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Nuclear Magnetic Resonance Spectroscopy Metabolomics in Idiopathic Intracranial Hypertension to Identify Markers of Disease and Headache

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Abstract

Background and Objective

We evaluated the metabolomic profile in CSF, serum and urine of participants with idiopathic intracranial hypertension (IIH) compared to controls and measured changes in metabolism associated with clinical markers of disease activity and treatment.

Methods

A case-control study compared women aged 18-55 years with active IIH (Friedman diagnostic criteria), to a sex, age and body mass index matched control group. IIH participants were identified from neurology and ophthalmology clinics from National Health Service hospitals and underwent a prospective intervention to induce disease remission through weight loss with re-evaluation at 12 months. Clinical assessments included lumbar puncture, headache, papilledema and visual measurements. Spectra of CSF, serum and urine metabolites were acquired utilizing proton nuclear magnetic resonance spectroscopy.

Results

Urea was lower in IIH (CSF; controls median ±IQR 0.196 ±0.008, IIH 0.058 ±0.059, p<0.001, urine; controls 5971.370 ±3021.831, IIH 4691.363 ±1955.774, p=0.009), correlated with ICP (urine p=0.019) and headache severity (CSF p=0.031) and increased by 12 months (CSF 12 months; 0.175 ±0.043, p=0.004, urine; 5210.874 ±1825.302, p=0.043). The lactate:pyruvate ratio was increased compared to controls (CSF; controls 49.739 ±19.523, IIH 113.114 ±117.298, p=0.023, serum; controls 38.187 ±13.392, IIH 54.547 ±18.471, p=0.004) and decreased at 12 months (CSF; 113.114 ±117.298, p<0.001). Baseline acetate was higher in IIH (CSF; controls 0.128 ±0.041, IIH 0.192 ±0.151, p=0.008), correlated with headache severity (p =

0.030) and headache disability (p = 0.003) and was reduced at 12 months (0.160 ±0.060, p = 0.007). Ketones 3-hydroxybutyrate and acetoacetate were altered in CSF at baseline in IIH (3-hydroxybutyrate; controls 0.074 ±0.063, IIH 0.049 ±0.055, p = 0.019, acetoacetate; controls 0.013 ±0.007, IIH 0.017 ±0.010, p = 0.013) and normalized at 12 months (0.112 ±0.114, p = 0.019, 0.029 ±0.017, p = 0.015 respectively).

Discussion

We observed metabolic disturbances that are evident in CSF, serum and urine of IIH participants, suggesting global metabolic dysregulation. Altered ketone body metabolites normalized following therapeutic weight loss. CSF:serum urea ratio was altered which may influence ICP dynamics and headache. Elevated CSF acetate, known to stimulate trigeminal sensitization, was associated with headache morbidity. These alterations of metabolic pathways specific to IIH provide biological insight and warrants mechanistic evaluation.

Trial Registration Information

Registered on ClinicalTrials.gov, NCT02124486 (submitted April 22, 2014) and NCT02017444 (submitted December 16, 2013).

Keywords: idiopathic intracranial hypertension; pseudotumor cerebri; metabolism; headache; ketone

Glossary:

^{1}H-NMR Proton Nuclear Magnetic Resonance

BMI Body mass index

CSF Cerebrospinal fluid

HIT-6 Headache Impact Test -1

HVF Humphrey visual field

ICP Intracranial pressure

IIH Idiopathic Intracranial Hypertension

LP Lumbar puncture

LP OP Lumbar puncture opening pressure

OCT Optical coherence tomography
PMD Perimetric mean deviation
RNFL Retinal nerve fiber layer

Introduction

Idiopathic Intracranial Hypertension (IIH) is characterized by raised intracranial pressure (ICP) which causes papilloedema and a risk of permanent visual loss, in addition to chronic headaches which significantly reduce quality of life.[1-4] IIH is becoming more common (indicated by a 350% increased incidence within a decade[5]), and is related to the escalation in worldwide obesity rates.[6,7] Disease modification can be achieved through weight loss.[8,9] The underlying cause remains unknown and this lack of knowledge hinders advances in IIH.

It is well established that IIH occurs almost exclusively (>90%) in women with obesity in association with recent weight gain and truncal adiposity.[9-11] Knowledge of the disease is, however, advancing and IIH is no longer considered to be exclusively a central nervous system disease, with mounting evidence indicating systemic metabolic perturbation in excess to that driven by obesity.[11-13] IIH patients are more insulin resistant in the context of hyperleptinemia and adipocyte leptin hypersecretion.[11] Additionally, omental and subcutaneous IIH adipose demonstrates a unique depot-specific lipogenic profile, with adipose tissue transcriptionally primed for increased calorie storage.[11] Systemic hormonal dysregulation has been noted in IIH with a distinct profile of androgen excess identified.[12] Additionally, metabolic targeting by blocking the cortisol generating enzyme 11β hydroxysteroid dehydrogenase type 1 has shown therapeutic potential in IIH.[14] Patients with IIH also have a doubled risk of cardiovascular disease as compared to those with obesity alone.[6]

Defining the etiology of IIH and identifying biomarkers to guide diagnosis were deemed top priorities for research, by both healthcare professionals and patients with IIH in a priority setting partnership.[15] Metabolites have not been previously quantified in IIH in comparison to controls matched for obesity. We suggest that

investigation of the metabolic pathways involved in IIH may shed light on disease pathogenesis and have relevance for developing targeted therapeutics.

This study used an untargeted metabolomic method to identify quantitative differences in metabolites in the CSF, serum and urine of active IIH participants using proton nuclear magnetic resonance spectroscopy, in comparison to an age, sex and body mass index (BMI) matched control group. Subsequently we sought to identify the relationship between metabolites and clinical measurements and finally to determine alterations in metabolites at 12 months following disease treatment.

Methods

Study design

A case-controlled study compared metabolite concentrations between control and IIH participants at baseline. A subgroup of IIH participants subsequently underwent a prospective intervention study, which evaluated methods of weight loss to achieve disease remission over 12 months. The clinical trial protocol and clinical results of the weight loss intervention have been reported elsewhere.[8,16]

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the National Research Ethics Committees from the West Midlands–The Black Country (14/WM/0011) and York and Humber-Leeds West approved (13/YH/0366)]. In accordance with the Declaration of Helsinki, all subjects gave written informed consent to participate in the study. The detailed trial methodology, protocol and statistical analysis plan have been published.[16-18] This sub-study is reporting on registered trials NCT02124486 and NCT02017444 and was an exploratory objective of the IIH:WT trial. Written informed consent was obtained from all participants in the study.

Participants

Inclusion and exclusion criteria

IIH participants were identified from neurology and ophthalmology clinics from 7 United Kingdom National Health Service hospitals between March 2014 and May 2017. Briefly the inclusion criteria for IIH participants featured: female sex, aged

between 18-55 years, BMI ≥35kg/m^2, clinical diagnosis of active IIH meeting the Friedman diagnostic criteria [19] (ICP ≥25 cmCSF >2 months duration and active papilloedema (Frisen grade >1 in at least one eye)), normal brain imaging (including magnetic resonance venography or CT with venography), apart from radiological signs of raised ICP, and ability to give informed consent.
Briefly exclusion criteria included: aged <18 or >55 years, pregnancy, having undergone optic nerve sheath fenestration, significant comorbidities including known endocrinopathies, receiving hormone manipulating medication, the inability to perform a visible field reliably, if there was a secondary cause of raised ICP or if the IIH had gone into remission (absence of papilloedema).

Controls participants were recruited through advertising on social media and included women aged between 18-55 years with obesity (BMI ≥35kg/m^2) and with analogous exclusion criteria to the IIH participants.

Assessments

At baseline, all participants underwent detailed medical history and examination. A headache diary recorded monthly headache days (days per month) and severity (numerical rating of scale 0-10 with 10 denoting maximum pain). Headache disability was evaluated using the Headache Impact Test-6 (HIT-6) questionnaire. BMI was calculated from weight and height and using the following formula: BMI = (weight (kg) / height (m)^2). A lumbar puncture (LP) was carried out in all participants in the lateral decubitus with knees bent at a 90° angle or more and lumbar puncture opening pressure (LP OP) recorded before CSF was collected (up to 15ml). Visual assessments included automated perimetry with the Humphrey Visual Field (HVF) Analyzer (Carl Zeiss Ophthalmic Systems, Inc) using a 24-2 Swedish Interactive Threshold Algorithm (SITA) standard test pattern. The worse eye was identified for each patient by defining the eye with the more severe perimetric mean deviation (PMD). The optic nerve head swelling was measured using spectral domain optical coherence tomography (OCT; Spectralis, Heidelberg Engineering) to evaluate the average peripapillary retinal nerve fiber layer (RNFL) thickness.

Sample collection and preparation

Participants were fasted overnight (from midnight) prior to CSF and blood serum sample collection at the study visit. Samples were transported on ice and centrifuged within 30 min (10 min 1500g for blood, 800g for CSF) at 4°C. 24-hour urine samples were also provided by participants on attendance to the research facility. All samples were stored at -80˚C and analyzed after a maximum of one freeze-thaw cycle.

Metabolite Extraction

For serum and CSF samples, metabolites were extracted prior to processing. In brief, methanol (-80°C) was added to all samples to quench metabolism. Samples were then incubated on wet ice and chloroform was added to aid the separation of non-polar metabolites. This mixture was vortexed and centrifuged at 4°C to enable separation of polar metabolites. 2 ml of the polar fraction was transferred to a new tube and dried. The polar phase was then reconstituted in sodium phosphate buffer (100mM sodium phosphate, 500µM 4,4-dimethyl-4-silapentane-1-sulfonic acid and 2mM Imidazole in 100% Deuterium oxide).

Urine samples were manually pH adjusted (target pH, 7.0) and mixed with concentrated NMR buffer, yielding an endpoint phosphate buffer concentration of 100mM, 10% D20, 0.5mM Sodium trimethylsilylpropanesulfonate (DSS) and 2mM imidazole.

Acquisition and pre-processing of ^{1}H-NMR spectra

One-dimensional ^{1}H-NMR spectra were obtained from all samples using a 600MHz Bruker Avance III spectrometer with a 1.7mm z-PFG TCI Cryoprobe at 300K. Solvent suppression was achieved, using the NOESY presaturation pulse sequence. Spectral width was set to 12.2 ppm and 16384 complex data points were acquired with a 4.0s interscan relaxation delay. All NMR spectra processed using the MetaboLab software.[21] Free induction decays were apodised using an exponential line broadening of 0.3 Hz and zero-filled to 131072 real data points prior to Fourier transformation. The DSS internal standard signal in each spectrum was referenced to 0.0 ppm, followed by manual phase correction and batch baseline correction using a spline baseline prior to export int Bruker format. Chenomx NMR Suite version 8.3 (Chenomx Inc, Alberta, Canada) was used to carry out comprehensive, untargeted metabolite annotation and quantification.

Normalization of data

Serum datasets were normalized prior to statistical analysis using probabilistic quotient normalization (PQN)[22] on MetaboAnalyst 4.0[23] to remove variation due to dilution factors. Normalization was not necessary for CSF as it is subject to tight homeostatic regulation and is unlikely to vary in concentration between individuals.[24]

Statistical analyses

This was a prospective evaluation, and we report the primary analysis of these data. All univariate statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp, Armonk, NY, USA). No pre-treatment of data were performed prior to these analyses.[25] Figures were produced using GraphPad Prism version 8.0 (GraphPad Software Inc, San Diego, CA, USA).

The normality of data were assessed using quantile-quantile plots and the Shapiro-Wilk test. Distributions which were normally distributed were compared using parametric tests (t tests) whilst non-parametric tests (Mann-Whitney test, Spearman rank correlation test, Wilcoxon signed ranks test for baseline and 12 month paired data) were used in the case of distributions which were not normally distributed. Continuous clinical characteristic data are reported as mean and standard deviation (SD), metabolites are reported median and inter-quartile range (IQR) and analyzed as non-parametric data. Where data points were missing, data were not imputed. Statistical significance is defined as $p<0.05$. Since the study is not aiming to delineate diagnostic biomarkers, but instead to understand metabolic pathways, deriving conclusions from multiple pathways and metabolites, data was not corrected for multiple testing.

Data Availability

Anonymized individual participant data may be made available along with the trial protocol. Proposals should be made to the corresponding author and will be reviewed by the Birmingham Clinical Trials Unit Data Sharing Committee in discussion with the Chief Investigator. A formal Data Sharing Agreement may be required between respective organizations once release of the data are approved and before data can be released.

Results

Metabolite concentrations in IIH participants relative to controls

All participants were female with a mean ± SD age of 36.60 ± 8.47 years in controls (n = 20) and 33.01 ± 7.11 years in IIH participants (n = 84) (Table 1). BMI-matched controls had a BMI of 43.74 ± 4.96 m^2/kg whilst IIH participants had a BMI of 42.24 ± 7.90 m^2/kg. As expected, controls had a significantly lower LP OP pressure (23.74 ± 3.81 cmCSF controls vs 34.03 ± 5.52 cmCSF, p<0.001, Table 1).

A distinct metabolic profile was identified in IIH participants at baseline in comparison to controls featuring 23 CSF metabolites, 12 serum metabolites and 9 urine metabolites (Table 2) that significantly differed in concentration between IIH and control subjects. We have discussed in detail the differential metabolites which were most consistently associated with clinical measures and normalized after 12 months.

Within the CSF analysis, we identified that acetate was significantly higher in IIH participants than controls (0.128 ± 0.041 control vs 0.192 ± 0.151, p = 0.008, Fig 1A). Moreover, the lactate:pyruvate ratio, a marker of mitochondrial dysfunction was significantly higher in IIH than in controls (49.739 ± 19.523 control vs IIH 113.114 ± 117.298, p = 0.023, Fig 2A). Individual lactate and pyruvate measurements, products of glycolysis, were significantly lower in IIH compared to controls (lactate 4.561 ± 0.666 control vs 3.949 ± 0.972 IIH, p = 0.001, Fig 2C, pyruvate 0.096 ±0.042 control vs 0.033 ±0.034 IIH, p = 0.001, Fig 2D). There was no difference in the lactate:pyruvate ratio or lactate concentrations in those taking acetazolamide compared to those who were not at baseline (medication use; eTable 1 in the Supplement). Fumarate, a citric acid cycle metabolite was also lower in IIH in comparison to controls (0.074 ± 0.020 controls vs 0.065 ± 0.023 IIH, p = 0.039). Urea an osmolar metabolite, was significantly lower in IIH than controls (0.196 ± 0.088 controls vs 0.058 ± 0.059 IIH, p <0.001, Fig 3A). Ketones 3-hydroxybutyrate and acetoacetate were also altered in IIH in comparison to controls (3-hydroxybutyrate 0.074 ± 0.063 controls vs 0.049 ± 0.055 IIH, p = 0.019, acetoacetate 0.013 ± 0.007 controls vs 0.017 ± 0.010, p = 0.013, Table 2).

Although there were fewer differential metabolites in the serum (12 metabolites, Table 2), there were similarities to the differential metabolites in the CSF, with the lactate:pyruvate ratio being significantly higher in the serum of IIH participants in comparison to controls (38.187 ± 13.392 control vs 54.547 ± 18.471 IIH, p = 0.004, Fig 2B). Moreover, individual pyruvate measurements were also significantly lower in IIH compared to controls (controls 0.405 ±0.157 vs IIH 0.277 ± 0.113, p <0.001).

Of note, of the 9 differential urine metabolites (Table 2), urea was significantly lower in IIH than controls (controls 5071.37 ± 3021.83 vs 4691.363 ± 1955.774 IIH p = 0.009, Fig 3B), a finding also observed in the CSF. Citrate, a citric acid cycle metabolite, is significantly lower in IIH in comparison to controls (419.131 ± 96.894 controls vs 289.070 ± 247.403 IIH, p = 0.001).

Metabolite changes at 12 month follow up

We sought to explore any changes in metabolite concentrations by prospectively re-evaluating IIH participants at 12 months following a therapeutic weight loss intervention. Over the follow-up period we noted a mean ± SD reduction in BMI of 6.18 ± 7.68 kg/m^2 and a reduction in LP OP of 10.3 ± 12.55 cmCSF (eTable 2 in the Supplement).

Of the 23 CSF metabolites which were significantly different between controls and IIH participants at baseline, 13 changed significantly at 12 months in IIH (Table 3). The elevated CSF acetate noted at baseline was reduced at 12 months (0.192 ± 0.151 baseline vs 0.160 ± 0.060 12 months, p = 0.007, Fig 1A). Importantly the lactate:pyruvate ratio, which was higher in IIH participants than controls at baseline, was reduced at 12 months follow up and no longer significantly different to controls (baseline 113.114 ± 117.298 vs 12 months 70.776 ± 39.050 p = 0.004, Fig 2A). The reduced CSF urea identified at baseline significantly increased at 12 months (0.058 ± 0.059 baseline vs 0.175 ± 0.043 12 months, p = 0.004, Fig 3A). Ketones acetoacetate and 3-hydroxybutyrate also normalized at 12 month follow up (acetoacetate 0.017 ± 0.031 baseline vs 0.029 ± 0.017, p = 0.015, 3-hydroxybutyrate 0.074 ± 0.063 baseline vs 0.112 ± 0.114 12 months, p = 0.019).

In serum, of the 12 differential metabolites identified in IIH at baseline, 11 were significantly altered at 12 months (Table 3). Interestingly, urea was significantly lower at 12 months in serum than at baseline (baseline 2.382 ± 1.085 vs 12 months 1.552 ± 0.576, $p < 0.001$, 3C). The CSF:serum urea ratio was significantly increased at 12 months, reflecting a decrease in serum urea and increase in CSF urea at 12 months (baseline 0.025 ± 0.083 vs 12 months 0.115 ± 0.058, $p <0.001$). Pyruvate was also significantly lower at 12 months than at baseline (baseline 0.263 ± 0.157 vs 12 months 0.189 ± 0.155, $p = 0.022$).

Of the 9 differential metabolites identified in urine, 5 were significantly changed at 12 months in IIH participants (Table 3). 2 of which were increased to concentrations akin to controls, including glycylproline (baseline 55.36 ± 25.96 vs 12 months 64.974 ±28.762, $p = 0.010$) and urea (baseline 4691.36 ± 1955.77 vs 12 months 5210.874 ± 1825.302 $p = 0.043$, Fig 3B).

Disease remission

15 of 45 patients with matched data had an ICP <25cmCSF at 12 months and therefore were in disease remission. As described, 3-hydroxybutryate a ketone body which was significantly lower in IIH than controls at baseline and significantly increased at 12 months, demonstrated a greater increase in the CSF of the disease remission group (0.080 ± 0.067 baseline vs 0.163 ± 0.109 12 months $p = 0.020$). Acetoacetate, another ketone body which was differential between IIH and controls at baseline and significantly increased at 12 months, exhibited a greater increase in the disease remission group (0.0148 ± 0.007 baseline vs 0.0377 ± 0.020, $p = 0.004$). Serum glucose which significantly decreased at 12 months demonstrated a greater reduction in those in disease remission (56.318 ± 19.046 baseline vs 40.989 ± 11.187 12m $p = 0.005$).

Associations between metabolites and clinical measurements

The relationship between the IIH specific metabolites and clinical features were then evaluated. ICP measured by LP was associated with galactitol in CSF, pyruvate and 2-hydroxyisobutyrate in serum and urea in urine (eTable 3 in the Supplement). ICP was significantly reduced at 12 months follow up (eTable 2 in the Supplement) and the change in ICP was associated with change in butanone in the CSF and o-

phosphocholine and N-phenylacetylglycine in the urine (eTable 4 in the Supplement).

Raised ICP in IIH drives papilloedema, the hallmark sign of disease activity with subsequent risk of visual loss. Papilloedema was evaluated using the OCT RNFL and measurements were associated with isobutyrate and O-acetylcarnitine in serum and 2-hydroxyisobutyrate in urine (eTable 3 in the Supplement). The change in RNFL thickness was associated with pyruvate change in CSF (eTable 4 in the Supplement). Visual function, measured by perimetric mean deviation, was associated at baseline with fumarate, lactate and threonate in CSF, 5-hydroxyindole-3-acetate in serum and citrate in urine (eTable 3 in the Supplement). Changes in the perimetric mean deviation were associated with changes in leucine in CSF, in addition to valine, trimethylamine, 3-methyl-2-oxovalerate and urea in serum (eTable 4 in the Supplement).

Headache in IIH is initiated by elevated ICP and causes significantly reduced quality of life, yet the underlying mechanism driving pain are not understood.[3,26] We explored the relationship between metabolites and headache morbidity. Headache disability, measured by the HIT-6 was associated with 3-hydroxybutyrate and acetate in CSF at baseline (acetate Fig 1B, eTable 3 in the Supplement). Headache severity was associated with acetate (Fig 1C) and urea in CSF and creatine phosphate in urine. Monthly headache days were also significantly associated with creatine phosphate in urine (eTable 3 in the Supplement). At 12 months follow up, changes in HIT-6 were associated with changes in butanone in CSF and urea in urine. Changes in headache severity were associated with changes in pyruvate and were significantly associated with changes in multiple metabolites in urine including butanone, 2-hydroxyisobutyrate and creatine phosphate (eTable 4 in the Supplement).

Obesity is a typical feature of IIH and has been implicated in disease etiology. We sought to evaluate which metabolites were associated with BMI. At baseline, BMI was significantly associated with multiple metabolites in CSF including 3-methyl-2-oxovalerate, isoleucine, methylsuccinate, propylene glycol and threonine (eTable 3 in the Supplement). In urine, 3-Indoxylsulfate, N- acetylornithine, N-

phenylacetylglycine, were also significantly associated with BMI. Following 12 months of weight loss intervention, changes in BMI were significantly associated with butanone in CSF, and importantly urea, 3-indoxylsulfare and N- phenylacetylglycine in urine (eTable 4 in the Supplement).

Discussion

Despite the phenotype of IIH being stereotyped in women with obesity, the etiology has remained elusive. We have employed untargeted, quantitative metabolite phenotyping in a large cohort of active IIH subjects. We have identified a profile of disease specific metabolites that are associated with clinical measures and markers of disease activity. The results provide biological insights and point towards remodeling of metabolite pathways in IIH. The dominating signals were dysregulation of the lactate:pyruvate ratio, a marker of respiratory chain metabolism, and altered ketone body metabolism. We also note metabolic changes in the urea CSF:serum ratio and acetate metabolism that maybe critical in contributing to the severe headache phenotype in IIH.

The lactate:pyruvate ratio was repeatedly altered in IIH and is an established marker of anaerobic metabolism as well as mitochondrial energy metabolism disorders, such as oxidative phosphorylation disorders and pyruvate dehydrogenase deficiency.[27,28] We identified an increased ratio at baseline in IIH compared to controls in both CSF and serum. Elevated CSF lactate:pyruvate ratio is a feature of numerous conditions of raised ICP including traumatic brain injury,[29] subarachnoid haemorrhage[30,31] and hydrocephalus[32], suggesting that these metabolic alterations may be a feature of raised ICP. Fumarate, a citric acid cycle intermediate is also reduced in the CSF of IIH patients, contributing toward the readout of dysfunctional respiratory metabolism in the brain (eFigure 1). Significant reduction in lactate:pyruvate ratio in CSF at 12 months is consistent with findings in hydrocephalus, in which ratios are highest in the setting of elevated ICP and reduced following ventriculoarterial shunt insertion in association with reduced ICP.[32]

Urea, a hyperosmotic metabolite, was noted to differ in IIH. Urea was reduced in IIH at baseline in CSF and urine. The urea levels normalized in line with disease

resolution and falling ICP at 12 months. In physiological conditions, CSF urea concentrations are slightly lower than in serum.[33] We suggest that in IIH the reduced CSF urea, relative to the serum, may represent a compensatory mechanism which increases the osmotic gradient allowing more fluid to move out of the CSF (Fig 4). Following treatment, in which ICP is reduced in IIH, the CSF:serum ratio normalizes. The osmotic properties of urea are used therapeutically with intravenous urea administration being used extensively to reduce brain swelling in a range of conditions, [34] and in particular was able to significantly reduce ICP in patients with acute brain injury especially in those with the highest ICP amongst the cohort (ICP ≥15 mm Hg).[35] Traumatic brain injury (TBI) models in mice have also demonstrated alterations in the expression of urea-transporters which was thought to be an adaptive modulation for changing urea levels.[36]

Interestingly, we also demonstrated a negative relationship between CSF urea and headache severity (with high CSF urea being associated with increased headache severity). These findings suggest that alteration of the CSF:serum urea gradient in IIH may be important in driving headache (Fig 4). The importance of urea and perturbed osmotic gradients is noted in individuals following hemodialysis who frequently experience headaches.[37]

We also identified alterations in acetate, a metabolite which is converted to Acetyl CoA for multiple metabolic reactions including ketone body production and the citric acid cycle. Acetate concentrations are significantly higher in IIH at baseline and are associated with headache disability (HIT-6) and headache severity. Acetate's conversion into acetyl-CoA via Acetyl CoA synthase yields AMP and adenosine, which mediates pain transmission via stimulation of nociceptive nerve terminals via adenosine A2A receptors [38] and release of pain mediators such as histamine by mast cells.[39] Acetate is also the main metabolite responsible for headache symptoms in 'hangover headache[40] and administration is able to increase sensitivity of headache pathways through the trigeminal system in rodent migraine models.[40] The use of acetate as a buffer for kidney dialysis also led to headaches experienced by patients.[41] IIH headaches are known to have migraine-like features.[26] Although we did not measure adenosine, higher concentrations of acetate could also contribute towards trigeminal sensitivity in IIH patients and may contribute to the

etiology of headaches in IIH (Fig 4). Acetate is also significantly reduced at 12 months in IIH participants following disease remission and reduction in headache measurements. Expression of Acetyl CoA synthase has been found to be suppressed in leptin deficiency. Leptin resistance has been demonstrated in IIH,[11] and may have a role in modulating expression of acetyl CoA synthase and its ability to metabolize acetate.

The ketone body acetoacetate is significantly higher in CSF at baseline in IIH, again indicating upregulation of ketone formation. Ketogenesis is similarly upregulated during fasting and insulin resistance. Previous IIH studies have identified insulin insensitivity in IIH,[11] which may lead to increased acetoacetate production. However, reduced 3-Hydroxybutyrate and acetone concentrations in the CSF suggests that either conversion of acetoacetate to these ketone bodies is inhibited, or that these ketone bodies are utilized as an alternative energy source. Following 12 months, acetone and 3-hydroxybutyrate are significantly increased in IIH. Increase in these ketone bodies is also exhibited early in patients following bariatric surgery in serum, and was thought to be due to an increased lipolytic activity.[42] As IIH participants have significant weight loss at 12 months, ketone body formation may continue as fat loss is achieved and lipolysis continues. Moreover, in a sub analysis we noted that acetoacetate was significantly different between the diet and bariatric surgery arm, with acetoacetate being significantly higher in the surgery arm at 12 months ($p<0.05$). It is possible that changes in ketones at 12 months may be driven by weight loss or the metabolic changes following bariatric surgery. We also noted a reduction in serum glucose at 12 months, which may reflect changes attributed to weight loss. However, since this was not associated with any changes in BMI we cannot be certain.

The study includes some potential confounds, one of which is the smaller number of control participants in comparison to IIH. Participants were matched by age, sex and BMI, which limited the number of controls eligible for inclusion. However, obtaining performing detailed phenotyping and CSF collections from healthy BMI matched controls is challenging due to ethical considerations and participant acceptability. Despite this, we present the largest group of controls matched for age, sex and BMI of any IIH studies. As this study was limited to including women only the results of

which may not be generalizable to children and men with IIH. We found that not all clinical markers (including papilledema) were correlated with metabolites this may relate to the severity of disease in participants, therefore performing this analysis in a more severe disease cohort would be of future interest. Another strength of our study is that we were able to review metabolite profiles at 12 month follow up. However this led to some missing data, decreasing the amount of paired data, this was due to the patients decision not have a sample taken at that timepoint. We acknowledge that a small number of patients were on medications and cannot exclude that this may have impacted the metabolite profile, however there was no significant changes noted to result from medications, for example acetazolamide. Therefore, it is unlikely that the medications meaningfully alter the inference of our data.

In this quantitative metabolomics study of IIH we report a distinct metabolomic profile in the serum, CSF and urine of IIH participants in comparison to sex, age and BMI matched control group. The results provide a preliminary mechanistic insight into the pathogenesis of IIH, implicating metabolic dysfunction which manifests in an increased lactate:pyruvate ratio and altered ketone body concentrations. We have also identified metabolic perturbations which are implicated in the mechanisms driving the severe chronic headaches in IIH. Acetate is elevated in IIH and known to stimulate trigeminal sensitization. Alterations in the urea CSF:serum ratio, a contributor to osmotic gradient and fluid shifts, was noted in IIH and was also associated with headache pain.

These findings extend our knowledge of IIH etiology and provide a roadmap for future mechanistic studies. Further work is required to validating our findings and to establish which metabolites may be the most clinically useful as biomarkers of disease diagnosis, progression and outcome. This may include instigation of the role of these key metabolites in IIH pathogenesis in animal and cell models.

Figure legends

Fig 1. Changes in acetate concentration and its association with headache in IIH participants. A- *Acetate concentration is significantly higher in CSF in IIH than controls and reduced at 12 months.* ***B-*** *Acetate is associated with headache disability (HIT-6) scores at baseline in IIH.* ***C –*** *Acetate is also associated with headache severity at baseline in IIH*

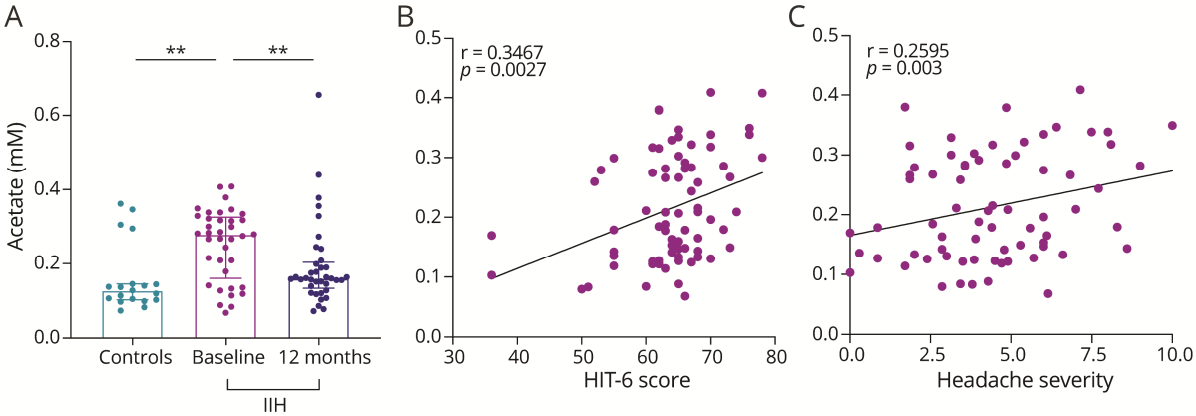


Fig 2. Lactate:pyruvate measurements in IIH in comparison to controls and at 12 months. A *– Lactate:pyruvate ratio in CSF in controls in comparison to IIH at baseline and 12 months.* ***B*** *- Lactate:pyruvate ratio in serum in controls and IIH participants at baseline.* ***C*** *– Lactate ratio in CSF in controls and IIH participants at baseline and 12months.* ***D*** *- Pyruvate ratio in CSF in controls and IIH participants at baseline and 12months.*

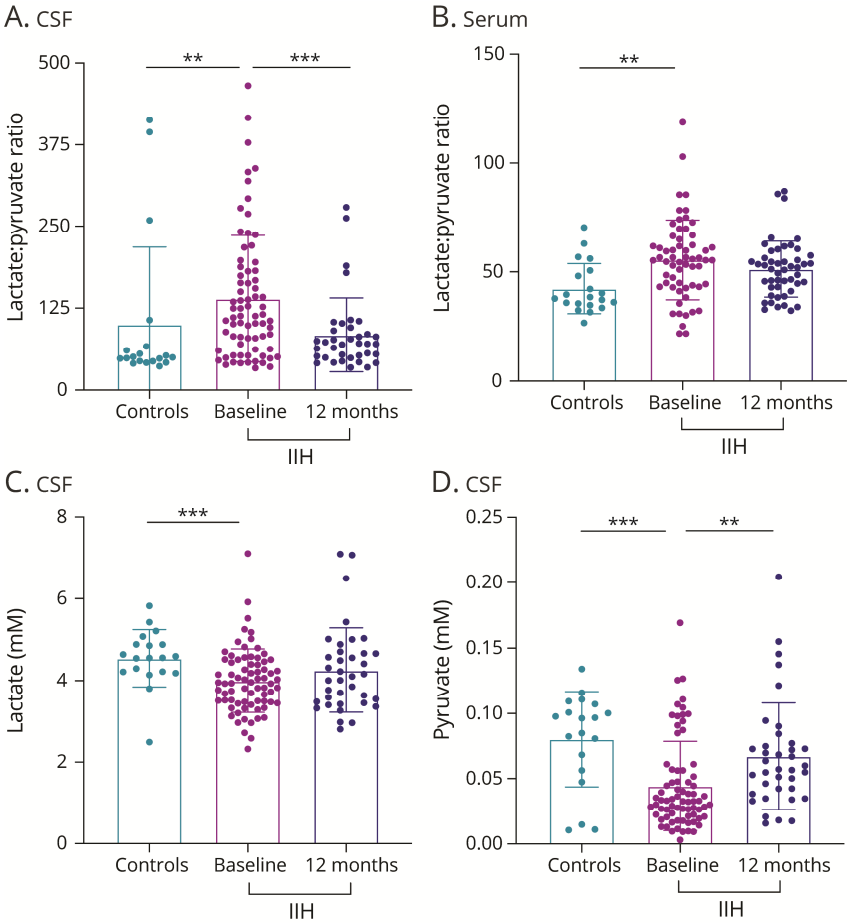


Fig 3. Urea concentrations in CSF, serum and urine and their correlations with clinical measurements. ***A –*** *CSF and* ***B****- Urine urea in controls and IIH at baseline and 12 months.* ***C –*** *Serum urea in IIH at baseline and 12 months.*

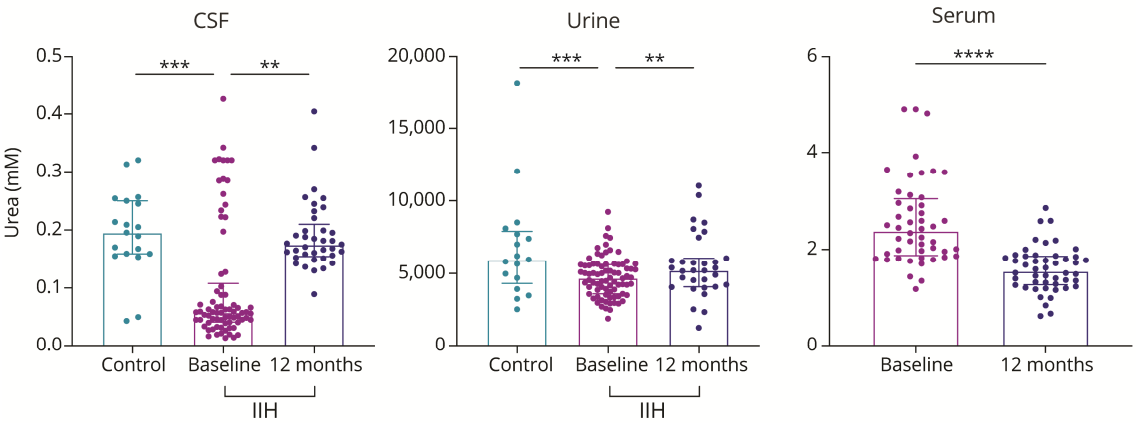
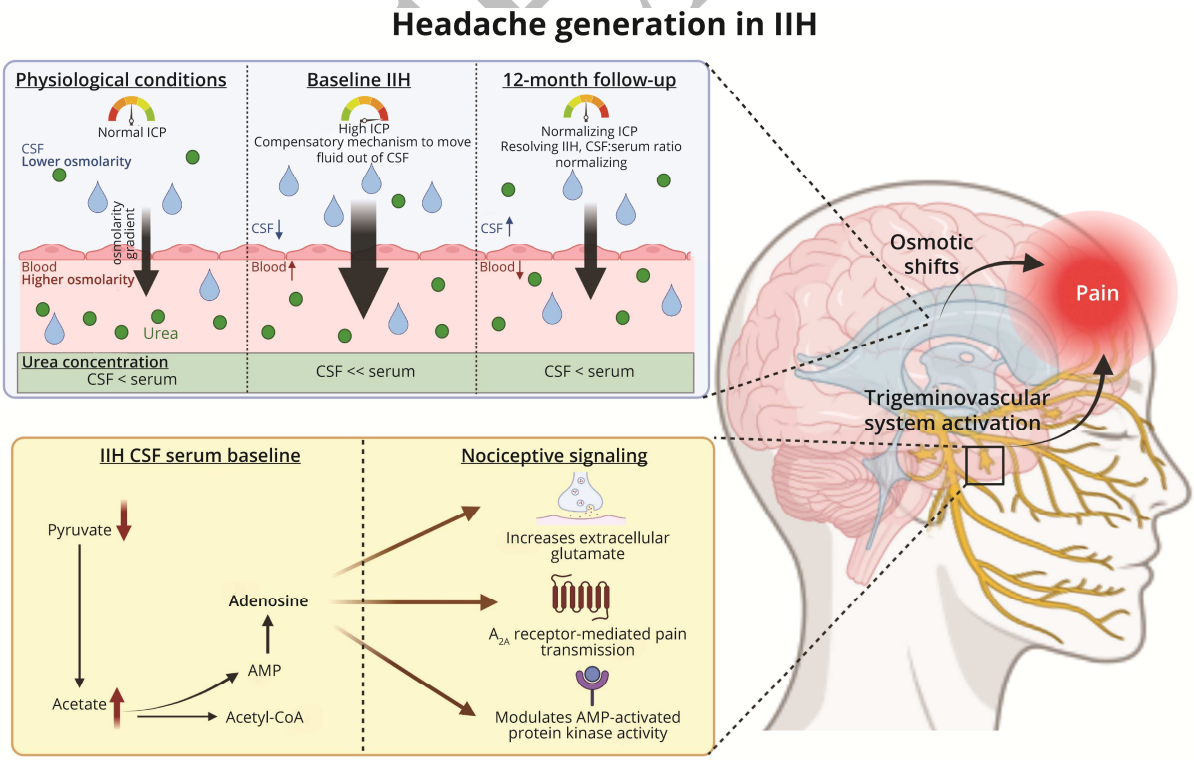


Fig 4. Perturbed metabolic pathways in IIH which may contribute toward headache generation. *Changes in urea concentration in IIH may indicate compensatory adaptive biological mechanisms to regulate fluid movement in the brain. Hypothesized compensatory mechanism in which urea, a hyper osmolar agent, directs movement of water from low to high concentrations. CSF:serum ratio is significantly increased at 12 months in IIH. Metabolic pathway of acetate production and its products which are implicated in trigeminal sensitizing pathways and may contribute toward headache generation in IIH.*



	Controls	IIH
Demographic characteristic	**Mean ± SD, n**	**Mean ± SD, n**
Age (years)	36.60 ± 8.47, 20	33.01 ± 7.11, 84
BMI (kg/m^2)	43.74 ± 4.96, 20	42.24 ± 7.90, 84
LP opening pressure (cmCSF) ****	23.74 ± 3.81, 19	34.03 ± 5.52, 84
Perimetric Mean Deviation (dB)	- 2.76 ± 5.16, 18	- 4.32 ± 5.04, 83
RNFL thickness (µm) *	95.71 ± 8.74, 14	143.12 ± 69.57, 77
Headache frequency per month ****	12.00 ± 9.60, 18	22.97 ± 7.76, 80
Headache severity ***	2.55 ± 2.34, 18	4.60 ± 2.23, 80
HIT-6 score ****	53.18 ± 9.46, 17	63.96 ± 7.69, 83

Table 1. Baseline characteristics of study population. *All values reported are shown as mean ± SD. Abbreviations: Body Mass Index (BMI); Lumbar puncture (LP); Retinal nerve fiber layer (RNFL); Headache Impact Test-6 (HIT-6). * denotes significant differences as tested by unpaired t test.*

CSF				Serum				Urine			
	Controls	IIH			Controls (n =20)	IIH (n = 60)			Controls (n = 17)	IIH (n = 79)	
Metabolite	Median ± IQR (mM), n	Median ± IQR (mM), n	p	Metabolite	Median ± IQR(mM)	Median ± IQR (mM)	p	Metabolite	Median ± IQR (mM)	Median ± IQR (mM)	p
2-hydroxyisobutyrate	0.026 ± 0.008, 17	0.006 ± 0.009, 74	< 0.001	2-Phenylpropionate	0.421 ± 0.054	0.487 ± 0.070	<0.001	2-Hydroxyisobutyrate	7.079 ± 0.946	6.271 ± 2.028	0.037
3-hydroxybutyrate	0.074 ± 0.063, 19	0.049 ± 0.055, 74	0.019	3-Hydroxyisovalerate	0.049 ± 0.011	0.064 ± 0.028	<0.001	3-Indoxylsulfate	31.573 ± 15.104	25.615 ± 15.780	0.032
3-hydroxyisovalerate	0.018 ± 0.004, 19	0.014 ± 0.006, 74	0.001	3-Methyl-2-oxovalerate	0.378 ± 0.067	0.333 ± 0.059	0.006	Citrate	419.131 ± 96.894	289.070 ± 247.403	0.001
3-methyl-2-oxovalerate	0.023 ± 0.006, 17	0.007 ± 0.004, 74	< 0.001	5-Hydroxyindole-3-acetate	0.110 ± 0.047	0.077 ± 0.055	0.026	Creatine phosphate	115.43 ± 28.267	84.190 ± 43.108	0.006
Acetate	0.128 ± 0.041, 19	0.192 ± 0.151, 74	0.008	Glycerol	3.337 ± 0.748	2.915 ± 1.111	0.005	Glycylproline	79.072 ± 41.022	55.358 ± 25.965	0.003
Acetoacetate	0.013 ± 0.007, 19	0.017 ± 0.010, 73	0.013	Isobutyrate	0.052 ± 0.022	0.035 ± 0.024	0.002	N-Acetylornithine	7.575 ± 5.325	5.325 ± 6.421	0.031
Acetone	0.009 ± 0.003, 19	0.005 ± 0.003, 69	< 0.001	Lactate:pyruvate	38.187 ± 13.392	54.547 ± 18.471	0.004	N-Phenylacetylglycine	42.426 ± 14.496	36.557 ± 18.559	0.045
Butanone	0.013 ± 0.005, 11	0.006 ± 0.005, 42	0.028	Methylamine	0.047 ± 0.055	0.058 ± 0.024	0.032	O-Phosphocholine	16.443 ± 9.488	10.345 ± 10.786	0.045
Caprate	0.072 ± 0.029, 15	0.057 ± 0.016, 34	0.007	O-Acetylcarnitine	0.102 ± 0.030	0.121 ± 0.042	0.027	Urea	5971.37 ± 3021.831	4691.363 ± 1955.774	0.009
Choline	0.016 ± 0.007, 19	0.010 ± 0.005, 74	< 0.001	Pyruvate	0.405 ± 0.157	0.277 ± 0.113	<0.001				
Dimethylamine	0.007 ± 0.066, 18	0.003 ± 0.005, 68	0.011	Succinylacetone	0.182 ± 0.035	0.156 ± 0.053	0.013				
Formate	0.047 ± 0.016, 19	0.039 ± 0.021, 74	0.010	Trimethylamine	0.053 ± 031	0.026 ± 0.023	0.0038				
Fumarate	0.074 ± 0.020, 19	0.065 ± 0.023, 39	0.039	Valine	2.191 ± 0.463	2.495 ± 0.422	0.0172				
Galactitol	0.114 ± 0.031,19	0.091 ± 0.043, 74	< 0.001								
Isoleucine	0.026 ± 0.014, 19	0.018 ± 0.008, 74	0.002								
Lactate	4.561 ± 0.666, 19	3.949 ± 0.972, 74	0.001								
Leucine	0.052 ± 0.015, 19	0.060 ± 0.019, 74	0.038								
Lactate:pyruvate	49.739 ± 19.523, 19	113.114 ± 117.298, 74	0.023								
Methylsuccinate	0.013 ± 0.004, 19	0.011 ± 0.005, 74	0.032								
Propylene glycol	0.026 ± 0.031, 18	0.015 ± 0.023, 69	0.014								
Pyruvate	0.096 ± 0.042, 19	0.033 ± 0.034, 74	0.001								
Threonate	0.582 ± 0.207, 19	0.424 ± 0.193, 74	< 0.001								
Threonine	1.478 ± 0.445, 19	2.154 ± 0.961, 74	< 0.001								
Urea	0.196 ± 0.088,19	0.058 ± 0.059, 74	< 0.001								

Table 2. Significantly different metabolites in CSF, serum and urine between IIH and control participants. *A total of 47 metabolites were detected and quantified in CSF. 23 metabolites were significantly different between the IIH patient and control groups at baseline.*

CSF				Serum				Urine			
	Baseline	12m			Baseline (n=60)	12m (n=48)			Baseline (n=79)	12m (n=31)	
Metabolite	Median ± IQR, n (mM)	Median ± IQR, n (mM)	p	Metabolite	Median ± IQR, n (mM)	Median ± IQR, n (mM)	p	Metabolite	Median ± IQR (mM)	Median ± IQR (mM)	p
2 -hydroxyisobutyrate	0.006 ± 0.009, 74	0.024 ± 0.010, 33	<0.001	2-phenylpropionate	0.421 ± 0.054	0.338 ± 0.129	<0.001	2-Hydroxyisobutyrate	6.271 ± 2.028	6.140 ± 2.073	0.003
3-hydroxybutryate	0.049 ± 0.055, 74	0.112 ± 0.114, 37	0.012	3-hydroxyisovalerate	0.049 ± 0.011	0.035 ± 0.013	<0.001	Creatine phosphate	84.19 ± 43.11	80.168 ± 41.020	0.034
3-methyl-2-oxovalaterate	0.007 ± 0.004, 74	0.021 ± 0.012, 37	<0.001	3-methyl-2-oxovalerate	0.378 ± 0.067	0.270 ± 0.114	0.004	Glycylproline	55.36 ± 25.96	64.974 ± 28.762	0.010
Acetate	0.192 ± 0.151, 74	0.160 ± 0.060, 37	0.007	Glucose	50.373 ± 16.171	41.779 ± 15.213	0.004	N-Acetylomithine	5.32 ± 6.421	6.000 ± 2.336	0.008
Acetoacetate	0.017 ± 0.010, 73	0.029 ± 0.017, 36	0.015	Glycerol	3.337 ± 0.748	2.441 ± 1.333	0.023	Urea	4691.36 ± 1955.77	5210.874 ± 1825.302	0.043
Acetone	0.005 ± 0.003, 69	0.009 ± 0.003, 37	<0.001	Isobutyrate	0.052 ± 0.022	0.051 ± 0.022	0.023				
Butanone	0.006 ± 0.005, 42	0.013 ± 0.006, 25	0.012	Methylamine	0.047 ± 0.055	0.029 ± 0.040	<0.001				
Choline	0.010 ± 0.005, 74	0.010 ± 0.005, 37	0.022	O-acetylcarnitine	0.102 ± 0.030	0.066 ± 0.045	<0.001				
Fumarate	0.065 ± 0.023, 39	0.058 ± 0.012, 32	0.027	Pyruvate	0.263 ± 0.157	0.189 ± 0.155	0.023				
Isoleucine	0.018 ± 0.008, 74	0.024 ± 0.008, 37	0.015	Succinylacetone	0.155 ± 0.053	0.110 ± 0.065	0.004				
Lactate:pyruvate	113.114 ± 117.298, 74	70.776 ± 39.050,	<0.001	Trimethylamine	0.026 ± 0.023	0.053 ± 0.022	<0.001				
Pyruvate	0.033 ± 0.034, 74	0.057 ± 0.033, 37	0.004	Urea	2.382 ± 1.085	1.590 ± 0.521	<0.001				
Urea	0.058 ± 0.059, 74	0.175 ± 0.043, 37	0.004	Valine	2.495 ± 0.422	1.552 ± 0.058	<0.001				

Table 3. Significantly different metabolites in CSF, serum and urine of IIH participants between baseline and 12 months follow-up

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