NeuroImage: Clinical 19 (2018) 213–221

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Hippocampal metabolites in asthma and their implications for cognitive function

Juliet L. Kroll[a,*], Ashton M. Steele[a], Amy E. Pinkham[b], Changho Choi[c], David A. Khan[d], Sheenal V. Patel[d], Justin R. Chen[d], Sina Aslan[b,e,f], E. Sherwood Brown[f], Thomas Ritz[a,*]

[a] Department of Psychology, Southern Methodist University, Dallas, TX, USA
[b] School of Behavioral and Brain Sciences, The University of Texas, Dallas, TX, USA
[c] Department of Radiology, The University of Texas Southwestern Medical Center, Dallas, TX, USA
[d] Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, TX, USA
[e] Advance MRI LLC, Frisco, TX, USA
[f] Department of Psychiatry, The University of Texas Southwestern Medical Center, Dallas, TX, USA

ARTICLE INFO

Keywords:
Hippocampus
^{1}H-MRS
Structural magnetic resonance imaging
Asthma
Cognition
N-acetylaspartate
Glutamate
Creatine

ABSTRACT

Emerging research indicates that individuals with asthma have an increased risk of cognitive impairment, yet the associations of asthma with neural correlates of memory remain relatively unknown. The hippocampus is the predominant neural structure involved in memory, and alterations in the hippocampal metabolic profile are observed in individuals with mild cognitive impairment. We therefore hypothesized that individuals with asthma may have altered hippocampal metabolites compared to healthy controls.

Structural magnetic resonance imaging (sMRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) were used to compare hippocampal volume and metabolites of otherwise healthy adults with and without asthma (N = 40), and to study the association of these measures with cognitive function and asthma-related variables. Participants underwent 3-Tesla sMRI and ^{1}H-MRS, with the volume of interest placed in the left hippocampus to measure levels of *N*-acetylaspartate (NAA), glutamate (Glu), creatine (Cr), and myo-inositol (MI), as indicators of neuronal viability, cellular activity, cellular energy reserve, as well as glial activation.

Individuals with asthma had lower hippocampal NAA compared to healthy controls. For all participants, poorer cognitive function was associated with reduced NAA and Glu. For individuals with asthma, poorer cognitive function was associated with reduced disease control. Additionally, short-acting rescue bronchodilator use was associated with significantly lower NAA, and Glu, whereas inhaled corticosteroid use was related to significantly higher Cr and in tendency higher NAA and Glu. All findings controlled for left hippocampal volume, which was not different between groups.

These findings highlight that asthma and/or its treatment may affect hippocampal chemistry. It is possible that the observed reductions in hippocampal metabolites in younger individuals with asthma may precede cognitive and hippocampal structural deficits observed in older individuals with asthma.

1. Introduction

Emerging research indicates that individuals with asthma across the life span suffer from higher rates of cognitive and memory impairment compared to the healthy population (Irani et al., 2017); however, biological correlates of these behavioral deficits remain unknown. A recent meta-analysis synthesizing over 4148 participants, found a medium effect sized relation between asthma and reduced cognitive function (Irani et al., 2017), and in a large community sample of individuals over 55 years, individuals with asthma had a 78% increased risk for the presence of mild cognitive impairment (Caldera-Alvarado et al., 2013). Despite an accumulation of studies indicating the risk of cognitive deficits in asthma, the impact of asthma on neural regions involved in memory and their chemistry remains relatively unexplored. Central nervous system (CNS) processes in asthma and dyspnea have recently attracted attention (Binks et al., 2014; Von Leupoldt et al., 2009a; Peiffer et al., 2008; Evans, 2010; Rosenkranz et al., 2012; Pattinson, 2015; Raux et al., 2013); however, any influence of asthma on neural regions involved in memory, has only been explored in one study, observing smaller hippocampal volumes in middle aged individuals with

* Corresponding authors at: Department of Psychology, Southern Methodist University, 6116 N. Central Expressway, Suite 1160, Dallas, TX 75206, USA.
E-mail addresses: jkroll@smu.edu (J.L. Kroll), tritz@smu.edu (T. Ritz).

https://doi.org/10.1016/j.nicl.2018.04.012
Received 20 November 2017; Received in revised form 7 March 2018; Accepted 11 April 2018
Available online 12 April 2018
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asthma (Carlson et al., 2017). The influence of asthma on neural chemistry has, to the best of our knowledge, not been explored.

The primary neural region involved in memory is the hippocampus, a structure integral for encoding episodic memory and memory consolidation (Dudek et al., 2016). Changes in hippocampal volume and chemistry often coincide with reduced cognitive performance (Brown et al., 2004; Kantarci et al., 2002); however, no studies have examined the hippocampal chemistry of patients with asthma.

Neuroimaging technology has advanced from purely structural insights and functional imaging to non-invasive identification of chemical metabolite markers of CNS activity through magnetic resonance spectroscopy (^{1}H-MRS), a technology offering valuable in-vivo neural information, which was once otherwise limited to animal models of memory (Maddock and Buonocore, 2012). MRS can capture the metabolite *N*-acetylaspartate (NAA), an indicator of neuronal density and integrity; myo-inositol (MI), a putative marker of glial activation; glutamate (Glu), an excitatory neurotransmitter; and creatine (Cr), which additionally includes phosphocreatine and is used as a marker for cellular energy and metabolism (Allaili et al., 2015). NAA is the second most concentrated molecule in the brain after Glu and provides a critical source of acetate for myelin lipid synthesis in oligodendrocytes. NAA facilitates energy metabolism in neuronal mitochondria, and is therefore used as a putative marker of neuronal number, health, and viability (Maddock and Buonocore, 2012). MI is preferentially concentrated in glial cells and is used as a putative marker of microglial activation. Neuroinflammation is characterized by an increase in MI, and an increase of MI in neural regions has been found to be associated with and precede dementia (Voevodskaya et al., 2016; Targosz-Gajniak et al., 2013). Glu is the most prevalent excitatory neurotransmitter and its role is critical for the establishment of long term potentiation (LTP), which strengthens neural connections leading to gains in working and declarative memory (Rupsing et al., 2011). Cr is a precursor of adenosine triphosphate and therefore an indicator of cellular energy metabolism and storage, thought to be a relatively stable neural metabolite in both health and disease (Maddock and Buonocore, 2012; Allaili et al., 2015). Abnormalities in these four neural metabolites (deficits, elevations, or changes in their ratios) are observed in those with cognitive impairment, and have even been found to predict disease progression and future cognitive decline (Tumati et al., 2013; Modrego et al., 2011; Voevodskaya et al., 2016; Targosz-Gajniak et al., 2013; Rupsing et al., 2011).

Hippocampal gray matter volume deficits and alterations in hippocampal metabolites are additionally observed in chronic pulmonary inflammatory disease states, including COPD (Shim et al., 2001; Li and Fei, 2013; Esser et al., 2016); however, to the best of our knowledge, there are no studies examining hippocampal metabolites in asthma. Patients with asthma may be uniquely influenced by natural sequelae of a chronic systemic inflammatory disease, moments of hypoxia, asthma medication, and/or behavioral influences commonly associated with asthma including diminished sleep quality. Outside of the context of asthma, these factors independently demonstrate detectable influences on the hippocampus and cognition in both human and animal studies (Guo et al., 2013; Takada et al., 2015; Brown, 2009; Elcombe et al., 2017). We therefore hypothesized that hippocampal metabolites would be altered in asthma compared to healthy controls, and further hypothesized that lower levels of NAA and Glu would be associated with poorer cognitive function. We additionally hypothesized that asthma medication would demonstrate influences on hippocampal metabolites. As prior research suggests that hippocampal volume can be reduced in asthma (Carlson et al., 2017), we also analysed hippocampal structure to exclude the possibility that differences in metabolite levels were secondary to hippocampal volume. Studies in patients receiving chronic corticosteroid treatment have found no significant differences between left and right hippocampal metabolites (Brown et al., 2004), consistent with reviews of hippocampal metabolites in mild cognitive impairment (Maddock and Buonocore, 2012). Individuals with greater cognitive deterioration have shown lower levels of NAA in the left temporal lobe (Maddock and Buonocore, 2012), and we therefore focused our analyses on the left hippocampus.

2. Methods and materials

Additional methodological details are provided in the online supplement.

2.1. Participants

Twenty patients with a physician's diagnosis of asthma were compared to twenty age- and gender-matched healthy controls. Exclusion criteria for all participants included: neurological or cardiovascular disease, any other chronic inflammatory disease, lung disease besides asthma, history of smoking, current major depressive episode, current or recent history (within one year) of substance related disorders including alcohol abuse, recreational drug use, history of any manic episode, and symptoms of schizophrenia, bipolar disorder, or psychosis. Individuals who used corticosteroids (oral and injected) within the past 3 months were additionally excluded. As a precaution for the scanning environment, participants with values of forced expiratory volume in one-second (FEV_1) % predicted < 70%, were excluded (National Heart, Lung, and Blood Institute (NHLBI), 2007). FEV_1 is a clinical standard measure of mechanical lung function captured by spirometry.

2.2. General procedure

Participants completed questionnaires followed by a trained experimenter presentation of the Montreal Cognitive Assessment (MoCA) and spirometry. Within one week, administration of the Asthma Control Questionnaire (ACQ) and spirometry were repeated immediately prior to magnetic resonance imaging. Individuals with asthma were asked to refrain from taking rescue inhalers the day of either session and were encouraged to reschedule if needed. This study was approved by the University of Texas Southwestern Medical Center (STU 082011-038) and Southern Methodist University (2015-007-RITT) Institutional Review Boards. Written informed consent was obtained from all participants.

2.3. Magnetic resonance spectroscopy acquisition and analysis

MR assessments were carried out on a whole-body 3 T scanner (Philips Medical Systems, Best, The Netherlands), equipped with a whole-body coil for RF transmission and an 32-channel phased-array head coil for reception. Water-suppressed point-resolved spectroscopy (PRESS) data were acquired with TR = 2 s, TE = 112 ms, sweep width = 2.5 kHz, number of sampling points = 2048, and number of signal averages (NSA) = 256. Water suppression was obtained with a vendor-supplied four-pulse variable-flip-angle sub-sequence. First and second order shimming was carried out, using the fast automatic shimming technique by mapping along projections (FASTMAP). The RF carrier frequencies of the PRESS sequence were set at 2.5 ppm and were adjusted for B_0 drifts in each excitation using a vendor-supplied tool (Frequency Stabilization). Unsuppressed water was acquired from the voxel for eddy current compensation and multi-channel combination. Spectral fitting was performed with LCModel software (Provencher, 1993), using in-house basis spectra that were computer simulated incorporating the PRESS volume localizing radio-frequency and gradient pulses. The basis set included NAA, Cr (creatine + phosphocreatine), Glu, glutamine, GABA, glycine, MI, lactate, glutathione, alanine, acetate, aspartate, ethanolamine, phosphorylethanolamine, scyllo-inositol, taurine, *N*-acetylaspartylglutamate, glucose, Cho (glycerophosphorylcholine + phosphorylcholine). The spectral fitting was conducted between 0.5 and 4.1 ppm. Cramér-Rao lower bounds (CRLB) were returned as percentage standard deviation by LCModel.

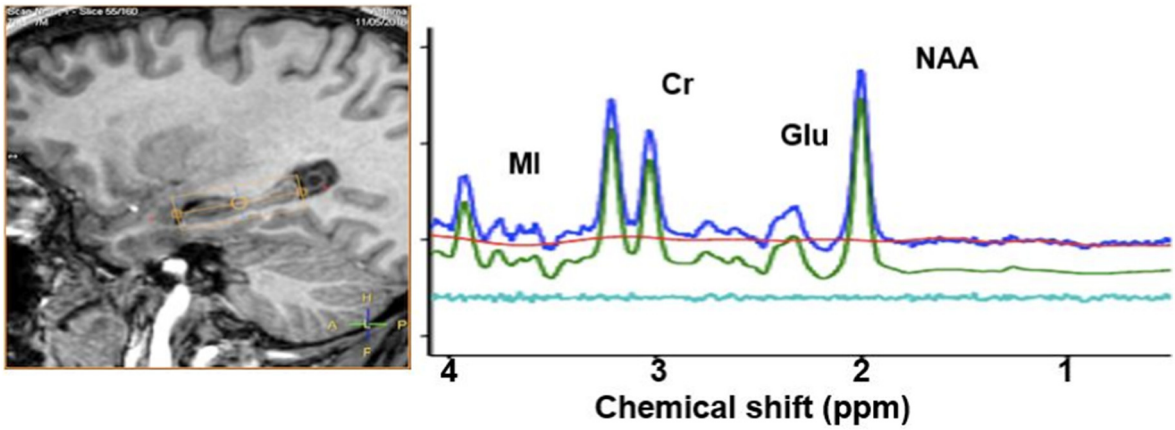

Fig. 1. MRI voxel placement and ^{1}H-MRS spectra from the hippocampus of a participant with asthma. Sagittal view demonstrates placement of the volume of interest in the left hippocampus. The area under the spectra represents the concentration of each metabolite in the identified tissue. Red line, baseline; blue line, in vivo data; green line, fit; light blue line, residual.
Abbreviations: Cr, creatine; NAA, *N*-acetylaspartate; MI, myo-inositol; Glu, glutamate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Metabolites were estimated with reference to both water and tCr (Fig. 1).

2.4. Magnetic resonance imaging acquisition and analysis

Structural MRI was conducted on the 3 T scanner described above, immediately prior to MRS imaging. A high-resolution T1-weighted image called magnetization prepared rapid acquisition of gradient-echo (MPRAGE) sequence was collected with the following parameters: FOV = 256 × 256 × 160 mm^3, TR/TE = 8.1 ms/3.7 ms, flip angle = 12°, 160 sagittal slices, voxel size = 1 × 1 × 1 mm^3 and duration of 4 min. The segmentation of left hippocampal region was performed via FSL-FIRST software (Schoemaker et al., 2016). Briefly, the images were initially registered to the MNI152 standard space template in a two-stage process, where the first stage was a whole-brain and the second stage was a subcortical-weighted 12 degrees of freedom linear fit. The registration was visually checked for each participant. The inverse of this transformation was applied to the segmentation model in order to bring it into the native space of the original (non-interpolated) T1-weighted image, where the segmentation was performed. In order to account for different brain sizes, we normalized the volume of the left hippocampus by the total intracranial volume (ICV). The ICV was calculated via FSL's BET and FLIRT routines.

2.5. Spirometry

FEV_1 was measured with a handheld spirometer (CareFusion Jaeger/Toennies AM-2) to capture the participants' best of three forced exhalations (National Heart, Lung, and Blood Institute (NHLBI), 2007).

2.6. Measures

Cognitive function was measured with the Montreal Cognitive Assessment (MoCA), a 10-minute global cognitive screening tool (Brown, 2009) designed to detect mild cognitive impairment (MCI) and assesses orientation, executive function, visuospatial skills, abstraction, language, memory, and attention. Scores range from 0 to 30, with those scoring above 26 extremely unlikely to meet MCI criteria (Nasreddine et al., 2005).

Asthma control was measured with the Asthma Control Test (ACT), a self-report measure assessing asthma control over the past month (Nathan et al., 2004), and the Asthma Control Questionnaire (ACQ), assessing asthma control over the previous week (Juniper et al., 1999). ACQ captures both self-report and lung function by spirometry (FEV_1% predicted).

2.7. Statistical analyses

Independent two sample *t*-tests calculated between group (asthma vs. healthy) differences in hippocampal metabolites and volume. Non-parametric Mann-Whitney test was calculated for group differences in Glu, as it violated assumptions of normality. Pearson partial correlations calculated relations among: hippocampal metabolites, cognitive scores, medication use and asthma control, controlling for hippocampal volume. IBM SPSS (Version 24) was used for analyses, and all variables demonstrated properties of normality. As group differences were observed in Cr, total metabolite values in reference to water, rather than in reference to Cr, were used for analyses according to recommendations of Tumati et al. (2013). MRS data was low quality for 5 subjects (3 asthma, 2 healthy), whose MRS data was excluded from analyses. Questionnaire and behavioral measures were retained for the entire sample.

3. Results

3.1. Participants

Participants were on average 25 years old and had a high education level (most were recruited from a local university). There was neither a statistically significant difference between the groups on demographics, years of education, nor physical characteristics (Table 1). Of those with asthma, 13 had well-controlled asthma with ACT values > 19, and only two participants reported a single lifetime occurrence of an asthma-related emergency room visit, none within the past year (Table 1). Asthma severity ranged from intermittent to severe, based on National Asthma Education and Prevention Program guidelines (National Heart, Lung and Blood Institute, 2007) (Table 1). Individual classifications of asthma severity, asthma control scores, and medication prescriptions are provided in the supplement.

3.2. Differences between asthma and healthy groups

Individuals with asthma had lower levels of both NAA, $t(33) = -3.23$, $p = 0.003$ ($d = 1.12$) and Cr, $t(33) = -3.31$, $p = 0.002$ ($d = 1.15$) compared to controls (Table 2), and demonstrated a non-significant trend in that direction with Glu, $U = 204.0$, $p = 0.096$ ($d = 0.53$) (Fig. 2). There were no statistically significant group differences in hippocampal volume, $t(38) = 0.42$, $p = 0.677$, MI, t

Table 1
Participant demographics and characteristics.

	Asthma n = 20			Healthy Controls n = 20			p
	Mean	SD	Min–Max	Mean	SD	Min–Max	
Age (years)	25.3	8.9	18–44	25.10	8.88	18–45	0.958
Gender (male)	50%			50%			1.00
Education (years)	14.9	2.5	12–23	15.10	3.06	12–25	0.800
Height (m)	1.7	0.1	1.5–1.9	1.70	0.09	1.52–1.85	0.893
Weight (kg)	71.3	17.5	45.4–127.0	76.04	17.21	53.5–117.9	0.391
BMI (kg/m^2)	24.5	4.8	17.7–38.0	26.39	5.38	18.5–37.7	0.261
MoCA (0 − 30)	27.1	2.5	21–30	28.05	2.01	23–30	0.190
FEV_1 (% predicted)	95.0	10.5	77.0–116.0	100.3	13.3	80.0–130.0	0.366
L hippocampal volume	2.4e^3	3.2e^4	1.5e^3–2.8e^3	2.4e^3	3.1e^4	1.7e^3–2.9e^3	0.677
Asthma duration (years)	17.10	8.03	7–44				
Lifetime asthma-related ER visits	0.10	0.31	0–1				
ACT (5–25)	23.30	3.76	12–25				
ACQ (0–6)	0.99	0.55	0.14–2.14				
Asthma severity[a] (%)							
Intermittent	22						
Mild (persistent)	44						
Moderate (persistent)	22						
Severe (persistent)	11						
Race (%)							0.617
White/Caucasian	60			45			
Asian	5			0			
Native American	0			0			
African American	5			30			
Other	10			5			
Ethnicity (%)							0.937
Hispanic	20			20			

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 s; MoCA, Montreal Cognitive Assessment; ACT, Asthma Control Test; ACQ, Asthma Control Questionnaire.
Note, independent samples (two-tailed) *t*-test found no statistically significant difference between groups on any variables. Ranges of questionnaire scores are provided in parentheses after each measure. ACT is scored with higher values indicating better control and ACQ is scored with lower values indicating better control.
[a] Asthma severity was calculated for participants on short-term medication and for those on maintenance medication whose asthma was well controlled according to NIH/NAEPP (2007) severity and medication step therapy guidelines. Two individuals who were on maintenance medication and did not have well-controlled asthma according to the ACT, were therefore excluded from severity classification.

(33) = −0.65, $p = 0.521$, or global cognitive function scores, t (38) = −1.34, $p = 0.19$.

3.3. Hippocampal metabolites and cognitive function

Controlling for hippocampal volume, MoCA scores were significantly correlated with total Glu ($r = 0.35$, $p = 0.038$) and demonstrated a trend in this direction with NAA ($r = 0.29$, $p = 0.092$) for all participants, suggesting that those with higher levels of these metabolites, in particular Glu, performed better on a measure of global cognitive function (Table 3, Fig. 3). In individuals with asthma only, the correlations controlling for hippocampal volume followed a similar pattern; however, they were not statistically significant (*Online Supplement*).

3.4. Hippocampal metabolites and asthma medication

Frequency of short-acting bronchodilator use in the past week was negatively correlated with total NAA ($r = -0.56$, $p = 0.020$) and Glu ($r = -0.58$, $p = 0.014$), controlling for hippocampal volume, and demonstrated a nonsignificant correlation in that direction with Cr ($r = -0.39$, $p = 0.138$), indicating that more frequent use of rescue inhaler was related to lower levels of hippocampal metabolites (Table 4, Fig. 4). On the other hand, inhaled corticosteroid use (yes/no) was positively correlated with total Cr ($r = 0.70$, $p = 0.002$) and demonstrated a positive trend with NAA ($r = 0.43$, $p = 0.082$) and Glu ($r = 0.40$, $p = 0.113$), indicating that those on inhaled corticosteroids, clinically recommended for long-term asthma control, had higher levels of Cr (Table 4).

Table 2
Total left hippocampal metabolites in reference to water.

	Asthma (n = 17)			Healthy controls (n = 18)			p	r
	Mean	SD	Range	Mean	SD	Range		
tCr**	9.15	1.46	6.85–11.70	10.57	1.06	9.04–12.71	0.003	−0.49
tNAA**	13.56	0.92	10.07–16.40	15.31	1.24	12.85–17.84	0.004	−0.49
tMI	11.20	2.16	7.35–14.90	11.72	2.56	6.95–15.93	0.521	−0.11
tGlu	9.52	2.01	6.19–12.65	10.33	0.75	9.00–11.41	0.096	−0.26

Independent samples (two-tailed) *t*-test between group (asthma vs. healthy controls). Non-parametric independent samples Mann-Whitney *U* test was used for tGlu.
Abbreviations: SD, standard deviation; *r*, effect size; tCr, total creatine; tNAA, total *N*-acetylaspartate; tMI, total myo-inositol; tGlu, total glutamate.
** Indicates significant difference between groups $p < 0.01$.

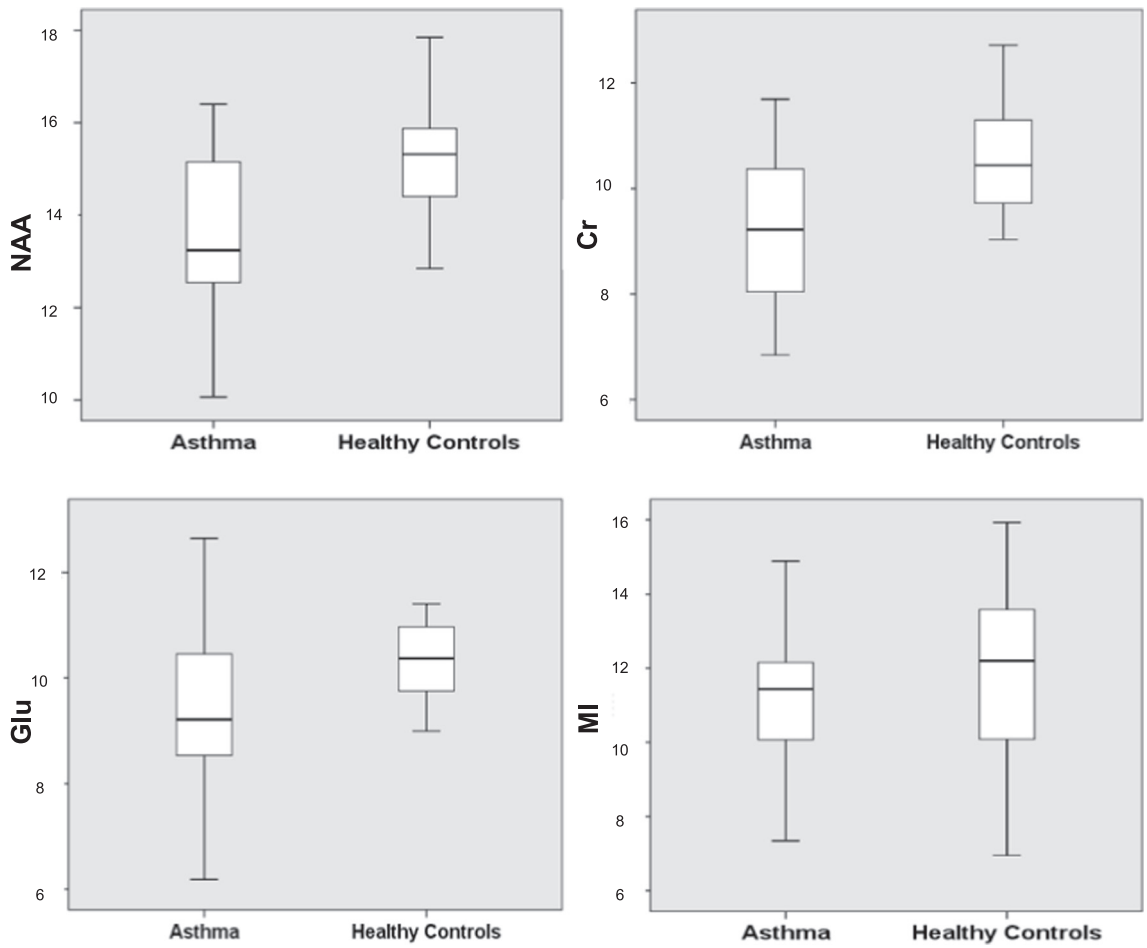

Fig. 2. Group differences on total values of left hippocampal metabolites. *Abbreviations*: Cr, creatine; NAA, *N*-acetylaspartate; MI, myo-inositol; Glu, glutamate.

Table 3
Correlations among hippocampal metabolites, global cognitive function, and asthma control.

Variables	1	2	3	4	5
1. tCr	–				
2. tNAA	0.58**	–			
3. tMI	0.33†	0.28†	–		
4. tGlu	0.65**	0.70**	0.32†	–	
5. MoCA	0.27	0.29†	0.16	0.35*	–
6. ACQ	−0.21	−0.14	−0.03	−0.31	−0.46*

Pearson partial correlations (two-tailed), controlling for hippocampal volume. All individuals included in correlations with MoCA. Only individuals with asthma.
(n = 17) are included in correlations with ACQ. Pearson correlation (two-tailed) included for relation of ACQ and MoCA.
Abbreviations: ACQ, Asthma Control Questionnaire, higher scores indicate poorer control; MoCA, Montreal Cognitive Assessment, higher scores indicate better cognitive function; tCr, total creatine; tNAA, total *N*-acetylaspartate; tMI, total myo-inositol; tGlu, total glutamate.
* Indicates $p < 0.05$.
** Indicates $p < 0.01$.
† $0.05 < p < 0.10$.

3.5. Hippocampal metabolites, asthma control, and cognitive function

Hippocampal metabolites were not significantly correlated with asthma control; however, asthma control for the week before the imaging assessment, measured by the ACQ, was correlated with the MoCA, ($r = -0.46$, $p = 0.040$), indicating that those with poorer asthma control had lower cognitive scores (Table 3, Fig. 5). This finding was no longer significant when controlling for age.

Additional results of metabolite/Cr ratios and exploratory correlations among metabolites, disease duration and asthma-related nocturnal awakenings are included in the *Online Supplement*.

4. Discussion

4.1. Key findings

The present work demonstrates that in younger individuals with asthma, there are substantial changes in the hippocampal chemical profile. Even in the absence of cognitive impairment, those with asthma had reductions in both hippocampal NAA, a marker of neuronal integrity and Cr, a marker of cellular energy metabolism. These metabolites, in particular Glu, were additionally related to lower cognitive performance, after controlling for hippocampal volume. There was no group difference in hippocampal volume in this cognitively healthy sample, which was on average 25 years younger than that of previous studies observing hippocampal volume deficits in asthma (Carlson et al., 2017). However, the changes already observed in hippocampal metabolites in asthma before the onset of gross structural changes, may inform our understanding of the biological contributions to cognitive impairment observed in individuals with asthma over the lifespan.

4.2. Hippocampal metabolites in asthma

Our findings of reduced hippocampal NAA, already present in younger patients with asthma compared to age and gender matched

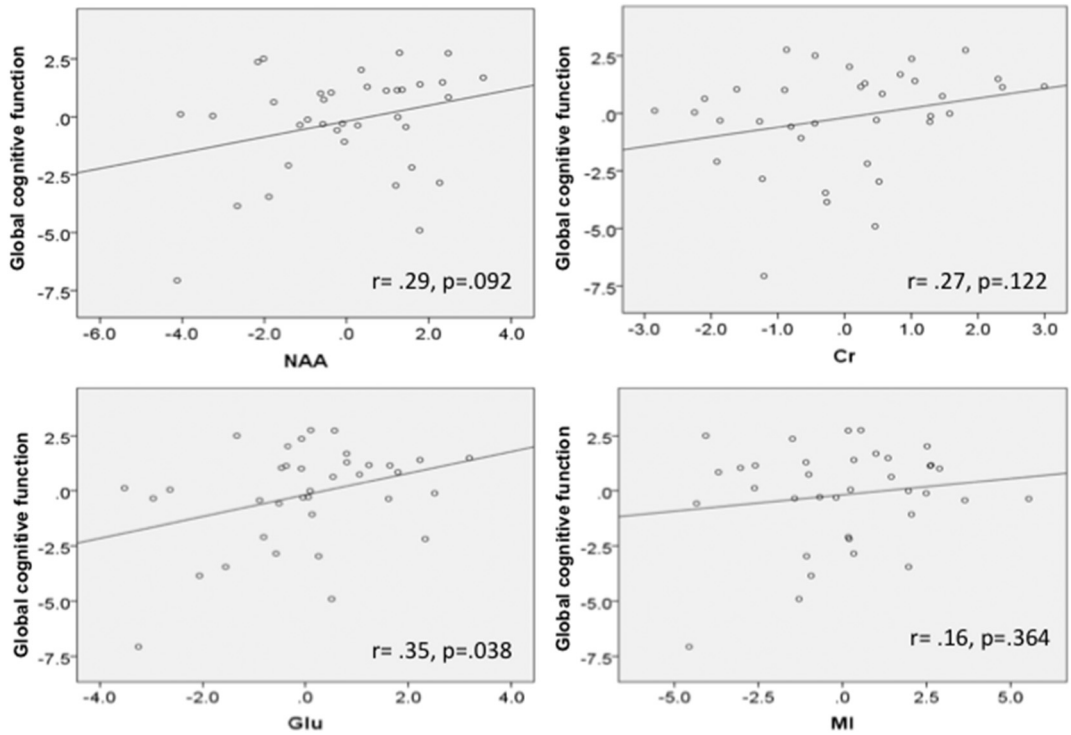

Fig. 3. Residual plots of left hippocampal metabolites and global cognitive function scores captured by the Montreal Cognitive Assessment, controlling for hippocampal volume in the entire sample. Statistics for Pearson partial correlations (two-tailed) displayed, controlling for left hippocampal volume. *Abbreviations*: Cr, creatine; NAA, *N*-acetylaspartate; MI, myo-inositol; Glu, glutamate.

Table 4
Correlations among hippocampal metabolites and asthma medication use.

Variables	1	2	3	4	5
1. tCr	–				
2. tNAA	0.59*	–			
3. tMI	0.33	0.42†	–		
4. tGlu	0.73**	0.76**	0.57*	–	
5. Inhaled corticosteroid use (yes/no)	0.70**	0.43†	0.38	0.40	–
6. Short-acting bronchodilator use (past week frequency)[a]	−0.39	−0.56*	−0.12	−0.58*	−0.19

Pearson partial correlations (two-tailed), controlling for hippocampal volume. *Abbreviations*: tCr, total Creatine; tNAA, total *N*-acetylaspartate; tMI, total myo-inositol; tGlu, total glutamate.

* $p < 0.05$.

** $p < 0.01$.

† $0.05 < p < 0.10$.

[a] Item from the Asthma Control Questionnaire (ACQ).

healthy controls, complement previous observations of hippocampal volume deficits (Carlson et al., 2017) and increased risks of mild cognitive impairment (Caldera-Alvarado et al., 2013) in middle-aged individuals with asthma and affirm the influence of this chronic inflammatory disease not only on hippocampal structure, but also on its chemical composition. NAA is considered the most reliable MRS marker of brain dysfunction in mild cognitive impairment (Tumati et al., 2013), and lower levels of NAA are additionally observed to predict future cognitive decline (Modrego et al., 2011). Despite the absence of cognitive deficits in this younger sample, there are already differences in hippocampal NAA in individuals with asthma, which may precede future cognitive decline.

While the exact comparison of total NAA values observed in the present study to those previously reported is limited due to fewer studies reporting total NAA, differences in scanner strength, sample age, volume of interest size, and post processing methods, the reduction of hippocampal NAA in individuals with asthma demonstrated a medium effect size, consistent with the magnitude of change observed in mild cognitive impairment studies (Tumati et al., 2013). Compared to other disease groups who show reductions in hippocampal NAA compared to healthy controls, this sample demonstrated a smaller effect size compared to those with multiple sclerosis (Llufriu et al., 2014), schizophrenia (Maddock and Buonocore, 2012) and treatment resistant depression in youth (Lefebvre et al., 2017). The present findings suggest that there is a reduction in the hippocampal neuronal integrity of younger patients with asthma, which may be an important component to understand neural mechanisms contributing to the increased rate of cognitive deficits observed with asthma.

In addition to NAA, reductions in hippocampal Cr were observed in individuals with asthma. Cr is a marker of cellular energy thought to be stable neural metabolite in health and disease (Maddock and Buonocore, 2012; Allaili et al., 2015). As such, it has been used routinely as a reference for other metabolites. However, individuals with cognitive impairment have demonstrated reductions in hippocampal Cr, raising concerns for a potential Cr confound if metabolite/Cr ratios only are reported (for review see Tumati et al., 2013). As MRS technology has developed, a water reference can now be captured in the same scan, minimizing a potential confound of Cr, if it is influenced by disease process. The present findings support that concern and suggest the need for future studies to additionally capture total metabolic levels in asthma.

Despite similar levels of cognitive functioning across groups, the present findings may indicate pre-clinical influences of asthma control on global cognitive function. Poorer asthma control was correlated with lower cognitive scores, consistent with previous studies identifying variability in diurnal peak flow (used as a proxy for airway hyperreactivity, which is a hallmark of asthma) as the strongest predictor of cognitive function (O'Byrne et al., 2013). While it cannot be ruled out that poorer cognitive function leads to poorer asthma control with this cross-sectional study design, these first findings extend hypothesized influences of asthma on cognition beyond asthma severity (Irani et al.,

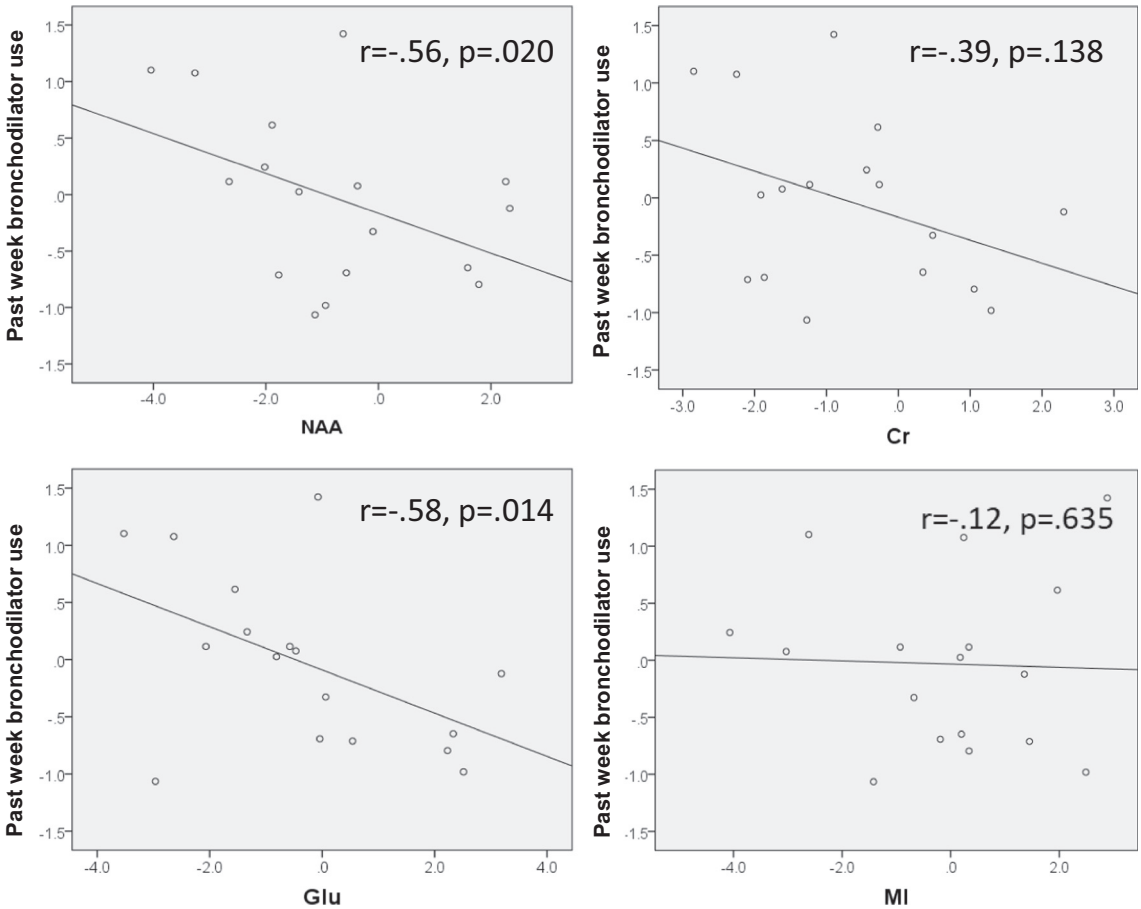

Fig. 4. Residual plots of left hippocampal metabolites and frequency of short-acting bronchodilator use in the past week for the asthma group only, controlling for left hippocampal volume. Statistics for Pearson partial correlations (two-tailed) displayed, controlling for left hippocampal volume. *Abbreviations*: Cr, creatine; NAA, *N*-acetylaspartate; MI, myo-inositol; Glu, glutamate.

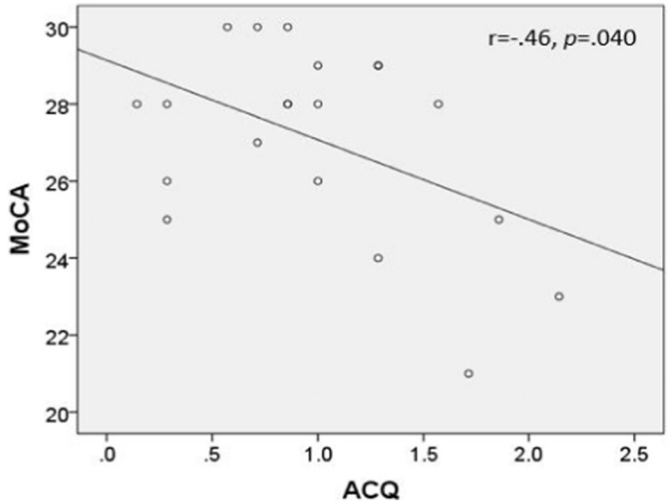

Fig. 5. Relations of Asthma Control Questionnaire (ACQ) and MoCA (Montreal Cognitive Assessment) scores. Pearson correlation (two-tailed) displayed.

2017).

While future longitudinal research is needed to identify specific mechanisms for cognitive impairment in asthma, the present findings of deficits in markers of hippocampal neuronal viability, cellular energy metabolism and their relations with poorer global cognitive scores provides first insight into neural processes that may contribute to poorer cognitive function observed in individuals with asthma (Irani et al., 2017).

4.3. Potential mechanisms

There are a number of potential explanations for the changes observed in these hippocampal metabolites.

First, inflammatory processes in asthma could affect hippocampal chemistry. Potential mechanisms include oxidative stress, which may influence both Th_2 and Th_1 immune responses and activate additional pro-inflammatory cytokines through NF-kB (Dozor, 2010). Active binding of peripheral cytokines to endothelial receptors may release additional mediators that impair blood-brain barrier integrity (Di Benedetto et al., 2017). Peripheral cytokines may also influence the CNS through stimulation of vagal sensory nerve or the sympathetic nervous system (Di Benedetto et al., 2017), which then induce central pro-inflammatory cytokine production, known to influence hippocampal chemistry (Barrientos et al., 2015).

Second, medication use may influence hippocampal metabolites. Systemic oral corticosteroid use has established dose-dependent influences on both poorer cognitive function and reduced hippocampal volume (Brown and Chandler, 2001; Brown, 2009), and individuals receiving chronic prednisone therapy have demonstrated reductions in hippocampal NAA/Cr (Brown et al., 2004). Participants in this study did not have recent exposure to systemic corticosteroids; however, use of such medications over a lifetime has demonstrated lasting influences on neurological and cognitive function (Shim et al., 2001). Although this study was not designed to elucidate the specific mechanisms of medication, exploratory analyses found evidence for positive effects of

inhaled corticosteroids on Cr, NAA, and Glu. In contrast to high systemic doses of corticosteroids, local administration of this medication in small doses leads to better control of asthma (O'Byrne and Parameswaran, 2006) and therefore may have helped avoid some of the adverse CNS asthma sequelae. The additionally observed associations between more frequent rescue inhaler usage and lower levels of Cr, NAA, and Glu would be consistent with that interpretation. When individuals with asthma followed clinically recommended medication guidelines to achieve proper control (e.g. use of maintenance medication rather than relying on more frequent use of rescue inhalers; National Heart, Lung and Blood Institute, 2007), their hippocampal metabolites were more in line with those observed in the healthy sample. However, medication manipulation studies are needed to draw any further claims beyond this cross sectional association.

Third, hypoxia could be speculated to play a role, given its demonstrated influence on hippocampal structure, function and cognitive behavior in both animal and human studies (Takada et al., 2015). While mechanistically probable, prolonged moments of hypoxia are less frequent in physician monitored cases of controlled asthma and lifetime visits to the emergency room due to asthma exacerbation in this sample were infrequent (Table 1).

It is additionally possible that reductions in NAA and Cr may be influenced indirectly by behavioral factors common in chronic disease states, rather than the direct influence of asthma or its treatment. Sleep disruption, which is common in asthma, can influence hippocampal chemistry independently (Cross et al., 2013) and one of our supplemental findings, demonstrating an association between asthma-related night-time awakening and higher MI levels, points in that direction (*Online Supplement*). Equally important may be psychological factors, including anxiety and depression, which can additionally be comorbid with asthma and separately affect hippocampal chemistry (Brown et al., 2004). In other chronic respiratory disease states such as COPD, hippocampal activation during the anticipation of increased dyspnea is associated with behavioral variables including symptoms of dyspnea, anxiety, and reduced exercise capacity (Esser et al., 2017). This may be contrary to the present findings with asthma; however, it nonetheless indicates the importance of studying behavioral factors and the hippocampus in those with respiratory disease.

4.4. Further considerations

This is the first study, to the best of our knowledge, to test differences in the hippocampal metabolic profile of individuals with and without asthma, and further explore the relations between disease variables, cognition, and neural chemistry. This study not only provides first evidence of absolute differences in hippocampal neuronal integrity, but additionally highlights their importance, along with asthma control, in cognitive function.

Limitations of this study include a modest sample size, which may have led to lower power to detect differences in smaller MRS signals, such as Glu, which demonstrated reductions with a smaller effect size (Table 2). Nevertheless, there was evidence of robust group differences in NAA and Cr, with medium effect sizes. Neither older individuals, nor those with cognitive impairment were represented in this sample and likely limited the strength of observable relations among asthma, hippocampal chemistry and cognition. However, our restricted range established a more conservative estimate of such influences and highlights the fact that reductions in the hippocampal neuronal integrity are already observed at a younger age and may precede cognitive impairments observed in older adults with asthma. The brevity and global nature of the MoCA likely limited the measure's sensitivity to detect uniform statistically significant relations with metabolites of a neural region primarily involved in memory. However, the consistent pattern of relations across multiple metabolites contributes to increased confidence in their relations to cognition. The associations of the MoCA and asthma control may indicate that additional cognitive domains, such as exectuive function, are more associated with asthma control, a finding that is consistent with studies observing poorer academic performance observed in childhood asthma (Liberty et al., 2010). As such, a more comprehensive cognitive assessment, specific to memory, may yield stronger relations between metabolites and cognitive performance and the association between asthma control and MoCA scores may indicate asthma control may be related to multiple domains of cognitive function, rather than memory alone. Although we observed a relation between use of inhaled corticosteroids and hippocampal metabolites in this sample, medication use was recorded here as a binary variable to capture the comparison between corticosteroid-naïve participants and those who were prescribed inhaled corticosteroids. A more invested measurement of medication adherence and dosage over time using electronic monitoring was beyond the scope and means of our study, but will be important to capture in future studies of medication influences on hippocampal chemistry.

Despite these limitations, our findings demonstrate, for the first time, changes in hippocampal chemical profile of individuals with asthma, and their relations with cognitive performance. Future studies will require: replication with larger samples; inclusion of both left and right hippocampal metabolites to assess for potential lateralization (Shipton et al., 2014); extensions to additional neural regions relevant to cognition, asthma, and dyspnea (e.g. prefrontal cortex, insula, cingulate cortex, periaqueductal gray (Takada et al., 2015; Von Leupoldt et al., 2009b); use of diffusion tensor imaging to determine integrity of white matter tracts in asthma (Dodd et al., 2012); markers of peripheral immune function; and comprehensive neuropsychological assessment exploring specific components of memory. Studies with a longitudinal perspective could further explore potential moderating variables including: medication use, disease duration, asthma control, and sleep. Studies that are designed to test longitudinal mediation models, particularly for medication usage, will be important to include in future research.

The present study indicates, for the first time, that hippocampal neuronal integrity is reduced in asthma compared to healthy controls, providing insight into neural mechanisms, which may ultimately influence cognitive deficits observed in individuals with asthma. These findings provide additional evidence to support the recommendations (Irani et al., 2017) to screen for presence of cognitive impairment in individuals with asthma, particularly those who have poor disease control.

Acknowledgments

This research was supported by National Institute on Aging (NIA) R24AG048024 and the Southern Methodist University Research Council (URC 413876). ESB holds the Aradine S. Ard Research Chair in Brain Science. The authors thank Binu Thomas, Lilly Yang, and Salvador Pena (The University of Texas Southwestern Medical Center, Advanced Imaging Research Center) for their help with imaging acquisition; Sharon Deol, Maryam Saifai, Julie Kim, and Steve Dorman (The University of Texas Southwestern Medical Center, Internal Medicine) for their help with data collection; and Brittany Mason and Alexandra Kulikova (The University of Texas Southwestern Medical Center, Psychiatry) for administrative help.

Financial disclosures

Dr. Brown reports having received research funding from Otsuka and lecture fees from Genentech. Dr. Steele reports stock ownership in GlaxoSmithKlein. Dr. Khan reports no biomedical financial interests or potential conflicts of interest. Dr. Pinkham reports no biomedical financial interests or potential conflicts of interest. Dr. Ritz reports no biomedical financial interests or potential conflicts of interest. Dr. Chen reports no biomedical financial interests or potential conflicts of interest. Dr. Patel reports no biomedical financial interests or potential

conflicts of interest. Dr. Choi reports no biomedical financial interest or potential conflicts of interest. Ms. Kroll reports no biomedical financial interests or potential conflicts of interest. Dr. Aslan reports no biomedical financial interests or potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2018.04.012.

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Online Data Supplement

Title: Hippocampal metabolites in asthma: implications for cognitive function

Authors: Juliet L. Kroll, Ashton M. Steele, Amy E. Pinkham, Changho Choi, David. A. Khan, Sheenal V. Patel, Justin R. Chen, Sina Aslan, E. Sherwood Brown, Thomas Ritz

DETAILED METHODS

This data was collected in the context of a larger study on cognition, emotion, and brain activity in asthma. Image acquisition was completed in one session including structural Magnetic Resonance Imaging and Proton Magnetic Resonance Spectroscopy. The study was approved by the Institutional Review Boards of University of Texas Southwestern Medical Center (STU 082011-038) and Southern Methodist University (2015-007-RITT), and all participants provided written informed consent. Participants were compensated $65 for their participation in the imaging session. All participants provided verbal consent for an initial phone screen and written consent before participating in the assessments.

Sample

Patients with asthma (n=20) and age and gender matched healthy controls (n=20) were recruited from the general community through flyer advertisements, online postings, previous research study participants who consented to be contacted for future studies, and a research pool of undergraduate students, between August 2014 and April 2017. Criteria for eligibility included: age between 18 and 55 years; English as first language; and a physician's diagnosis of asthma (for the asthma group only). Exclusion criteria included: endorsement of current clinically significant levels of depression; current or recent history (within 1 year) of substance

related disorders including alcohol abuse (5 or more drinks on one occasion), recreational drug use; any manic episode, symptoms of schizophrenia, bipolar disorder, dementia or psychosis; cardiovascular disease; neurological disorder; any lung disease other than asthma; current smoking or history of smoking; and use of corticosteroids (oral or injected) in the previous 3 months. A clinical psychology doctoral student screened all of the patients over the phone for eligibility and asthma diagnosis was confirmed with the participant's physician through medical records.

All participants were additionally screened for the presence of orthopedic circumstances or metallic inserts deemed unsafe for MRI or metallic inserts that may interfere with the image (braces or othodonture). Female participants completed a urine test to exclude pregnancy.

Protocol

Participants completed two study sessions. At the first session, participants underwent a brief cognitive test and provided self-report questionnaires and measurements of spirometric lung function. At the second session, participants completed additional self-report questionnaires and measurements of lung function, followed by a structural MRI and MRS scan, with volume of interest placed in the left hippocampus.

For all participants with asthma, their rescue inhaler was on hand and an allergist medical fellow was present in the control room in case of adverse event. No participants required the use of their inhaler or experienced asthma exacerbations during the study. All participants were asked to refrain from eating or drinking anything besides water or exercise for 1 hour before the study session. The MRS sequence was conducted as the final scan, on average 60 minutes after participant arrived.

Airway assessment

Forced expiratory volume in one second (FEV_1) was measured by handheld spirometer (*Jaeger/Toennies AM-2*) to capture the participants' best of three forced exhalations and the percentage of predicted FEV_1 was scored. FEV_1 was additionally used as a safety precaution to ensure no individuals with values ≤70% predicted entered the scanning environment. As no participants presented with FEV1% predicted ≤70, all subjects progressed through the entire protocol.

Questionnaire measures

The Hospital Anxiety and Depression Scale, HADS: Symptoms of anxiety and depression over the past week were assessed with the HADS (1), a 14-item self-report questionnaire with separate scales for symptoms of anxiety and depression.

Perceived Stress Scale, PSS: Symptoms of perceived stress over the past month were assessed with the PSS (2), a 10-item self-report questionnaire designed to capture self-perception of psychological stress.

The Montreal Cognitive Assessment, MoCA: Cognitive function was assessed with the brief cognitive screening tool, MoCA (3), designed to detect mild cognitive impairment (MCI). This 10 minute screening tool has demonstrated adequate test re-test reliability, with correlations ranging from 0.75-0.96 depending on population re-test interval length ranging from 2 to 8 weeks (4) . The MoCA additionally demonstrates sufficient internal consistency, yielding a Cronbach alpha of .83 for individual tasks. Composite scores reliably discriminate between patients with mild cognitive impairment and normal controls while controlling for effects of age and education (3). Individuals are scored on a 30-point scale, with those scoring

above 26 “extremely unlikely to meet clinical and neuropsychological criteria for MCI even after extensive evaluation” (3). Subsequent large studies determined mean MoCA total scores in a population without cognitive decline at 23.36, indicating that individuals scoring above 23 may lack any cognitive impairment (5). The MoCA demonstrates very good to good specificity for identifying normal controls (87%) and excellent sensitivity in identifying MCI (90%) with sensitivity calculated as the percentage of normal controls who scored at or above the cut off score of 26 (3).The MoCA specifically assesses eight cognitive domains: short-term memory recall, visuospatial ability, executive functioning (trail making, phonemic fluency, and verbal abstraction), attention, concentration, working memory, language, and orientation to time and place (3). In comparison to the Mini Mental Status Examination (MMSE), the MoCA has demonstrated superior sensitivity to detecting MCI (3; 6) and was selected for the present study due to the brevity of administration and for continuity with previous large samples demonstrating an association with asthma diagnoses and hippocampal biomarkers (7; 8). The MoCA was scored by two raters to ensure accuracy, with a high *ICC*=.99, indicating excellent inter-rater reliability for the MoCA ratings.

Asthma Control Questionnaire (ACQ): Asthma control over the previous week was measured by the ACQ, a brief 7-item instrument that has demonstrated high reliability (ICC=0.90) and adequate validity with other measures of general health status and asthma-related quality of life (9). The ACQ consists of 7 multiple choice items reflecting participants’ experience of asthma symptoms in the past week (e.g., “How much of the time did you wheeze?”, “How much shortness of breath did you experience because of your asthma?”), symptom interference (e.g., “How often were you woken by your asthma during the night?”, ”How bad were your asthma symptoms when you woke up in the morning?”, “How limited

were you in your activities?”) and medication use (“How many puffs of a short-acting bronchodilator have you used each day?”). Participants ranked items from a 0 – 6 scale, with 0 indicating the absence of symptoms and 6 indicating most severe ranking of symptoms. The last item is the participant’s lung function scored a 0-6 scale according to rankings of percentages of norm values. All items on the ACQ were equally weighted and a mean score calculated and used for analyses.

Asthma Control Test (ACT): Asthma control over the past four weeks was measured by the ACT, a 5 item self-report instrument which has demonstrated good internal consistency (α=.84) and moderate correlation between a specialist’s ratings (10). The ACT consists of 5 multiple choice questions, reflecting the participants’ experience of asthma symptoms (e.g. “How often have you had shortness of breath?”), daytime interference (“How much of the time did your asthma keep you from getting as much done at work, school, or at home?”), nighttime interference (“How often did your asthma symptoms wake you up at night or earlier than usual in the morning?”), medication use (“How often have you had to use your rescue inhaler or nebulizer medication?”), and perception of asthma control (“How would you rate your asthma control during the past 4 weeks?”). All items are equally weighted with total scores ranging from 5 (poor control) to 25 (total control) (10). Both the ACT and ACQ are designated as core instruments for National Institutes of Health initiated clinical research trials in adults (11).

MRS Acquisition and Analysis:

MR assessments were carried out on a whole-body 3T scanner (Philips Medical Systems, Best, The Netherlands), equipped with a whole-body coil for RF transmission and a 32-channel phased-array head coil for reception. Water-suppressed point-resolved spectroscopy

(PRESS) data were acquired with TR = 2 s, TE = 112 ms, sweep width = 2.5 kHz, number of sampling points = 2048, and number of signal averages (NSA) = 256. Water suppression was obtained with a vendor-supplied four-pulse variable-flip-angle sub-sequence. First and second order shimming was carried out, using the fast automatic shimming technique by mapping along projections (FASTMAP). The RF carrier frequencies of the PRESS sequence were set at 2.5 ppm and were adjusted for B_0 drifts in each excitation using a vendor-supplied tool (Frequency Stabilization). Unsuppressed water was acquired from the voxel for eddy current compensation and multi-channel combination. Spectral fitting was performed with LCModel software (12), using in-house basis spectra that were computer simulated incorporating the PRESS volume localizing radio-frequency and gradient pulses. The basis set included NAA (N-acetylaspartate), tCr (creatine+phosphocreatine), glutamate, glutamine, GABA, glycine, myo-inositol, lactate, glutathione, alanine, acetate, aspartate, ethanolamine, phosphorylethanolamine, scyllo-inositol, taurine, N-acetylaspartylglutamate, glucose, tCho (glycerophosphorylcholine + phosphorylcholine). The spectral fitting was conducted between 0.5 and 4.1 ppm. Cramér-Rao lower bounds (CRLB) were returned as percentage standard deviation by LCModel. Metabolites were estimated with reference to both water and tCr.

Additional Results:

Correlations among Sleep, Cognitive Function and Hippocampal Metabolites

Exploratory analyses also found that asthma-related sleep interruption captured by the ACQ was positively correlated with MI (r=.50, p=.049), controlling for hippocampal volume, and night-time asthma-related awakenings captured by the ACT, scored in the opposite direction from the ACQ, were negatively correlated with the MoCA (r=.60, p=.005), suggesting

that asthma-related sleep disturbances are related to higher glial inflammation and poorer cognitive function (Table 5).

Hippocampal Metabolites, Age and Cognitive Function

As differences were observed in the neurochemical profile between the two groups, secondary analyses tested correlation of age in each group. As predicted, age was negatively correlated with NAA in asthma only (r=-.52, p=.039), controlling for hippocampal volume, which indicated that older patients with asthma had lower levels of NAA, a marker of neuronal integrity. There was no significant association of age with NAA in healthy controls. In in asthma only, age was negatively correlated with MoCA (r=-.47, p=.039), indicating older participants with asthma had poorer global cognitive function. There was no significant association of age with cognitive function among healthy controls.

Additional Discussion

Behavioral patterns are known to influence CNS structure and function, including the hippocampus. Exploratory analyses indicated the potential influence of asthma-related sleep interruptions on poorer cognitive function, consistent with literature on sleep and cognition (13). Sleep interruptions have demonstrated influences on hippocampal function (14), and in the present study asthma-related sleep interruptions were correlated with increased hippocampal MI, a putative marker of glial inflammation and a metabolite whose elevations are observed to predict future cognitive decline (15, 16). These findings combined with previous studies examining the role of sleep in reductions hippocampal neuronal integrity (17), point to the likely role of sleep behavior influencing hippocampal chemistry of asthma patients.

The present findings additionally point to a differential influence of aging on hippocampal inflammation and global cognitive function in individuals with asthma. Exploratory analyses revealed significant associations between age and NAA, a marker of neuronal integrity, in the asthma sample only. NAA is facilitates energy metabolism in neuronal mitochondria, with reductions observed in healthy aging as well as in individuals with mild cognitive impairment (15). While the association of age and hippocampal NAA is established in healthy aging, it is surprising that these relations were already present in a younger asthma sample, with no evidence of this relation in the age matched healthy controls. Age was additionally correlated with poorer cognitive scores, in the asthma sample only. These findings may be cautiously interpreted as a potential indication that neurochemical changes, typical of an aging process, may be stronger or accelerated in individuals with asthma.

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Table 1. Participant demographics, medication status, lifetime emergency room visits, and current asthma severity and control

Participant	Age	Sex	Asthma Medication (usage)	Lifetime Asthma-Related Emergency Room visits	Asthma Severity (Intermittent to Severe)	ACT Score (5-25)
1	21	M	SABA (as needed)	0	Intermittent	22
2	24	M	LABA and ICS (daily); SABA (as needed)	1	Severe*	15
3	34	F	SABA (daily)	0	Moderate	19
4	44	F	SABA (daily)	0	Mild	14
5	20	F	SABA (as needed)	0	Mild	22
6	26	F	SABA (2x daily)	0	Intermittent	23
7	22	M	SABA (infrequent)	0	Intermittent	23
8	19	F	SABA (as needed)	0	Mild	21
9	20	F	SABA (as needed); ICS (daily)	0	Mild	21
10	35	M	SABA (as needed)	0	Moderate	12
11	18	M	SABA (as needed)	0	Mild	24
12	19	F	SABA (as needed); LABA, ICS	0	Moderate	21
13	20	M	LABA and ICS (2x daily)	0	Severe	24
14	19	F	SABA (as needed); LABA and ICS (daily)	0	Severe	20
15	21	F	SABA (as needed)	0	Mild	17
16	44	M	LABA and ICS (as needed)	1	Moderate*	16
17	40	F	SABA (as needed)	0	Mild	19

18	22	M	SABA (as needed, infrequent)	0	Mild	24
19	19	M	LABA and ICS (daily)	0	Moderate	25
20	18	M	SABA (as needed; infrequent)	0	Intermittent	24

Abbreviations: Short-acting beta-adrenergic bronchodilator (SABA); Long-acting beta-adrenergic bronchodilator (LABA); inhaled corticosteroid (ICS); Asthma Control Test (ACT) with scores >19 indicating well controlled asthma. Asthma severity was calculated for patients on rescue medication and for those with well controlled asthma on maintenance medication based on NHLBI/NAEPP (2007) step medication and asthma severity guidelines.

*Participants on maintenance medication whose ACT scores specify poorly controlled asthma, indicating that severity classification is based on a lower medication dosage than that potentially needed to achieve asthma control. These two participants were not included in overall sample severity classification.

Table 2. Metabolite ratios in reference to Cr

	Asthma (n = 17)			Healthy (n = 18)		
	Mean	SD	Min - Max	Mean	SD	Min - Max
NAA/Cr	1.50	0.19	1.20 - 1.93	1.46	0.17	1.14 - 1.74
MI/Cr	1.24	1.24	0.78 - 1.66	1.11	0.26	0.69 - 1.60
Glu/Cr	1.04	0.15	0.80 - 1.33	0.98	0.10	0.77 - 1.14

Note: no significant differences between groups were found at $p<.05$ level for metabolite ratios.
Abbreviations: NAA/Cr, ratio of NAA standardized to Cr; MI/CR, ratio of MI standardized to Cr; Glu/Cr, ratio of Glu standardized to Cr

Table 3. Means (SDs) of questionnaire measures

	Asthma n = 20			Healthy n = 20		
	Mean	SD	Min - Max	Mean	SD	Min - Max
HADS-A (0-21)	6.80	4.11	0 - 15	5.90	2.31	3 - 12
HADS-D (0-21)	2.85	2.45	0 - 9	2.11	1.85	0 - 9
PSS (0-40)	16.20	6.88	3 - 28	16.45	6.10	7 - 27

Note. No significant differences were found between groups for self-report measures at $p<.05$ level. Ranges of possible scores are provided after each measure in parentheses. *Abbreviations:* HADS-A, Hospital Anxiety and Depression Scale, Anxiety; HADS-D, Hospital and Anxiety Depression Scale, Depression; PSS, Perceived Stress Scale.

Table 4. Correlations of metabolites ratios standardized to creatine with age, asthma duration, disease control, and cognitive function

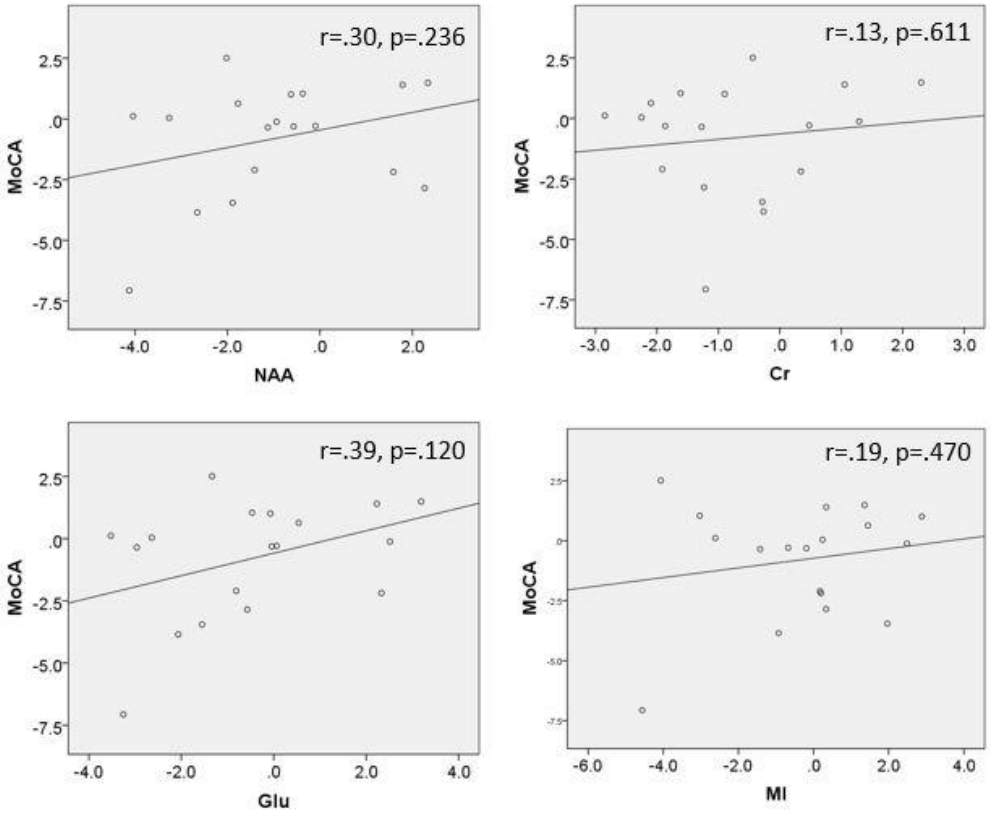
Variables	1	2	3	4	5	6	7
1. NAA/Cr	-	.15	.64**	-.17	-.52*		
2. MI/Cr	.47	-	-.10	-.01	-.25		
3. Glu/Cr	.59*	.49*	-	-.07	-.26		
4. MoCA	.21	.13	.42†	-	.19		
5. Age	-.32	.24	-.30	-.47*	-		
6. lnAsthma Duration[a]	-.56*	.-29	-.44†	.02	.66**	-	
7. ACQ	. 06	.09	-.21	-46*	.55*	-.28	-

Pearson-partial correlations (two-tailed), controlling for hippocampal volume, for asthma sample (n=17) are presented below the diagonal and correlations for the healthy sample (n=18) are presented above the diagonal.
*Indicates $p<.05$, **indicates $p<.01$, and †indicates $.05 < p < 0.1$
[a] Pearson partial correlation controlling for age and hippocampal volume. Asthma duration was additionally log transformed to reduce skewness.
Abbreviations: ACT, Asthma Control Test (higher values indicate higher control); ACQ, Asthma Control Questionnaire (higher values indicate lower control, which is opposite to the ACT); MoCA, Montreal Cognitive Assessment; NAA/Cr, ratio of NAA standardized to Cr; MI/CR, ratio of MI standardized to Cr; Glu/Cr, ratio of Glu standardized to Cr.

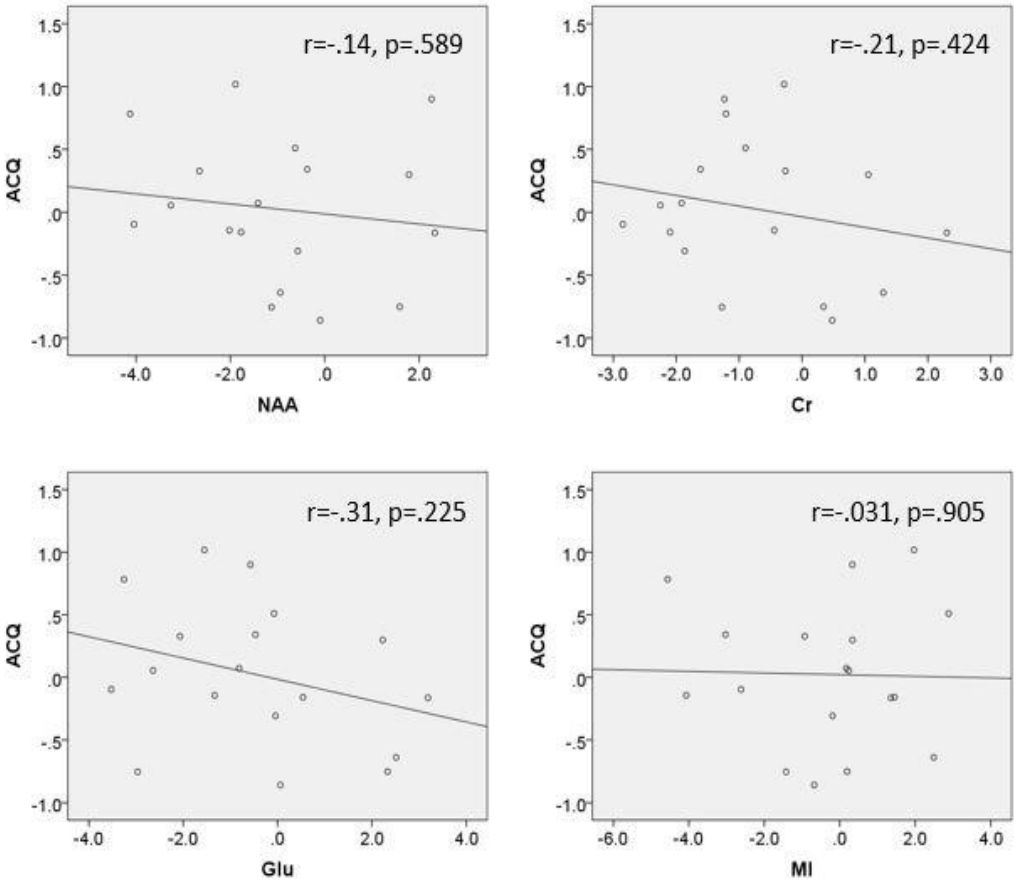
Table 5. Correlation of total metabolite levels with cognitive scores, medication use, and sleep

Variables	1	2	3	4	5	6	7	8	9
1. tCr	-								
2. tNAA	.58**	-							
3. tMI	.33	.28†	-						
4. tGlu	.65**	.70**	.32†	-					
5. MoCA	.27	.29†	.16	.35*	-				
6. Inhaled corticosteroid use (yes/no)	.70**	.43†	.38	.40	-.21	-			
7. Short-acting bronchodilator use (past week frequency)[a]	-.39	-.56*	-.32	-.58*	-.05	-.19	-		
8. Asthma-related nighttime awakenings (past week frequency)[a]	.15	.05	.50*	-.06	-.41†	.28	*.43*†	-	
9. Asthma-related nighttime awakenings (past month frequency)[b]	-.02	.05	-.27	.14	.60**	-.18	-.50*	-.79**	-

Pearson-partial correlations for all metabolites controlled for hippocampal volume. * $p<.05$, ** $p<.01$, † $p < 0.125$
[a] Item taken from the ACQ, higher values indicate lower control, [b] Item taken from the ACT, higher values indicate higher control
Abbreviations: MoCA, Montreal Cognitive Assessment; t, total metabolite level standardized to water



Supplement Figure 1. Residual plots of left hippocampal metabolites and MoCA scores (higher values indicate better cognitive functioning) separately for the asthma sample, controlling for hippocampal volume. Pearson-partial correlations (two-tailed), displayed controlling for left hippocampal volume. *Abbreviations*: Cr, creatine; NAA, N-Acetyl Aspartate; MI, myo-inositol; Glu, glutamate; MoCA, Montreal Cognitive Assessment



Supplement Figure 2. Residual plots of left hippocampal metabolites and Asthma Control Questionnaire (higher values suggesting lower control), controlling for hippocampal volume. Pearson partial correlations (two-tailed), controlling for hippocampal volume displayed. *Abbreviations*: Cr, creatine; NAA, N-Acetyl Aspartate; MI, myo-inositol; Glu, glutamate; ACQ, Asthma Control Questionnaire