielsweise Hinweise auf Veränderungen der Mitochondrienfunktion.[53 54 55] Coenzym Q10 und Riboflavin, beide wichtig für die mitochondriale Energiegewinnung, schützen vor oxidativem Stress, der auch mit Migräne in Verbindung gebracht wird.[56 57] Zudem konnte mit Hilfe von bildgebenden Verfahren in einigen Hirnarealen von Migränikern eine verminderte Zuckerverwertung (Hypometabolismus) nachgewiesen werden.[58] Da grundlegende Hausarbeiten der Nervenzellen, wie das Aufrechterhalten des Membranpotentials zwischen dem Zellinneren und dem extrazellulären Raum, etwa die Hälfte des Energiebedarfs ausmacht, könnte ein Energiemangel auch einen Teil der Übererregbarkeit erklären, die für Migräne (und Epilepsie) so typisch ist:[59 60 61 62 63] Sinkt das Membranpotential, weil nicht genügend ATP vorhanden ist, feuert die Nervenzelle viel leichter, ähnlich wie ein niedriger Damm eher von Wellen überspült wird als ein hoher.

Oft sind es die Migränepatienten selbst, die sich auf den Weg machen, um die Hintergründe ihrer Krankheit besser zu verstehen und nach Möglichkeiten zu suchen, wie sie sich vor neuen Attacken schützen oder sie doch zumindest abmildern können. Ein wichtiger Vorreiter im

deutschsprachigen Raum war der Ingenieur Peter Mersch, der selbst über 30 Jahre lang an schwersten Migräneattacken litt – bis er eine Lösung fand. Sein detailreiches und lesenswertes Buch Migräne – Heilung ist möglich können wir nur empfehlen.[64] Ihm verdanken wir auch die Einsicht, dass es in der Migräneprophylaxe nicht darum gehen sollte, das Leben möglichst un-abwechslungsreich zu gestalten, sondern dass auch hier die metabolische Flexibilität entscheidend ist: Wir müssen die Ketolysefähigkeit unseres Gehirns erhalten und pflegen, damit es jederzeit flexibel zwischen der Verwertung von Glukose und Ketonen hin- und herschalten kann.

Dazu ist es nötig, zumindest phasenweise in Ketose zu sein und zu wissen, wie man diesen Zustand erreicht. Zu diesem Thema interviewten wir die Neurowissenschaftlerin und Psychologin Elena Gross, die in Oxford studierte und in der klinischen Forschung an der Uni Basel tätig war. Als Selbstbetroffene, die zum Teil chronisch an schwerer Migräne litt und der niemand helfen konnte, beschloss sie, selbst zur Migräneforscherin zu werden. So kam es, dass sie heute in der Neurologie mit Schwerpunkt Migräne-Prävention tätig ist.

» Interview mit Elena Gross, Neurowissenschaftlerin, Keto- und Migräneexpertin, Basel



Elena Gross vertritt, wie einige andere Forscher auch, die Hypothese, dass es sich bei Migräne, zumindest zum Teil, um ein Energiedefizitsyndrom handelt.[65] Daher gilt ihr Hauptinteresse den metabolischen Aspekten von Migräne und anderen neurologischen Erkrankungen und hier vor allem der Ketose. Sie promoviert im Rahmen einer randomisierten, Placebo-kontrollierten, doppelt-blinden Phase 2 Sicherheits- und Wirksamkeitsstudie mit dem Titel Exogene Ketonkörper in der Migräneprävention, kurz MigraKet genannt.[66]

Frau Gross, wie sind Sie auf die ketogene Ernährung beziehungsweise auf die Ketone gekommen und wie dachten Sie zunächst darüber? Was hat Sie letztlich bewogen oder überzeugt, sie auszuprobieren und zu erforschen?

Im Rahmen meines Masterstudiums der Neurowissenschaften im englischen Oxford hatte ich das große Glück, an Migräne forschen zu dürfen: einmal mit iPSCs (induzierten pluripotenten Stammzellen) von Migränepatienten und einmal mit chronischen Migränepatienten im Rahmen eines Projektes mit bildgebenden Verfahren. Eines Tages musste eine meiner Migränepatientinnen aufgrund eines technischen Fehlers den Scan wiederholen. Sie war – wie die meisten meiner Patienten – fast jeden Tag von Migräne geplagt und konnte vor Schmerz, Übelkeit und Kraftlosigkeit kaum noch am Leben teilnehmen. Als sie zwei Wochen später für die Wiederholungsmessung ins Labor zurückkam, erkannte ich sie erst nicht wieder.

Sie ging aufrecht, unbeschwert, hatte abgenommen und strahlte bis über beide Ohren. Auf meine Frage, was passiert sei, berichtete sie mir, sie habe in den vergangenen zwei Wochen nichts gegessen und in dieser Zeit keine einzige Migräneattacke erlitten. Sie hätte abends sogar ein Glas Rotwein getrunken, der ja als wichtiger Migräneauslöser gilt. Doch während dieser Fastenzeit hätte selbst das bei ihr keine Migräne verursacht. Wie war das möglich? Nach zwei Wochen gänzlich ohne Nahrung sollte doch das

Gehirn, das angeblich so sehr auf Glukose angewiesen ist, komplett streiken oder etwa nicht? Und dann auch noch Rotwein! In meinen Augen klang das wie das perfekte Migräneauslöserezept.

Nach dem Scan aß die Patienten ein paar der von uns angebotenen Kekse. Schon in derselben Nacht suchte sie die Migräne erneut heim, so heftig wie selten. Dabei hatte sie doch nun gegessen, sogar viel Glukose, die das Gehirn ja mit schneller Energie versorgt. Oder etwa nicht?

Einige Monate später saß ich in der Bibliothek und blätterte zur Ablenkung von der Masterarbeit das Wissenschaftsjournal Nature durch. Vielleicht war es Schicksal, jedenfalls enthielt es ein *Epilepsy Special*, in dem auch die älteste Therapiemethode für Epilepsie beschrieben wurde: die ketogene Diät (KD). Als ich die möglichen Mechanismen der KD durchlas, konnte ich mich kaum noch auf dem Stuhl halten, denn ich war mir sicher, des Rätsels Lösung in meinen Händen zu halten! Ich wusste bereits, dass Migräne und Epilepsie genetische Gemeinsamkeiten haben, und damit war eigentlich klar, dass die beschrieben Mechanismen einer KD auch bei Migräne relevant sein mussten.

Die Ketose erklärte zwanglos das mir bisher als paradox erschienene Fastenereignis meiner Patientin. Und ich war am Ende meines Studiums der Neurowissenschaften angelangt, ohne jemals etwas von Ketonkörpern gehört zu haben! Am medizinischen und psychologischen Institut der University of Oxford hatte man uns gelehrt, dass das Gehirn quasi ausschließlich Glukose metabolisiert. Bis heute frage ich mich, wie das möglich ist und je mehr ich über Ketonkörper lerne, desto absurder erscheint es mir.

Aber noch ein Gedanke schoss mir damals durch den Kopf: Es ist durchaus möglich, dass das Gehirn verhungert, egal wie viel wir essen! Denn wenn der Glukosestoffwechsel oder der Glukosetransport in die Hirnzellen nicht richtig funktioniert, können wir von diesem Treibstoff so viel essen wie wir wollen, im Gehirn wird dennoch nicht genug Energie ankommen. Noch am selben Tag habe ich mich mit Nüssen und Fett eingedeckt und mit dem ersten Keto-Selbstexperiment begonnen, denn ich war ja selbst chronische Migränepatientin. Allerdings wusste ich noch zu wenig darüber, wie ich am besten in Ketose komme und habe durch meine ersten Versuche die Migräne teilweise sogar noch schlimmer gemacht. Aber ich habe nie daran gezweifelt, dass die Ketose, richtig praktiziert, eine Lösung für Migräne darstellen könnte. Damit war auch klar, dass ich über das Thema Ketose als Migräneprävention promovieren wollte.

Inzwischen sind fünf Jahre vergangen – und ich habe kaum noch Migräne. Allerdings habe ich immer noch großen Spaß an (Selbst-)Experimenten, sei es mit verschiedenen Formen der ketogenen Diät oder mit verschiedenen ketogenen Substanzen. Ich bin sehr dankbar dafür, mittlerweile vielen Patienten eine Lösung für ihr Problem anbieten zu können. Meine ersten Migränepatienten in Oxford vergesse ich allerdings bis heute nicht. Ich konnte ihnen in ihrem großen Leid nicht helfen und Millionen Menschen weltweit geht es ähnlich. Aus eigener Erfahrung weiß ich, dass die meisten Pharmazeutika, die gegen Migräne eingesetzt werden, mit oft unerträglichen Nebenwirkungen verbunden sind. Daher lohnt es sich besonders, für einfache Stoffwechselprodukte wie die Ketone, die tatsächlich Linderung bringen können, zu kämpfen.

In welchen Fällen setzen Sie die ketogene Diät beziehungsweise Ketone heute ein und welche Ergebnisse erzielen Sie damit?

Die ketogene Diät empfehle ich vor allem, wenn die Triggerfaktoren (= Auslöser) einzelner Attacken mit dem Stoffwechsel beziehungsweise mit oxidativem Stress verbunden sind. Typische Migräne-Trigger wären in diesem Fall:

- Sport,
- Auslassen einer Mahlzeit oder unregelmäßiges Essen,
- unregelmäßiges Trinken,
- Alkohol, der oxidativen Stress fördert und die Glukoneogenese einschränkt,
- Stress, egal, ob physischer oder psychischer Natur,
- unregelmäßiger Tag-Nacht-Rhythmus,
- Migräne nach hohem Zucker- beziehungsweise Kohlenhydratkonsum,
- ggf. kann auch eine menstruelle Migräne mit oxidativem Stress in Verbindung gebracht werden, denn Östrogen wirkt protektiv, während ein fallender Spiegel vor der Menstruation das Migränerisiko verstärken kann,
- intensive sensorische Reize,
- Hypoxie, also ein Sauerstoffmangel, der zur sogenannten Höhenmigräne führen kann
- Wetterumschwünge zu niedrigem Luftdruck, auch dann ist weniger Sauerstoff in der Luft, was die oxidative Phosphorylierung einschränken kann.

Auch der Zeitpunkt der Migräneattacke kann aufschlussreich sein. So ist eine Migräne, die in den frühen Morgenstunden beginnt, eher mit Hypoglykämie in Verbindung zu bringen als eine Attacke ab mittags.

Oft liegt der Auslöser einer Migräneattacke viele Stunden zurück. Deshalb müssen die Patienten sich erst noch einmal aktiv beobachten und Tagebücher führen, um einen möglichen Zusammenhang klären zu können. Da oft mehrere Triggerfaktoren infrage kommen und in der Regel ein Trigger nicht zu jeder Zeit und verlässlich die Migräne auslöst, kann dies einige Wochen dauern.

Mit einer KD, die gut an die Patienten, ihren Stoffwechsel und ihre Bedürfnisse angepasst ist, manchmal aber auch ›nur‹ mit einer Low-Carb-Diät beziehungsweise einer Ernährung mit niedrigem glykämischem Index (GI) lassen sich verschiedene positive Effekte erzielen. Allerdings treten nicht alle Effekte bei allen Patienten ein und die nötige Umstellungsphase, die der Körper braucht, um sich optimal an die Ketonkörper-Bildung und -Benutzung anzupassen, kann ebenfalls unterschiedlich lang ausfallen. Einige brauchen mehrere Wochen bis Monate für die Umstellung. Positive Effekte stellen sich allerdings in der Regel schon viel früher ein, meist nach etwa zwei Wochen.

Die unterschiedlichen Effekte sind vermutlich auf verschiedene Mechanismen zurückzuführen, die sich in zwei Hauptgruppen unterteilen lassen: Effekte, bedingt durch die Abwesenheit beziehungsweise starke Einschränkung von Kohlenhydraten sowie Effekte, bedingt durch die Anwesenheit von Ketonkörpern. Diese Unterscheidung halte ich deshalb für wichtig, weil die Effekte der ersten Kategorie bereits durch eine Ernährung mit geringem GI beziehungsweise eine Low-Carb-Ernährung erreicht werden können. Bei leicht bis mittelschwer betroffenen Migränikern kann das oft schon ausreichen.

Low-Carb- und Low-GI-Effekte

Erreichbar bereits durch das Weglassen von Einfachzuckern, Auszugsmehlen, Kartoffeln und Reduktion von süßem Obst, Milchprodukten und stärkehaltigen Gemüsen und Hülsenfrüchten:

- Blutzuckerschwankungen und die für Migräniker typische reaktive Hyperinsulinämie mit nachfolgender Unterzuckerung lassen sich allein durch eine Reduktion der stark blutzuckerwirksamen Kohlenhydratträger in der Regel recht gut eindämmen. Wir haben bei unseren Patienten beobach-

ten können, dass besonders nach kohlenhydratreicher Nahrung der Blutzucker zeitversetzt stark absinkt. Das bereitet dem Gehirn großen Stress. Stabile Blutzuckerverläufe haben dagegen einige positive Effekte zur Folge, wie zum Beispiel:

- eine bessere Energiebereitstellung für das Gehirn und andere Organe,
- dadurch reduzieren sich häufig Zittrigkeit, Brainfog (»vernebeltes« Hirn), Heißhunger auf Süßigkeiten, nächtliches Erwachen mit Albträumen, das Nachmittagstief und teilweise auch schon die Migräne am Morgen und am Nachmittag, auch die sportinduzierte Migräne kann damit schon eingeschränkt werden,
- reduzierter oxidativer Stress,
- geringere Ausschüttung von Stresshormonen wie Kortisol und Adrenalin, die benötigt würden, um im Falle einer Hypoglykämie den Blutzucker so rasch wie möglich anzuheben,
- der Insulinspiegel sinkt und der Körper hat die Chance, seine Fettreserven besser mobilisieren zu können, auch das unterstützt eine bessere Energieversorgung des Körpers, denn die meisten Organe (abgesehen vom Gehirn und wenigen anderen Ausnahmen) können auch Fettsäuren und Aminosäuren verstoffwechseln,
- Hormone, insbesondere Östrogen und Progesteron, interagieren mit Insulin, sodass ein niedriger Insulinspiegel sich oft positiv auf die hormonelle Regulation auswirkt. Auch ein höherer Verzehr von gesättigten Fettsäuren kann sich positiv auf die Hormonproduktion auswirken. So kann manchen Patientinnen mit menstruell-bedingter Migräne mit einer Ernährungsumstellung anstelle einer Hormontherapie geholfen werden.

→ Allein der Verzicht auf – insbesondere glutenhaltige – Getreide kann bei manchen Patienten schon zu einer Verbesserung der Migräne führen, vermutlich zum Teil bedingt durch eine Reduktion der Entzündungen im Körper.

→ Milchprodukte, vor allem aus Kuhmilch und besonders jene, die nicht aus Heumilch hergestellt wurden, können für einige Patienten ein Problem darstellen. Neben der Laktose ist das Milcheiweiß Casein für sie problematisch und kann Autoimmunprozesse verstärken. Ein Verzicht auf diese Milchprodukte kann protektiv wirken.

→ Stabile Blutzuckerverläufe wirken sich auch positiv auf die Elektrolyt-Balance und damit auf Ionenkanäle und die Erregbarkeit des Hirns aus. Bei Migränepatienten finden sich häufig Polymorphismen (genetische Variationen), die sich auf die Funktion von Ionenkanälen auswirken und das Migränikergehirn zusätzlich anfällig für einen Zusammenbruch des Membranpotentials der Neuronen machen. Im Englischen ist die Migräne auch als Ionchannelopathie bekannt, als Erkrankung der Ionenkanäle. Intrazellulär wirken sich sowohl zu hohe als auch zu niedrige Glukosespiegel negativ auf das Membranpotential und auf die kortikale Erregbarkeit aus. Für die Aufrechterhaltung des Membranpotentials werden Energie und Rohstoffe benötigt, das heißt ATP und Elektrolyte wie Natrium, Kalium und Chlorid. Schwindet das Potenzial, sei es durch Mangel an ATP oder Elektrolyten, werden die Neuronen übererregbar. Je nach genetischer Prädisposition kann das in einer Migräneattacke oder in einem epileptischen Anfall enden.

Ketonkörper-Effekte

→ Ketonkörper, egal ob von außen zugeführt oder im Körper produziert, bieten insbesondere dem Gehirn eine alternative und stabilere Energieversorgung. Das hat diverse potentiell positive Effekte bei beziehungsweise gegen Migräne. Ketone helfen, Probleme mit dem Glukosetransport, mit Pyruvatdehydrogenase-Defiziten und am Komplex I der Atmungskette (siehe Seite 25) zu umgehen. Auch der geringere oxidative Stress und ihre antioxidative Wirkung können sich positiv auswirken.

- → Dass Ketonkörper die Bildung neuer Mitochondrien fördern, für mehr GLUT1- und MCT-Transporter sorgen (bei länger anhaltendem niedrigem Blutzuckerspiegel und/oder erhöhtem Ketonkörperspiegel werden auch mehr GLUT1-Transporter in die Zellmembranen eingebaut), sind weitere Vorteile.
- → Ketone senken die kortikale Erregbarkeit, indem sie zum Beispiel das Gleichgewicht zwischen Glutamat und GABA in Richtung GABA verschieben, den Glutamattransport bremsen und dafür sorgen, dass mehr Kalium-Kanäle entstehen.
- → Ketonkörper reduzieren die Geschwindigkeit einer Depolarisierungswelle (Cortical Spreading Depression), die sich über die Großhirnrinde ausbereitet und die mit dem Auftreten einer Aura sowie mit Entzündungen in Verbindung gebracht wird.[67]

Welche Probleme erleben Sie bei der Einführung einer ketogenen Ernährung oder auf längere Sicht? Unter welchen Umständen würden Sie zum Abbruch raten?

Migräniker sind aufgrund ihrer genetisch bedingten Prädisposition zur Übererregbarkeit ihres Gehirns sehr empfindlich gegenüber Elektrolytmangel oder -imbalancen. Wenn sich bei der Ernährungsumstellung zu Low Carb oder einer ketogenen Diät die Glykogenspeicher leeren, geht dies mit einer verstärkten Ausscheidung von Mineralstoffen einher. Dies kann zu Beschwerden führen (siehe Seite 122), die sich durch erhöhte Salzzufuhr und die Substitution weiterer Elektrolyte verhindern lassen. Neben Natrium und Chlorid (= Salz) ist insbesondere Magnesium wichtig. Auch ausreichend Kalium sollte vorhanden sein und bei Bedarf ergänzt werden. Zur Statusbestimmung bitte ausschließlich Analysen aus Vollblut verwenden, da Serumanalysen nicht sehr aussagekräftig sind.

Ich empfehle gerne Himalaya-Salz, das, im Gegensatz zu Meersalz, kein Mikroplastik enthält, dafür jedoch Natrium und Chlorid und weitere Mineralstoffe. Ein weiterer Vorteil des Himalaya-Salzes ist, dass das Verhältnis von Natrium zu Chlorid etwa 2 zu 3 beträgt. Der höhere Chloridanteil stabilisiert das Membranpotential und mindert so die Übererregbarkeit des Gehirns. In Ruhe sind die beiden Ionen vor allem außerhalb der Zellen anzutreffen. Mit steigender Natrium-Konzentration sinkt das Membranpotential, weil der Extrazellulärraum dann positiver wird. Mit Zunahme der negativ geladenen Chloridionen steigt das Membranpotential und damit unser »Migränedamm«.

Insbesondere bei einer schnellen Änderung der Ernährung kann es vorkommen, dass dem Gehirn während der Umstellungsphase weder ausreichend Glukose noch genug Ketonkörper zur Verfügung stehen. Dies kann zu schlimmer, lang anhaltender Migräne führen. Durch exogene Ketonkörper, insbesondere Keton-Salze (siehe Seite 44), können diese Symptome zumindest teilweise umgangen werden.

Bei starker Nebenniereninsuffizienz, die im Endstadium einen Kortisolmangel zur Folge hat, kann eine ketogene Diät oder auch eine Low-Carb-Diät kontraindiziert sein, weil Kortisol essentiell für die Glukoneogenese ist. Um eine Unterzuckerung zu vermeiden, kann der Körper unter diesen Umständen gezwungen sein, permanent Adrenalin zu bilden. Das stresst ihn nicht nur enorm, es erhöht auch die Migräneneigung. Ist eine solche Problematik bekannt und geht es dem Patienten bei erhöhten BHB-Werten schlecht (zittrig, Hunger, Migräne, gereizt etc.), ist eine ketogene Diät wenig hilfreich. Eine Ernährung mit komplexen Kohlenhydraten beziehungsweise mit einem niedrigen GI sind hier die bessere Wahl.

Eine ketogene Diät ist nicht für jeden geeignet. Wenn sie nicht die gewünschten Ergebnisse erbringt, würde ich zunächst eventuelle Nahrungsmittelunverträglichkeiten und typische Fehlerquellen (siehe Seite 123) ausschließen. Dazu gehört auch der richtige Umgang mit Alkohol. Generell sollten Migräniker alkoholische Getränke nur maßvoll und idealerweise zu einer Mahlzeit genießen beziehungsweise

immer etwas dazu essen. Die Leber kann nicht gut gleichzeitig Alkohol entgiften, Glukoneogenese betreiben und Ketone zur Verfügung stellen. Das erhöht den oxidativen Stress und führt unweigerlich zu Energiemangel, der wiederum Migräne auslösen kann. Am Anfang einer Ernährungsumstellung ist es aus meiner Erfahrung ratsam, ganz auf Alkoholisches zu verzichten. Später kann man dann herausfinden, wo die individuelle Toleranzgrenze liegt. Vorsicht, viele alkoholische Getränke enthalten Zucker oder andere Kohlenhydrate.

Manche Patienten kommen nicht mit der Aussicht zurecht, ihr ganzes Leben lang auf viel Obst oder ein Stück Brot zu verzichten. In diesem Fall hilft es, Etappenziele zu setzen, zum Beispiel: »Wir probieren es jetzt einmal für vier Wochen aus und wenn es bis dahin keine Besserung bringt, brechen wir ab.« Auch die Aussicht auf eine zyklische ketogene Diät kann unterstützend wirken und sie hilft zusätzlich, die metabolische Flexibilität wiederherzustellen und zu erhalten.

Zum Abbruch würde ich unter folgenden Umständen raten:

- wen trotz der oben genannten Maßnahmen eine anhaltende Gewichtszunahme bis ins Übergewicht eintritt,
- wenn die Cholesterinwerte, insbesondere das small-dense LDL, stark ansteigen,wenn das hochsensitive CRP und/ oder die Triglyzeride ansteigen,
- wenn ein Patient ständig hungrig ist, auch nach sechswöchiger Adaptationsphase, oder wenn das Befinden des Patienten anhaltend schlechter wird,
- wenn sich Leber- und Nierenwerte verschlechtern,
- wenn eine Nebenniereninsuffizienz im letzten oder vorletzten Stadium vorliegt,
- und natürlich, wenn die Migränesituation schlechter wird oder gleich schlecht bleibt.

Was ist aus Ihrer Sicht entscheidend für den Erfolg?

Die Patienten müssen die Ernährungsweise verstehen und auch ein gewisses Grundverständnis über Lebensmittel haben. Nur wer weiß, was Kohlenhydrate, Proteine und Fette sind, auch gesättigte und ungesättigte Fettsäuren, und worin sie enthalten sind, kann sie gezielt meiden oder konsumieren. Da viele Fertigprodukte wegfallen, hilft eine Grundkenntnis beim Kochen, damit die ketogene Diät nicht an fehlender Vielfalt scheitert.

Je nach Typ empfehlen sich zwei Herangehensweisen, die den Start in die Ketose erleichtern und – je nach Charakter – erfolgsentscheidend sein können:

- Direkt ins kalte Wasser springen, also eine komplette Ernährungsumstellung von heute auf morgen. Diese Variante, auch als cold turkey bekannt, ist kurz, aber oft auch schmerzhaft. Denn bei Migränikern führt sie fast immer zunächst zu einer rapiden Zustandsverschlechterung und einigen Tagen mit schlimmen Attacken. Aber dafür sind die Verbesserungen auch schneller zu spüren.

- Einigen Patienten hilft es, das Ganze eher langsam und graduell anzugehen. Sie verzichten zunächst auf Zucker und einfache Kohlenhydrate und nähern sich der Ketose dann Stück für Stück. Diese Variante hilft dem Körper, den Kohlenhydrat-Entzug besser zu verkraften. Dafür dauert es allerdings länger, bis man am Ziel ist. Diese Methode hat aber wiederum den Vorteil, dass man erkennt, ob gegebenenfalls eine Low-Carb-Ernährung schon ausreichend Besserung bringt.

Unter den Süßstoffen steht besonders Aspartam im Verruf, Migräne auslösen zu können. Am besten ist es aus meiner Sicht, Süßstoffe nach einiger Zeit komplett wegzulassen. Der Süßhunger verschwindet damit auf längere Sicht eher und der Körper verlangt nicht mehr ständig nach etwas Süßem. Das Süßempfinden verändert sich und schnell wird einem vieles dann sogar zu süß.

Auch ein gewisses Fehlermanagement gehört zum Durchhalten: Wer mal vom Wagen gefallen ist, sollte sich einfach den Dreck abklopfen und wieder aufspringen! Eine große Hürde, der ich auch oft zum Opfer gefallen bin, ist das radikale Denken: alles oder nichts.

Ganz wichtig für das Durchhalten, besonders der schwierigen ersten Wochen, ist auch der soziale Support. Wer sich alleine fühlt und keine Unterstützung im Familien- und Freundeskreis findet, hat weniger Chancen, diesen Lifestyle lange durchzuhalten. Deshalb am besten gleich von Anfang an Familie und Freunde mit an Bord holen, erklären, warum Ketose so wichtig ist und wie sie gegen die Migräne, diesen kaum aushaltbaren Alptraum, helfen kann. Wer in seinem direkten Umfeld auf Unverständnis stößt, findet im Internet viele Supportgruppen. Auch auf Facebook gibt es Selbsthilfegruppen, die mentale Unterstützung und viele praktische Tipps und Tricks parat haben.

Wo sehen Sie Forschungsbedarf?

Die Mechanismen der ketogenen Diät bei verschiedenen Krankheitsbildern sind erst zu einem relativ kleinen Teil untersucht, auch wenn es mittlerweile fast täglich neue, spannende Studienergebnisse gibt. Ich würde mich besonders über neue Erkenntnisse freuen, die zeigen, welche weiteren Krankheiten und Krankheitsverläufe durch eine Ketose positiv beeinflusst werden können. Und natürlich über die pathophysiologischen Mechanismen dahinter. Auch über die Konditionen, in denen eine ketogene Diät kontraindiziert ist, wissen wir noch zu wenig.

Die Wirkungen exogener ketogener Substanzen sind beim Menschen noch fast gar nicht untersucht. Hier würde mich vor allen Dingen der Vergleich zur ketogenen Diät interessieren. Solche Studien würden es erlauben, die Mechanismen der Abwesenheit oder starken Einschränkung von Kohlenhydraten von jenen der Anwesenheit von Ketonkörpern besser abgrenzen zu können. Die bisherigen Studien zur ketogenen Diät erlauben diese Trennung leider nicht.

Ernähren Sie sich selbst derzeit ketogen?

Ich habe fünf Jahre lang Low Carb oder Low GI gegessen, dann zweieinhalb Jahre lang ziemlich streng ketogen, die letzten sechs Monate davon zusätzlich paläo, also auch ohne Milchprodukte. Auch exogene Ketonkörper habe ich probiert, über ein paar Monate regelmäßig oder episodisch, etwa vor der Periode, während der meine Ketonkörperspiegel aufgrund der hormonellen Veränderungen immer stark absinken. Immer mal wieder habe ich vorsichtig versucht, mehr Kohlenhydrate oder Eiweiß zu essen, hauptsächlich in Form von Obst, Gemüse, Nüssen oder Fleisch. Allerdings merkte ich schnell, dass sich die Migränefrequenz dann wieder erhöht.

Derzeit bin ich in einem neuen Selbstversuch und esse nur noch Low GI. Zu meinem Erstaunen ist die Migräne dadurch nicht zurückgekommen. Von pädiatrischen Epilepsiepatienten ist bekannt, dass viele nach zwei bis fünf Jahren strenger Ketose ›geheilt‹ sind und ›normal‹ essen können, ohne dass sich die Anfallshäufigkeit wieder erhöht. Kinderhirne sind noch sehr plastisch, bei Erwachsenen gibt es meines Wissens hierzu noch keine Daten. Allerdings deuten die Daten aus Tierexperimenten darauf hin, dass einige der durch längere Ketose induzierten Veränderungen der Genexpression die Ketose selbst über-

dauern. Vielleicht bin ich ein Beispiel dafür, dass auch der Stoffwechsel eines erwachsenen Menschen flexibler sein kann, als wir denken.

Künftig möchte ich mich einige Wochen oder Monate im Jahr ketogen ernähren und auch weiterhin moderat das intermittierende Fasten praktizieren, damit mein Körper sich die Fähigkeit der Fettverbrennung und Ketonkörperproduktion und -verwendung erhält. Eine zyklische Ketose halte ich für den evolutionsbedingt wahrscheinlichsten metabolischen Zustand meiner (westeuropäischen) Vorfahren. Es ist auch für mich persönlich der Weg des geringsten Widerstands, denn gerne genieße ich im Sommer frisches buntes Obst und Gemüse, um dann im Winter wieder auf ketogene Nahrung umzusteigen.

Können Sie uns einen konkreten Fall schildern?

Vielen Patienten geht es mit der Migräne ähnlich wie mir: Mit einer richtig praktizierten ketogenen Diät kann die Häufigkeit der Anfälle stark reduziert werden. Doch auch exogene Ketone sind eine ergänzende Option, an deren Erforschung ich mit beteiligt bin. Noch haben wir hierzu nur vorläufige Daten, doch einige davon kann ich gerne vorstellen.

Mit einer kleinen unverblindeten Machbarkeitsstudie wollten wir zum einen die Pharmakokinetik, also die Aufnahme, Verteilung und den Abbau einer Dosis von 10 Gramm eines BHB-Mineralsalzes** bestimmen. Außerdem untersuchten wir den Effekt einer vierwöchigen Einnahme von täglich 20 Gramm des Keton-Salzes im Vergleich zu einer achtwöchigen Periode ohne Einnahme. Dazu rekrutierten wir therapieresistente Migränepatienten, vier Frauen und einen Mann, die zwischen 25 und 61 Jahre alt waren und durchschnittlich an 6 bis 24 Tagen im Monat an Migräne litten. Die Probanden lösten jeweils morgens und abends 10 Gramm des Keton-Salzes in Wasser auf und tranken es. Einmal pro Woche wurden ihre BHB- und Glukosewerte im Blut gemessen, jeweils in nüchternem Zustand sowie während der Intervention 30 und 60 Minuten nach Einnahme des aufgelösten Pulvers. Die Patienten führten während der drei Monate dauernden Studie ein Migränetagebuch.

Die 10 Gramm BHB-Salz führten zu einer schnellen Steigerung der Blutketonwerte von 0,16 auf 0,62 mmol/l innerhalb einer Stunde. Die Nebenwirkungen waren hauptsächlich gastrointestinaler Natur, wie leichte Durchfälle oder Übelkeit, welche eine Patientin dazu veranlassten, die Studie abzubrechen. Nach einer Eingewöhnungsphase und mit der Einnahme nach dem Essen verschwanden die Nebenwirkungen bei den anderen Teilnehmern fast vollständig. In der dritten Einnahmewoche halbierten sich die maximalen Blutketonspiegel, ohne dass die Dosis verändert wurde. Wie kann das sein? Ich vermute, dass es ein Zeichen der Ketoadaptation sein könnte: Die Ketonkörper werden von nun an intensiver verwertet. Diese Annahme wird dadurch gestützt, dass einige Patienten berichteten, erst nach 10 bis 14 Tagen einen positiven Effekt der BHB-Einnahme zu bemerken.

Während der vierwöchigen Einnahme des BHB-Salzes reduzierten sich die Migränetage im Vergleich zur Phase ohne Supplementierung um die Hälfte. Ein Teilnehmer berichtete, er könnte sich gar nicht mehr daran erinnern, wann er das letzte Mal zehn Tage am Stück keine Migräne hatte. Das klingt zwar sehr gut, ich muss allerdings darauf hinweisen, dass es sich hierbei um vorläufige Daten handelt, die mit großer Vorsicht zu betrachten sind. Nicht nur, dass die Stichprobe klein und heterogen und die Intervention sehr kurz ist, bei Migräne ist gewöhnlich auch der Placebo-Effekt sehr groß: Im Schnitt beträgt er 30 Prozent, teilweise auch bis zu 50 Prozent. Zudem wurde ein Razemat, also ein Gemisch aus D- und L-BHB (siehe Seite 45) verwendet. Die Daten von zwei unserer Patienten deuten an, dass die D-Form des BHB nicht nur verträglicher ist, sondern dass seine größere ketogene Potenz auch ungefähr proportional zur Wirkung gegen Migräne sein könnte.

** D- und L-BHB gemischt = Razemat

3.4 Need for new review of article on ketogenic dietary regimes for cancer patients

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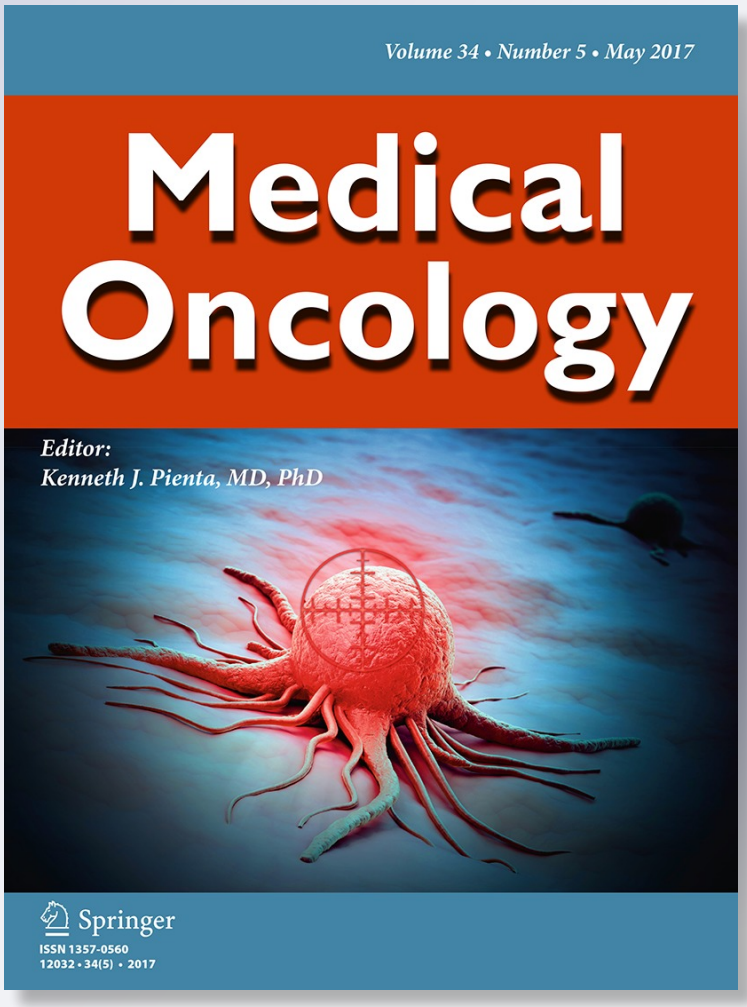

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LETTER TO THE EDITOR

Need for new review of article on ketogenic dietary regimes for cancer patients

Rainer J. Klement[1] · Richard D. Feinman[2] · Elena C. Gross[3] · Colin E. Champ[4] · Dominic P. D'Agostino[5] · Eugene J. Fine[2,6] · Ulrike Kämmerer[7] · Angela Poff[5] · Jong M. Rho[8] · Thomas N. Seyfried[9] · Adrienne C. Scheck[10]

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To the editor,

In a recent article entitled "Systematic review: isocaloric ketogenic dietary regimes for cancer patients" [1], Erickson et al. provide their summary of the use of ketogenic diets for treating cancer patients. As active researchers in this field, we find the Erickson paper to be inaccurate in its characterization of ketogenic diets. The writing is highly biased and contains a significant number of errors, some of an elementary nature. The overall tenor of the paper, rather than that of a balanced review, gives the impression of established experts warning patients about the risks of a new method. In fact, Dr. Erickson and co-authors are not acknowledged experts and, as far as we know, have no record of clinical or research experience in this area. We detail below the paper's faults and suggest the positive side of the issue which we feel remains ignored. We think that the pertinent subject matter was not considered in the manuscript. In essence, failure to address these questions means that the paper could not have received adequate peer review. We suggest that some form of re-review of the paper be instituted. As it stands, Erickson et al. are likely to be misleading to patients and practitioners alike.

Erickson et al. are correct in describing the limited number of studies and the somewhat preliminary nature of work in the field. They nonetheless evaluated these papers as if they were part of an established, well-defined discipline and have largely tried to find fault. Their analysis has ignored the real promise and logical rationale behind the undertaking [2–4]. Equally important is the unstated implication that there exist effective, reliable cancer and epilepsy therapies, dietary or otherwise, with acceptable impact on quality of life. We do not think that this is the case. A balanced review would sensibly focus on the potential of ketogenic diets to go beyond the limitations on efficacy and adverse effects of current standards of treatment.

Our specific objections:

1. The methodology was based on rote library work with no analysis beyond the size and scope of each study and without recognizing differing foci. We find this a reflection of the long-standing bias against diets based on carbohydrate restriction of which KDs are an extension. Erickson et al. introduce the KD as a "cancer diet" equating it with non-scientific diets such as the Breuss cure. The metabolic anti-tumor effects of KDs are supported by significant preclinical data and preliminary clinical results. The KD should be characterized as a potential metabolic therapy and not simply a diet [2–5].

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2. Erickson et al. state that "ketogenic diets for cancer patients are implemented with the aim to reduce the energy production of cancer cells, thus decreasing tumor proliferation" (page 2). While this is one rationale employed by some of the included studies, several other mechanisms have been displayed in these studies, such as reducing inflammation, enhancing the efficacy of standard therapies, or accounting for the altered substrate utilization of cancer patients. Erickson et al. should know this, e.g., from reading their own reference No. 2 which focuses specifically on increased reactive oxygen species production and weakening of the anti-oxidative capacity of tumor cells [2]. This narrow view understates the promise of KDs which rests with the effectiveness of the method in other disease states, particularly diabetes and epilepsy, and depends, as well, on the associated anabolic role of insulin and IGFs [3, 6]. And, again, beyond the promise of improving the efficacy of current methods, a metabolic approach can avoid or reduce the adverse effects and, in many cases, debilitating declines in quality of life, that characterize chemo- and radiotherapy alone.
3. In the results section, Erickson et al. criticize the included studies for not having a "methodological rigorous design." This follows from their assumption that all therapeutic methods are the same if they come up in a search on their key words. This appears somewhat disingenuous in that, as above, they have pointed out that this is a new approach with a relatively small number of studies where researchers are trying to find out what the key parameters are. As in many negative reviews on low-carbohydrate therapies that suggest "more work needs to be done," the negative tone ensures that it will be difficult for that work to ever be done.
4. On body mass, Erickson et al. cite weight loss as a negative effect. An example is the mean weight loss of 1.5 kg after 2–3 days on the KD in the study by Tan-Shalaby et al. [7] (not "Tan and Shalaby" as written in the article—there are several such minor typos). KDs frequently show an initial weight loss in the form of water bound to glycogen. The longer-term weight loss of 7.5 ± 5.8 kg is mentioned, but the authors do not state that all the patients in this study were overweight in the beginning (mean BMI 29.46 ± 5 kg/m^2). Reporting study results without considering the context indicates authors' bias. In fact, there is evidence that KDs may counteract weight loss in the context of cachexia as was shown in the clinical trial by Fearon et al. [8] and mechanistically investigated in preclinical work [9].
5. The Discussion section warns against the application of KDs for cancer patients but provides no support from the extracted studies. The authors cite putative side effects, most of which derive from epilepsy studies in children. A strict KD in children with epilepsy cannot be generalized. None of us has personally experienced side effects such as hypoglycemia or metabolic acidosis during our studies or care of cancer patients. Table 3 "Reported adverse effects of KD" would be substantially shorter if restricted to events actually observed and reasonably attributed to KD in the cancer trials. Any real or potential side effects which derive mainly from the pediatric population are minor compared to the side effects of standard cancer treatments and are readily prevented and/or managed by a trained dietician. Finally, a great deal has been learned about the KD and potential side effects in cancer patients in recent years, which is not reflected in older papers on the subject.
6. Erickson lists a highly objectionable collection of parochial "concerns" that are conjectural, have never materialized but have continued to dog serious research in low-carbohydrate strategies:
    1. "it is important to know that all forms of the KD are considered nutritionally inadequate." (page 5) This is without substance. No experimental evidence is supplied.
    2. "…the long-term application of the KD has been correlated with calcium deficits…" This is not true. If there are particular cases, the evidence is not presented.
    3. "…can exacerbate bone loss," is particularly objectionable. It is not true, has never been seen and has been raised and answered innumerable times.
    4. "…and the metabolic state of acidosis…" is the most serious lapse. This is a constant feature of criticism found mostly in the popular media, and the statement is an elementary error in biochemistry. KDs do not cause acidosis. Blood pH is regulated. Ketoacidosis occurs only in untreated type I diabetes or in other states of absolute or relative insulin deficiency.
    5. The paper cites failure to conform to official dietary guidelines as fault, but ketogenic dietary approaches explicitly seek to avoid and to improve on the guidelines. In any case, at least in the USA, the guidelines have been strongly criticized and the increase in diabetes and obesity concomitant with the institution of the guidelines is commonly cited as evidence of their stature and effectiveness.

7. It is important to point out that researchers and clinicians in this field—again, in distinction to popular diets—have been circumspect about the current state of knowledge and have emphasized that KDs may be valuable as adjuvant to other modalities [2, 10, 11], and therefore, one must be careful in attributing adverse effects to the KD alone. Along these lines, Erickson et al. suggest that Klement and Sweeney [11] (not Sweeny as written) would have underrated the side effects of the KD in their study on six cancer patients. However, the two patients who experienced nausea and the one patient with diarrhea underwent radio-chemo therapy, a more likely cause of these side effects. Furthermore, no mention was made that all six patients reported subjective well-being and improvements in quality of life during the KD.
8. Erickson et al. conclude that "evidence on benefits regarding tumor development and progression as well as reduction in side effects of cancer therapy is missing." Extraordinary responses of some cancer patients to the KD are reported [7, 12, 13] so that its efficacy must be considered a likely hypothesis in these cases.

An important trend in the medical literature is concerns about the actual quality and reliability of that literature [14–16]. We think Erickson et al. represent an example of the problem and an obvious cause: highly biased analysis without adequate editorial and reviewer oversight. The critiques of the literature recommend, as one potential remedy, post-publication review or, more generally, a more flexible method of publication where initial publication is more tentative. We think this paper should be re-reviewed and editors should obtain input from workers in KDs. The paper should be republished after all opinions—particularly from those authors whose work is cited—are examined. A novel format or mechanism for effecting this change in published papers may be required, but it seems necessary and the journal would receive approbation for bringing it about.

In summary, Erickson et al. represent an almost totally negative analysis of the ketogenic approach to therapy, and, while no particular credentials are required to summarize the literature, such a negative judgment is inappropriate given the authors' lack of direct experience with KD research, and lack of any communication with workers in the field. The net effect is to encourage patients to avoid a potential therapy that has significant promise and few side effects. The authors declare "This article does not contain any studies with human participants or animals performed by any of the authors and therefore did not require ethical approval." We think that this lack of experience is precisely why there is an ethical question.

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Compliance with ethical standards

Conflict of interest All other authors declare that they have no conflicts of interest.

Ethical approval This article does not contain original data from human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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3.5 Preliminary data on exogenous ketone bodies in migraine prevention

Abstract: '**Gross, E.C.,** Sandor, P. & Fischer, D. (2017). Preliminary data on exogenous ketone bodies in migraine prevention' at *International Headache Conference, Vancouver 2017 & Metabolic Health Summit, Los Angeles 2019*

Preliminary Data on Exogenous Ketone Bodies in Migraine Prevention

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Background and Objective

Currently available prophylactic migraine treatment options are limited and are associated with many – often intolerable - side-effects. Various lines of research suggest that abnormalities in energy metabolism are likely to be part of migraine pathophysiology. Previously, fasting or a ketogenic diet (KD) have been reported to lead to a drastic reduction in migraine frequency. An alternative method to a strict KD is inducing a mild nutritional ketosis (0.4-1 mmol/l) with exogenous ketogenic substances. The aim of this open label pilot study was to 1) assess the pharmacokinetics of a one-time dose of 10g beta-hydroxybutyrate (βHB) – one of the three physiological ketone bodies - in mineral salt form and 2) the effect of a one month supplementation with daily 20g βHB on migraine days compared to a one month baseline period.

Methods

Five treatment refractory patients (age range: 25-61 years, 1 male, attack frequency range: 6-24 migraine days/months) received 20g/day of sodium and calcium βHB (n=5) in two oral doses for the duration of 4 weeks. Blood βHB and glucose concentrations were assessed using an Abbot Freestyle Neo blood ketone and glucose meter once a week in a fasted state at 3 different time points: before βHB (baseline) and 30 mins and 60 mins after ingestion.

Results

10g βHB (n=5) lead to a quick elevation in blood βHB levels (peak 0.62mmol/l after 1 hour, SEM=0.08). No serious side-effects were reported. Adverse events observed included diarrhoea, nausea or gastrointestinal upset. These led to one drop-out. During the one month of intervention with 20g of βHB per day, an average reduction of 51% in migraine days compared to baseline could be observed (mean baseline = 16.25 days, SEM= 3.71; mean after βHB= 8 days, SEM= 2.92). This perceived benefit from βHB seemed to coincide with a drop in average peak βHB blood levels from 0.62 mmol/l to 0.3 mmol/l after 1-2 weeks of ingestion.

Conclusion

The drop in average peak βHB blood levels after 1-2 weeks of ingestion is likely to be a consequence of adaptation, enabling a quicker uptake and usage of βHB. While not much conclusion can be drawn from such few and heterogeneous case series patients, these preliminary results might warrant the conduction of a randomised, placebo-controlled, double-blind efficacy and safety trial to assess the potential of exogenous ketogenic substances in migraine prevention, which is currently undertaken at the University Hospital of Basel, Switzerland.

4. Discussion

4.1 Metabolism / mitochondrial functioning in migraine

4.1.1 Aim 1: Highlight the metabolic abnormalities in migraine.

The *Nature Neurology* review discussed the evidence for a variety of metabolic abnormalities in migraine obtained from clinical, biochemical, genetic, therapeutic and pathophysiological data. Experimental data was discussed to elaborate on potential mechanisms by which such metabolic abnormalities can generate migraine attacks.

Briefly, it was shown that:

- Prevalent migraine attack triggers have unbalanced cerebral energy metabolism and/or oxidative stress as common denominators.
- Magnetic resonance spectroscopy (MRS) studies show decreased mitochondrial phosphorylation potential and ATP in the brain of migraineurs between attacks. Glucose (and lipid) metabolism and mitochondrial functions are abnormal in the peripheral blood.
- Migraine patients have increased prevalence of various single nucleotide polymorphisms in non-coding and in nuclear-encoded mitochondrial DNA. Common migraine GWAS variants are functionally involved in mitochondrial metabolism.
- Metabolic enhancers like riboflavin or co-enzyme Q10, and dietary or pharmacological ketogenesis reduce migraine attack frequency, but novel more efficient metabolic strategies are warranted.
- Experimental studies provide a possible link between cerebral energy disequilibrium and migraine attack generation including cortical spreading depression and trigemino-vascular system activation via pannexin-1 and/or TRP/acid sensing ion channels (ASICs).
- CGRP, a major actor in the migraine headache, could also be part of an antioxidant response and metabolic changes, which might help restore energy homeostasis.
- Migraine can be regarded as a conserved (mal)adaptive response pattern that occurs in genetically predisposed individuals with a mismatch between the brain's energy reserve and workload.
- Finally, potential treatments targeting cerebral metabolism, such as antioxidants, nutraceuticals, pharmacological or dietary ketogenesis might represent promising treatment avenues, that can be individualised.

In summary, a large number of clinical, biochemical, genetic and therapeutic studies in migraine point towards a variety of different metabolic abnormalities, all related to energy homeostasis. It is likely that the interaction between the environment and a combination of different metabolic-endocrinological abnormalities, possibly together with an abnormal cerebral responsivity, determines the migraine attack threshold and hence diseases severity in a given patient.

To the best of my knowledge, this is the first review paper that mechanistically links cerebral energy deficiency and / or increased oxidative stress to migraine headache and associated symptoms, via a decrease in CSD threshold as well as TRP and ASIC channels activation, which stimulate CGRP and PACAP release. In addition, we are the first to hypothesize that they induce in parallel an antioxidant response and a variety of metabolic changes, which together with energy-conserving behavioural changes, decrease oxidative stress levels and increase glucose and ketone body availability for the brain to help restore energy homeostasis.

While this assumption has to be verified experimentally, the findings described in the *Nature Neurology* review could have several potential therapeutic implications. Should CGRP play a part in oxidative stress reduction, the consequences of blocking its action long-term on the endogenous anti-oxidant system should be carefully monitored.

Taking into account the key findings of the review would like to propose a 4-step approach to improve mitochondrial functioning and energy metabolism in migraine and hence reduce migraine frequency and severity:

1) Individualizing supplementation of micronutrients

2) Reducing oxidative stress and increasing antioxidants

3) Stabilizing blood glucose

4) Providing an alternative energy substrate for the brain

The next section will focus on specific markers of oxidative stress and mitochondrial functioning abnormalities in migraine.

4.1.2 Aim 2: Examine some potential peripheral biomarkers of metabolism and oxidative stress in migraine that have produced mixed results or have not yet been examined.

As discussed in great detail in the *Nature Neurology* Review and briefly in the previous section, increasing evidence points towards a role of mitochondrial functioning, energy metabolism and oxidative stress in migraine. However, when it comes to the specific peripheral markers showing such abnormalities, previous research has produced inconclusive results and several markers have not yet previously been examined. In this original research paper (submitted to Cephalalgia), seven potential peripheral biomarkers of mitochondrial functioning, energy metabolism and oxidative stress have been analysed in the serum of 32 higher frequency migraineurs episodic migraineurs (5-14 migraine days/ months, 13 MO and 19 MA): alpha-lipoic acid (ALA), thiols, total plasma antioxidant capacity (TAC), lipid peroxide (PerOx), oxidised LDL (oxLDL), HbA1c and lactate). To the best of our knowledge ALA has not previously been assessed in migraine patients, despite the fact that it has having been tested as a migraine preventative. Serum lactate has not recently been looked at as well. HbA1c and oxLDL have only been examined in one study. Thiols, TAC and PerOx have previously provided mixed results.

In brief, it was shown that:

- The majority of patients had abnormally low ALA and lactate levels (87.5% and 72%, respectively).
- 46.9% of the patients had abnormally high PerOx values
- For thiols and TAC over one third of patients had abnormally low values (31.2% and 37.5%, respectively).
- 21.9% had abnormally low HbA1c and no patient exceeded the healthy reference range.
- oxLDL was normal in all but one patient.

This study provides further evidence for a role of oxidative stress and altered metabolism in migraine pathophysiology, which might represent a suitable therapeutic target. ALA, being too low in almost 90% of patients, might represent a potential biomarker for migraine; however, further research on ALA levels in other diseases is needed to determine, whether this is a migraine specific finding, or a general marker of oxidative stress involvement in other neurological diseases.

As discussed in the manuscript, the major limitation of this study is the lack of a control group. We cannot determine whether any of these biomarkers might have significantly differed from a control group. It is likely, however, that the low ALA levels found in almost 90% of patients are a finding of clinical significance and the same might be true for low serum lactate. As discussed in the paper lactate can be used by the brain as an energy substrate in addition to or as an alternative to glucose and ketone bodies. When glucose levels are low and ketone bodies are almost non-existent, it would make sense for the brain to utilize lactate instead.

Secondly, the sample size was fairly small, which is particularly problematic for a correlation analysis. No evidence for a correlation between any of the seven mitochondrial function / oxidative stress markers and migraine severity, an effect of migraine prophylaxis or an effect of previous / coming migraine has been found.

Further research is needed to replicate these results in the presence of a control group. To establish the migraine specificity of such findings, studies looking at ALA and lactate levels in other neurological diseases would be justified. In addition, there are various other recently available mitochondrial function / oxidative stress biomarkers that might be interesting to asses in migraine, such as: isoprostane (the result of damaged arachidonic acid by free radical), 8-hydroxy-desoxyguanosine (resulting from oxidative damage to DNA bases), SOD, glutathione and glutathione peroxidase (e.g. measured by Ganzimmun Diagnostic AG, Mainz, Germany). Very recently it has even become possible to directly assess mitochondrial membrane potential (a measure for mitochondrial activity), intracellular ATP levels (before and after stress) and mitochondrial ROS production before and after antioxidant therapy (biovis Diagnostik M V Z GmbH, Limburg, Germany). Furthermore, the important co-factors for SOD and glutathione peroxidase zinc, copper, manganese and selenium might be worth examining. Future research looking at these markers at different time points during the migraine cycle and in different migraine types would be interesting and would assist individualised treatment. It would be of further interest to correlate treatment response to a metabolic treatment with improvement in such markers or even with genetic analysis of key anti-oxidant genes, such as SOD2, CAT, Glutathione-S transferase (GST)-M1, GST-P1, GST-T1 amongst others.

Taking into account the aforementioned metabolic abnormalities and the findings of our mitochondrial function biomarker study, the next section of the PhD thesis focuses on a potential new migraine preventative treatment and its potential mechanism.

4.2 Ketone bodies in migraine prevention

4.2.1 Aim 3: Review the potential therapeutic mechanisms of ketosis in migraine.

Migraine is a very heterogeneous disease; a multitude of fairly common genetic polymorphisms and pathophysiological mechanisms contribute to the migraine phenotype. In complex diseases such as migraine a therapy that can target multiple possible pathogenic pathways seems advantageous. Ketone bodies seem to be able to do exactly that. In the *Nutrients* review we have highlighted 8 different potentially migraine relevant therapeutic mechanisms of ketosis:

1) Hypoglycaemia / hypometabolism

2) Glucose transport

3.) Mitochondrial functioning

4.) Oxidative stress

5.) Cerebral Excitability

6.) CSD

7.) Inflammation

8.) the microbiome

KBs are an alternative fuel source for the brain and are hence likely able to circumvent some of the abnormalities in glucose metabolism and transport found in migraine. Its metabolic action is relevant for the first two potential mechanisms. Additionally, recent research has shown that KBs – D-βHB in particular – are more than metabolites [235]. As signalling molecules, they have the potential to positively influence other pathways commonly believed to be part of migraine pathophysiology: mitochondrial functioning, oxidative stress, cerebral excitability, inflammation and the gut microbiome (relevant for mechanisms 3-8).

Nevertheless, as discussed in the review, several uncertainties remain. A lot of the mechanistic effects of ketosis and / or presence of βHB have been examined in animals and more clinical research is needed to validate those effects. Such future clinical research could additionally help determine, whether and to what extent all the aforementioned potentially disease-modifying effects of ketosis are actually also relevant in migraine patients. Most importantly, it remains to be determined whether the absence / restriction of dietary carbohydrates, the presence of KBs, both or third factors are of primary importance for the potentially migraine protective effects of the KD that has previously been demonstrated.

Nevertheless, an elevation of KBs, D- βHB in particular, which has been shown to potentially influence all of the aforementioned migraine pathophysiological mechanisms, might offer a long-needed relatively side-effect free remedy for at least a proportion of migraine sufferers. The potential preventative anti-migraine effect of the supplementation with βHB without a strict dietary change is currently being examined in our randomised placebo-controlled efficacy and safety cross-over trial [236], which aims to answer some of these questions and is reviewed in the last chapter of the discussion. In order to find a suitable ketogenic IMP, we examined the pharmacokinetics of different ketogenic supplements and their relative strength and weaknesses. We also collected preliminary data on their concerning effect on migraine frequency. This will be discussed in the next section.

4.2.2 Aim 4: Examine the pharmacokinetics of various ketogenic supplements and the potential efficacy of exogenous ketone body substances in migraine.

As outlined in the introduction, currently available prophylactic migraine treatment options are limited and are associated with many – often intolerable - side-effects. The previous sections have discussed the role of energy metabolism in migraine pathophysiology and how ketosis might be beneficial. A KD has been shown to lead to a drastic reduction in migraine frequency (about 80% after one months of intervention [146]). An alternative method for getting patients into ketosis is to induce a mild nutritional ketosis (0.4-2 mmol/l) with exogenous ketogenic substances. In order to find the best ketogenic IMP at the time (i.e. 2015) we have examined the effect of various ketogenic substances, such as L-Leucine (LL), L-Lysine (LY), racemic and D-βHB on blood KB levels (pharmacokinetics), tolerability and migraine attack frequency. MCTs were not used due to known problems with tolerability at the high doses that are needed to reach BHB levels above 0.4mmol/l. Due to their very foul taste and their impact on liver redox state, we have also not looked at ketone esters.

As shown in the figures of the patent application, LL, but not LY lead to a very small increase (up to 0.35 mmol/l with 13 g of LL) in blood βHB over approximately 4 hours. However, the ketogenic amino acids were not well tolerated, it was impossible for the patients to ingest 26 g of amino acids per day and we were weary of potential long-term consequences of such high one-sided amino-acid consumption. The bitter taste further contributed to the problem.

In comparison, βHB mineral salts were generally better tolerated and had a comparatively strong effect on blood KB levels. Racemic βHB salts led to an increase in βHB blood levels of approximately two-fold compared to LL (up to 0.62 mmol/l); however, the half-life was very short, with levels dropping back to baseline after 2 hours. In addition, a substantial drop in blood glucose levels was observed. Tolerability and palatability of the racemic βHB was problematic, in particular gastrointestinal upset and nausea.

Surprisingly, the D-βHB isomer (10g on an empty stomach after an overnight fast) led to a more than threefold elevation in blood βHB levels (up to 1.94 mmol/l) as compared to the racemic version (up to 0.62mmol/l). This could be due to L- βHB somewhat competing with the uptake of D- βHB while only the latter enantiomer is measured. Levels remained elevated for over 4 hours. In addition, there was no concomitant drop in blood glucose, as observed with the racemic mix. Participants reported better taste (less foul), and no gastrointestinal side-effects were observed, even with 2 months consumption.

Exogenous KBs in quantities much lower than those produced by the liver during a KD, fasting or starvation (20 g instead of around 150 g) were found to have a migraine preventive effect. 20 g of the racemic mineral βHB salts daily were found to reduce average migraine day frequency by 51% in 5 higher frequency treatment refractory migraineurs. This reduction ranged from 25-80%. Interestingly, the reported migraine reduction correlated with the time point of reductions in peak βHB levels, suggesting that a keto adaptation also takes place with exogenous KBs. Despite fairly good efficacy gastrointestinal side-effects lead to one drop-out. An increased dose of 40 g racemic βHB lead to a further reduction of 72% in migraine days. Nevertheless, this increased dose exacerbated the side-effects and was intolerable for most patients. Further very preliminary efficacy data suggested that as little as 10 g of D-βHB daily could match the efficacy of 40 g of the racemic mix, with an average of 68.5% reduction in migraine days with 10 g of D-βHB compared to 72% reduction with 40 g racemic βHB in 2 patients.

The major limitation of this preliminary analysis is that due to time and financial constraints the sample sizes for the individual PK and efficacy experiments were very small. PK studies were conducted on 5 participants, while 8 would have been the ideal minimum. The very preliminary efficacy data was based on 10 patients, with groups of n=2-5 depending on substance tested (see patent for details). In addition, there was no control group and it is known that the placebo effect can be quite strong in migraine (20-40 % [237]). In order to overcome these limitations, we conducted MigraKet: a randomised, placebo-controlled, double-blind, crossover, single-centre efficacy and safety trial on beta-hydroxybutyrate (βHB) mineral salts in migraine prevention, which is discussed in the next section.

4.2.3 Aim 5: Plan and conduct an efficacy and safety phase 2 trial on exogenous ketones bodies (beta-hydroxybutyrate mineral salts) in migraine

The main objective of the MigraKet trial was to demonstrate safety and superiority of βHB in mineral salt form over placebo in migraine prevention. To this end, 45 episodic migraineurs (5-14 migraine days/months), with or without aura, aged between 18 and 65 years, were recruited at headache clinics in Switzerland, Germany, and Austria and via internet announces. As discussed in the *Trials* paper a conservative effect size was assumed, and a study population of moderate- to high-frequency episodic

migraineurs was chosen. This was done in order more easily demonstrate a sufficiently large effect size within a short timeframe, without introducing any confounders associated with chronic migraine. In addition, we chose a crossover design, as it greatly improves statistical power and participation rates, without increasing costs.
The primary outcome (mean change from baseline in number of migraine days during the last 4 weeks of intervention compared to placebo) followed the EMA (European Medical Association) and IHS guidelines for migraine prevention trials. The same was true for the secondary outcomes (mean changes in headache days of any severity, acute migraine medication use, migraine intensity and migraine and headache related disability).
In order to find out more about potential mechanisms of action of KBs in migraine prevention and exogenous elevation of KBs in general, we chose to include exploratory outcomes (in addition to routine laboratory analysis): genetic profiling and expression analysis, oxidative and nitrosative stress markers, as well as serum cytokine analysis, blood βHB and glucose analysis (pharmacokinetics) and markers of fat, protein and glucose metabolism. We chose to also examine single nucleotide polymorphisms (SNPs), in order to determine a potential effect of genotype on treatment response. In addition to that, we also plan to conduct gene-expression analysis (with a focus on but not limited to genes coding for mitochondrial related enzymes and anti-oxidant enzymes (such as GSTs, SOD2, N-acetyltransferase 2; NAT2). We will also examine serum concentrations of markers of mitochondrial functioning and energy metabolism, such as HbA1c, insulin, cortisol, lactate and thyroid functioning pre and post treatment.

Optionally, patients also receive an Abbott FreeStyle Libre Blood Glucose Monitoring System for a total of 8 weeks (4 weeks during active intervention and 4 weeks during placebo, respectively) which will allow permanent tissue glucose monitoring without finger pricking. This sub-study (MigraGlu) allows us to examine a potential association between blood glucose levels (hyper- or hypoglycaemia) and migraine attacks and the potential effect of racemic KB salt on glucose levels in the longer term.

To the best of our knowledge, this is the first controlled clinical trial using exogenous KB salts world-wide. If proven effective, βHB might offer a new prophylactic treatment option for higher frequency migraine patients or at least a subgroup thereof. We hope to not only shed light on its potential migraine protective effects and mechanisms of actions, but also establish the safety of supplementary KBs to pave the way for clinical trials assessing their use in related diseases (i.e. in other disorders with a metabolic component). This study will hopefully also help teasing apart the effects of KBs per se versus the absence / restriction of dietary carbohydrates, which is impossible to achieve with a KD intervention. Furthermore, should the racemic BHB be safe and effective, D-BHB should certainly be as well and could be examined in future RCTs in migraine or related diseases.

The major limitation of this trial is that due to limited availability and costs of D- βHB at trial onset, the much less potent racemic BHB was used. It only has about one third of the potency of D-βHB and a short half-life of about 2 hours. We tried to mitigate this constraint somewhat by using 3 daily doses. However, this still means that patients are not in ketosis at all during the night, which could be enough to trigger an energy deficit and subsequent migraines.

In addition, the racemic βHB seems to have much stronger gastrointestinal side-effects. We tried to mask this effect by using mannitol as a placebo, which also has laxative properties at higher doses. Unfortunately, some patients seemed to react rather strongly to it, which meant we had to reduce the doses in this case until the dose was tolerated. As the complete clinical team was blinded, we do not know whether we reduced mostly placebo doses or also some of the IMP.

4.3 Further research and directions:

In terms of future migraine research, it would be very interesting to compare the therapeutic efficacy of a KD to exogenous KBs in an RCT with three treatment arms. The potentially additive effect of exogenous KBs as an adjunct to a KD or a low glycaemic index / low carb diet would also be worth examining. Furthermore, the assessment of the potential migraine aborting effect of high dose KBs in acute migraine treatment would be interesting. Anecdotes already suggest that an acute migraine attack can be mitigated or even completely aborted, if it is caught early enough (before the pain phase has started, unpublished observations and [238]). Moreover, in order to test the assumption that treatment response to the exogenous KBs works via an improvement of brain energetics, assessing brain metabolism using P-MRS before and after treatment with BHB would be of great interest. As an endogenous molecule, BHB has no systemic side-effects and can most likely be administered as an adjunct therapy. However, further research is needed to definitely exclude potential interactions with other migraine preventatives, such as antidepressants, anticonvulsants and beta-blockers.

Recent research suggests that ketosis might also be beneficial in the treatment of the following diseases:

Neurodegenerative diseases

- Alzheimer's Disease [239–243]
- Parkinson's Disease [244,245]
- Multiples Sclerosis [246–249]

Neurological diseases

- Epilepsy [250–255]
- Autism [256–261]
- Depression and affective disorders [262,263]
- Anxiety [264,265]
- Traumatic brain injury [266–269]

Metabolic disorders

- Cancer [270–275]
- Glycogen Storage Disease [276]
- Acyl-CoA Dehydrogenase Deficiency [277,278]
- GLUT1 Deficiency Syndrome [279–281]

Clinical trials on the potential efficacy of exogenous KBs in these diseases either as a stand-alone or adjunct therapy to a more or less strict KD seem warranted. In the case of Alzheimer's, where adherence to a strict diet might be problematic even in a care home setting, the easily implemented addition of a potent KB supplement could have great therapeutic potential [133,239,240,242]. In contrast, in the case of cancer the concomitant reduction of dietary carbohydrates (as well as possibly the addition of a glutamine inhibitor) seems essential; here exogenous KBs could be helpful as an adjunct therapy to decrease the glucose-to-KB index [220,282].

Further research is also needed to determine which exogenous KB formulation might be best suited for targeting a particular disease or condition. For example, as discussed in the previous chapter, due to the glucose lowering effect of the racemic BHB, it might be particularly useful in a disease such as cancer, but possibly not ideal in a disease such as migraine. When it comes to the primary mechanisms of KB

elevation very little research exists in humans and more studies examining its effects on (cerebral) metabolism, oxidative stress, mitochondrial functioning, (cerebral) excitability, gut microbiota, inflammation and gene expression are needed.

4.4 Conclusion

This PhD thesis has highlighted various known metabolic abnormalities in migraine and introduced a potential novel migraine preventative treatment and its potential mechanisms of action: KBs. While more research is needed to confirm mechanisms of action, treatment efficacy and potential subgroups that respond, a therapy that can target multiple possible pathogenic pathways seems to be very advantageous in complex, heterogenous, multigenic diseases such as migraine.

In sum, KBs might offer a badly needed, well tolerated treatment alternative, at least for a subset of patients with a clear involvement of energy metabolism, mitochondrial dysfunction and / or oxidative stress.

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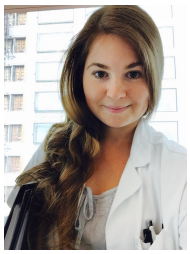
Curriculum Vitae Elena C. Gross



E-mail: elena.gross@oxfordalumni.org **Phone**: +41 76 479 87 62 **Nationality:** German

Address: Sperrstrasse 10, 4057 Basel, Switzerland **Civil status**: unmarried **DOB:** 23.12.1988

Current position

August 2017 -ongoing	CEO /CSO and founder of KetoSwiss AG

Education

Sept 2019- July 2020	Diploma of Entrepreneurship at ***University of Cambridge,*** *Cambridge Judge Business* School
July-Aug 2016	Research Fellowship at the ***University of Harvard*** *Medical School* with Prof Rami Burstein; focus on the role of inflammation in migraine, assessed with genetic and epigenetic analysis amongst others and shadowing at the headache clinic
Feb. 2015- Sept. 2019	PhD in clinical research at ***University of Basel***, Department of Neurology and Neuropediatrics, University Hospital and Children's Hospital Basel
	Clinical and basic research projects on: **The metabolic face of migraine** – Metabolism, mitochondrial (dysfunction) and the potential therapeutic benefit of exogenous ketone bodies in migraine and their potential mechanisms of action
Sept. 2013 – Sept. 2014	Master of Science in Neuroscience at the ***University of Oxford***, Lincoln College
	Final grade: **Distinction**, among top 3 students in year group
	(For the 2 dissertations see MSc lab internship 1 and 2)
September 2009 – July 2012	Bachelor of Science in Psychology at the ***University of York***
	Final grade: **79 (First with Distinction);** rank 1 out of 142 students (= best 0.7%),
	Best average grade since existence of university (5o years)
February 2005 – June 2008	Gymnasium Oberursel, German High School, **Abitur**
	Final grade: **1.0 (A-level equivalent of A*)**, rank 1 out of 145 students (= best 0.7%; Majors: Chemistry and English
August 2005 – Dec. 2005	Stay abroad: Fossil Ridge High School in Fort Worth, Texas, USA, average: **A**
August 1999 – July 2005	Gymnasium Oberursel, German High School, average 10th grade: **A***

Awards, scholarships and grants

Sept. 2017	***Best Poster Prize*** at International Headache Conference, Vancouver, Canada
Aug 2017	**Stipend** to attend the *iHEAD / IHA academy* in Vancouver, Canada, by the International Headache Society (IHS)
March 2017	*Swiss National Science Foundation (SNSF)* **project grant** for PhD project: "**Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A randomised, placebo-controlled, double-blind study" (525'000 CHF)**
Dec 2016	**PhD project stipend** for excellent young scientists from the *Free Academic Society (FAG)* Basel (12'000 CHF)
Nov 2016	**Project award** by the *Clinical Trial Unit, Department of Medicine*, Basel University Hospital (150 hours data - and on-site management)

Oct 2016	Second place at **BioBusiness 2016 start-up pitch**
Oct 2016	**iHEAD 2016 winner** of the young headache researcher debate
Aug 2016	**Stipend** to attend the *iHEAD academy 2016* in London, UK, by the International Headache Society (IHS)
June 2016	**UKBB Research Fellowship (Brian Fowler Fund)** to stay at the laboratory of Prof. Rami Burstein, Professor of Anesthesia and Neuroscience at Harvard Medical School (3'000 CHF)
April 2016	**Scholarship to attend BioBusiness,** a 5 day program on BioEntrepreneurship at the USI, an exclusive learning platform and network where academia, industry and venture capitalists interact fruitfully.
Jan 2016	**antelope@university stipend** aimed at highly qualified female doctoral students to systematically plan and promote their careers and prepare them for future leadership and management assignments in academia
Oct 2015	**Hans Ruedi Isler Prize** of the Schweizer Kopfschmerzgesellschaft (SKG) for the best migraine research project (5'000 CHF)
Sep 2015	**PPHS PhD Top-Up stipend** for highly qualified graduates to fund PhD student initiated research projects (19'250 CHF)
Aug 2015	*Novartis Biotechnology Leadership Camp 2015* **individual winner**
Aug 2015	*Novartis Biotechnology Leadership Camp 2015* **group winner**
Nov 2014	**Stipend** to attend and present at the *iHEAD academy 2014* in Leiden, Netherlands, by the international headache society (IHS)
Oct 2014	*Lincoln College Oxford* **prize for the best students** of the year
Feb 2014	**Full scholarship** from the Nuffield Department of Clinical Neuroscience for a DPhil in neuroimaging of migraine at the *University of Oxford (*among the 3 best applicants)
April 2013	**Full scholarship** for the fast-track neuroscience program (1 year MSc +3 PhD) at the *Graduate School of Neuroscience (GSN)* in Munich
March 2013	**Clarendon scholarship** (including tuition fees, college fees and living costs) and **Santander Graduate Award** for the MSc in Neuroscience at the *University of Oxford* (among best 3 applicants)
20 July 2012	**Fee waiver** for the Master of Cognitive Neuroscience at the *University of York*
13 July 2012	***British Psychology Society*** **Undergraduate Award**
	Departmental Prize for the **best student** of the year, *University of York*
	Departmental Research Prize (**best research project**), *University of York*
01 June 2012	**Best BSc Dissertation presentation**, *University of York*
December 2009	**Best Mini-Project**-Presentation of year 1 in BSc, *University of York*
06 June 2008	**Best Abitur** (German A-level equivalent) in cohort, *Gymnasium Oberursel*

Patents and Inventions

April 2016	Patent Application **WO 2018/115158 Al**; ***Migraine prevention and treatment***
	Inventors: Elena Gross, Prof. Dr. Dirk Fischer

Presentations and Posters

June 2019	Oral presentation at *Ketogenic Diet – Future Prevention and Treatment of Non-communicative Diseases,* Bergün, Switzerland,
	'The metabolic face of migraine and why (both endogenous and exogenous) ketosis might represent effective treatment strategies`
Feb 2019	Poster presentation at *3nd Metabolic Therapeutics Conference*, Los Angeles, California; 'Preliminary Data on Exogenous Ketone Bodies in Migraine Prevention'
Sept 2017	Oral and poster presentation at *International Headache Conference*, Vancouver, Canada; 'Preliminary data on exogenous in Migraine Prevention'
Feb 2017	Poster presentation at *2nd Metabolic Therapeutics Conference*, Tampa, Florida; 'Preliminary Data on Ketogenic Supplements in Migraine Prevention'
Oct 2015	Oral presentation at the annual meeting 2015 of the *Swiss Neurological Society*: 'Ketone bodies in migraine prevention', Swiss Headache Society Prize presentation
June 2014	Oral presentation at '*Deutsche Migräne und Kopfschmerzgesellschaft (DMKG) Jungforschertreffen 2014*', Migraine and Headache conference in Tutzing, Germany; 'Preliminary analysis of potential structural and functional neural correlates of chronic migraine'
March 2014	Poster presentation at *5th annual Oxford Neuroscience Symposium;* 'Preliminary analysis of potential structural and functional neural correlates of chronic migraine'

Professional membership of societies

From 2016	Junior committee member of the International Headache Society (IHS; Trainees and Residents SIG)
From 2014	Deutsche Migräne und Kopfschmerzgesellschaft (DMKG)
From 2014	International Headache Society (IHS)

Research and work experience

July-Aug. 2016	Research Fellowship at the *Harvard Medical School* with Prof Rami Burstein: Migraine-related research projects (focus on inflammation and photophobia)
From Jan 2015	Migraine related neuroimaging, genetic and clinical research projects within the course of the PhD in clinical research
	Supervision: Prof. Dirk Fischer and Prof. Ludwig Kappos
April – September 2014	*MSc lab internship 2 (at University of Oxford):* Development of an in vitro blood brain barrier model to examine the **effect of migraine drugs (prophylactic topiramate) on a cellular (induced pluripotent stem cell (iPSC) derived) brain endothelial model of migraine**, which will involve culturing iPSC, endothelial and neuronal cell lines, treating with drug or control, harvesting RNA, immunofluorescence and qPCR amongst others.
	Supervision: Dr Satyan Chintawar and Dr Zameel Cader
January – April 2014	*MSc lab internship 1 (at University of Oxford):* **Structural and functional neuroimaging in chronic migraine** using a region of interest approach, including voxel-based morphometry (VBM), diffusion tensor imaging (DTI), resting state BOLD functional connectivity, arterial spin labelling (ASL) at rest and spectroscopy (MRS) of the brainstem. Analysis were conducted with FSL.
	Supervision: Prof. Irene Tracey, Dr Zameel Cader and Dr Andy Segerdahl

January – June 2013	Research internship at the *Biological Psychology Department of the University of Freiburg*, Germany; focus on stress research, the psychobiology of stress, epigenetics, the role of stress in changing gene expression (gene x environment interactions), the role of oxytocin as a stress moderator, therapy of stress related disorders and stress management. *Supervision*: Prof. Robert Kumsta
January 2013	Analysis of questionnaire data for a local care home ("*Haus am Urselbach"*)
July – August 2012	Internship in the *Private Psychotherapeutic Practice Backhaus and 'Psyconomy',* schema-therapy, individual- and couple- consulting, business coaching, intensive short time therapy, amongst others; promotion on website, marketing techniques, personal secretary, first patient contact. *Supervision:* Dipl.-Psych. Christine Backhaus
June 2012	Full-time research assistant in the *University of York, Psychology Department* in the area of 'Perception'(psychophysics), extended work on dissertation especially on the 'normalisation' process using PsychoPy. *Supervision:* Prof. Peter Thompson
October 2011 – February 2012	Part-time (30%) research assistant in the area of 'Personality Psychology' in the *University of York, Psychology Department,* including developing and improving online questionnaires, general literature research and work on the departmental homepage. *Supervision:* Dr. Marcel Zentner
July – August 2010	Research internship in the *Max Planck Institut* for brain research in Frankfurt a.M., Germany; first-hand experience with single-cell recording (with monkeys and cats) in the area of visual perception, analysis with Excel and introducing 'Mendeley' (a new reference system) to the research staff. *Supervision*: Prof. Wolf Singer

Welfare / Community Activities

Aug 2016 (ongoing)	Junior committee member of the International Headache Society (IHS; Trainees and Residents SIG)
Aug. 2015 (ongoing)	Department representative of planning committee for a graduate academy (GRACE) for the *University of Basel* with the vice-chancellor of research (Prof. Edwin Constable)
July 2015 (on going)	German lessons/ private tutoring
Sept. 2013 – Sept 2014	Course representative for the MSc Neuroscience
July – September 2011	Internship and social work in the *Psychiatry "Antara"* in Kolkata, India; providing mental support and organising activates for the patients, shadowing psychiatrists and psychologists, neuropsychology assessments and visits to outreach clinics.
September 2011 – June 2012	Social Secretary of the *University of York German Society*; organising in/formal dinners, socials and other events within the club (weakly 'Stammtisch', Oktoberfest etc.), ensuring a welcoming atmosphere and integration of new members.
October 2010 – January 2012	Social Secretary of the *University of York Volleyball Club*; organising in/formal dinners, socials and other events within the club, ensuring a welcoming atmosphere and integration of new members

Additional Skills

Languages: *German* – excellent (native speaker);

English – proficient (both written and spoken);

French – basic knowledge

IT skills: Advanced Excel, Word and PowerPoint, good SPSS, R and FSL

Publications

In submission: **Gross, E.C.,** Putananickal, N., Orsini, A-L., Vogt, D., Sandor, P.S., Schoenen, J. & Fischer, D. (2019). Mitochondrial function and oxidative stress markers in higher-frequency episodic migraine. *Cephalalgia.* (Impact factor= 3.9)

Article in press: **Gross, E.C.**, Lisicki, M., Fischer, D. et al. (2019). The Metabolic Face of Migraine. *Nature Reviews Neurology*. (Impact factor= 20.3)

Gross, E.C., Klement, R. Schoenen, J. et al. (2019). Potential protective mechanisms of ketone bodies in migraine prevention. *Nutrients*. (Impact factor= 4.3)

Abstract: '**Gross, E.C.,** Sandor, P. & Fischer, D. (2019). 'Preliminary data on exogenous ketone bodies in migraine prevention' at *MHS, Los Angeles 2019*

Gross, E.C., Putananickal, N. Orsini, A.L..; et al. (2019). Safety, tolerability and efficacy of exogenous ketone bodies for preventive treatment of migraine: A single-centre, randomised, placebo controlled, double-blind crossover trial. *Trials.* (Impact factor= 2.1)

Abstract: '**Gross, E.C.,** Sandor, P. & Fischer, D. (2017). Preliminary data on exogenous ketone bodies in migraine prevention' at *IHC, Vancouver 2017*

Klement, R.J., Feinman, R.D., **Gross, E.C.,** et al. (2017). Need for new review of article on ketogenic dietary regimes for cancer patients. *Medical Oncology*. (Impact factor = 2.5)

Abstract: '**Gross, E.C.,** Sandor, P. & Fischer, D. (2017). Preliminary data on ketogenic supplements in migraine prevention' at *Nutritional Ketosis & Metabolic Therapeutics Conference, Tampa 2017*

Seifert, C. L., Schonbach, E.M., Magon, S., **Gross, E.,** et al., (2015). Headache in acute ischemic stroke – a lesion mapping study. *Brain.* (Impact factor= 9.2)

Seifert, C. L.*, **Gross, E.*,** Magon, S., & Sprenger, T. (2016). Neuroimaging findings on supratentorial deep grey matter abnormalities in migraine. *Headache.* (Impact factor= 3.2)

**Equally contributing first authors*

Abstract*: '***Gross, E.C.,** Segerdahl, A. & Tracey, I. (2015*).* Preliminary analysis of potential structural and functional neural correlates of chronic migraine' at *International Headache Conference,* Valencia 2015.

Sprenger, T. & **Gross, E.** (2015). Migränetherapie- Update. Auf dem Weg zu neuen Applikationswegen und neuen Therapieprinzipien, *Hausarzt Praxis,* 10(1).

Abstract: '**Gross, E.C.** & Thompson, P. (2012*).* Normalisation and Orientation Tuning in the Tilt After-Effect: A new approach' published at *European Conference of Visual Perception 2012*

Trainings

Subject-specific courses

Sept 2017	iHEAD / IHA international headache workshop for promising headache specialists
May 2017	Young Headache Researcher Workshop by DMKG 2017

April 2017, Basel	Project management in medicines development part 2(PMP and CAPM)
Nov 2016, Lugano	BioBusinness BioEntrepreneurship 5 day course
April 2016, Tutzing	Stress management with headache patients, manual examination of headache patients
March 2016, Basel	Project management in medicines development part 1 (PMP and CAPM)
Jan 2016, Basel	Data-processing pipelines in Life Science
Oct 2015, Bern	Schweizerische Neurologische Gesellschaft (SNG) Jahresversammlung
Aug 2015	MOOC: R Programming by John Hopkins University (Distinction)
Aug 2015, Basel	Novartis International Leadership BioCamp 2015 (group and individual prize)
July 2015	MOOC: The Data Scientist's Toolbox by John Hopkins University (Distinction)
July 2015, Basel	MRI safety course
May 2015, Basel	MD PhD Seminar Novartis
Feb and April 2015	Good Clinical Practice course (GCP)
April 2015, Wittenberg	Deutsche Migräne und Kopfschmerz Gesellschaft (DMKG) Jungforscher-Workshop
Feb 2015	MOOC: Introductory Human Physiology by Duke University
From Jan 2015, Basel	Journal Club Health Sciences
Jan 2015, Basel	Advanced statistics R course
	Transferable skills courses
Sept.-Dec. 2017	CTI entrepreneurship course module 2: Business Concept
Dec 2016	Workshop: Get Your Video Abstract (video making and editing course)
Oct 2016	Make The Most Out of Your Science – Patenting and Spin-Off Workshop
June 2016	Antelope@university: How to use social media for science?
April 2016	Successful self-marketing (branding and promotion)
March 2016	Advanced Studies UNIBAS: Project Management in Medicines Development part 1 (PMP and CAPM)
Feb 2016, Basel	Coaching on finances in academia
Sept. - Nov. 2015, Basel	Graduate academy (GraCe) planning committee with the vice chancellor of research
Nov 2015, Basel	PPHS Workshop: Recognizing, assessing and documenting your skills as a doctoral candidate in Health Sciences
Oct 2015, Basel	Communication and presentation skills
May 2015, Basel	PPHS Workshop: Funding in health sciences
Feb 2015, Basel	Advanced Excel

8. Courses and credits

No.	Course format	Course title	Semester	CP	Grade	Status	Date of assessment	Recognized	Level
	Advanced skills	CTI Entrepreneurship Training Business Concept (Module 2), including poster and oral presentation	fs 2017	2	PASS	Completed with Success	22.12.2017		Doctoral studies
	Advanced skills	18th International Headache Society Conference Vancouver 2017 (including poster and oral presentation)	fs 2017	2	PASS	Completed with Success	22.12.2017		Doctoral studies
	Advanced skills	iHEAD / International Headache Acadamy (IHA) 2017, including oral presentation	fs 2017	1	PASS	Completed with Success	22.12.2017		Doctoral studies
	Advanced skills	International Congress of Integrative Medicine, particularily the ketogenic diet in neurological diseases	ss 2017	1	PASS	Completed with Success	22.12.2017		Doctoral studies
	Advanced skills	ProjectManagement_MedicinesDevelopment_Part1<(>& <)>2	ss 2017	4	PASS	Completed with Success	22.12.2017		Doctoral studies
	Advanced skills	Young Headache Researcher Workshop of the German Migraine and Headache Society (DMKG) 2017	ss 2017	1	PASS	Completed with Success	22.12.2017		Doctoral studies
	Course	Make The Most Out of Your Science – Patenting and Spin-Off Workshop (Transferable Skills Program for Doctoral Candidates and Postdocs)	fs 2016	1	PASS	Completed with Success	10.01.2017		Doctoral studies
	Course	Get Your Video Abstract - Your Research in a Film (Transferable Skills Program for Doctoral Candidates and Postdocs)	fs 2016	1	PASS	Completed with Success	10.01.2017		Doctoral studies
	Advanced skills	BioBusiness - adavanced 5 day program on BioEntrepreneurship	fs 2016	2	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	International Headache Academy (iHEAD) 2016	fs 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	Ketogenic Diet Sympsium	fs 2016	1	PASS	Completed with Success	22.12.2017		Doctoral studies
43020	Seminar	Data-processing pipelines in Life Science	ss 2016	0	NE	Completed Unsuccessfuly	26.07.2016		Doctoral studies
	Advanced skills	Dreiländertagung und Workshops Kopfschmerz 2016	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	MOOC: R Programming	ss 2016	1	6.0	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	Antelope Trainings and Coachings	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	Swiss annual Neurology Congress and prize presentation	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	International Leadership BioCamp Novarits 2015	ss 2016	1	6.0	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	Good Clinical Practice course (GCP)	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	MOOC: The Data Scientist's Toolbox by John Hopkins Univeristy	ss 2016	1	6.0	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	Young Headache Researcher Workshop of the German Migraine and Headache Society (DMKG) 2015	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
		Applied Statistic R Course by CTU	ss 2016	1	PASS	Completed with Success	16.03.2017		Doctoral studies
41943	Practical course	Kommunikations- und Präsentationskompetenz	fs 2015	3	PASS	Completed with Success	23.12.2015		Doctoral studies
43027	Seminar	Data-processing pipelines in Life Science	fs 2015	2	PASS	Completed with Success	26.01.2016		Doctoral studies
36754	Lecture	Clinical Neurosciences	ss 2015	0	FAIL	Completed Unsuccessfuly	26.07.2018		Doctoral studies
	Advanced skills	Young Headache Researcher Workshop of the German Migraine and Headache Society (DMKG) 2014	ss 2015	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Advanced skills	PPHS and uni courses	ss 2015	1	PASS	Completed with Success	16.03.2017		Doctoral studies
	Guided self-study	MOOC: Introductory Human Physiology by Duke University	ss 2015	3	6.0	Completed with Success	16.03.2017		Doctoral studies

Total: **36 ECTS**