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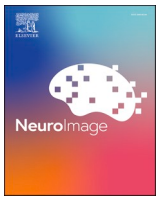

The effect of age and sex on brain metabolites: from childhood to adulthood

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ABSTRACT

Abnormalities in the concentration of brain metabolites have been found across neuropsychiatric conditions. This cross-sectional study set out to examine the relationship between levels of neurometabolites and age and sex —key modulators of brain function and structure—, continuously from childhood to early adulthood, in areas relevant to the study of psychiatric disorders.

Magnetic resonance spectroscopy (^{1}H MRS) data was acquired in the dorsomedial prefrontal region (dmPF) and the medial temporal lobe (mTL) in 128 healthy individuals aged 7 to 34 years, 68.5 % females ($n_{dmPF} = 124$; $n_{mTL} = 75$). Absolute concentrations of glutamate (Glu), glutamate and glutamine (Glx), myo-inositol (mIns), N-acetyl-aspartate and N-acetyl-aspartyl-glutamate (tNAA), glycerophosphocholine and phosphocholine (tCho), and creatine and phosphocreatine (tCr) were determined, and tested for the effects of age, sex and their interaction.

In the dmPF, there were linear, age-related decreases in Glu and Glx. The association between levels of both tNAA and tCr and age adjusted to a quadratic model, consisting of a positive association until ages 20.79 and 22.82, respectively, and a negative relationship thereafter. There was a significant effect of sex in the mTL, whereby concentrations of Glu, Glx and mIns were lower in females than in males. No age by sex interactions were detected.

These findings highlight the importance of accounting for both linear and non-linear age-related effects and for the potential effect of sex when interpreting disease-related differences in ^{1}H MRS-quantified metabolites from childhood through to adulthood.

1. Introduction

Brain structural and functional magnetic resonance imaging (MRI) abnormalities have been described in severe mental health disorders (Ching et al., 2020; Hettwer et al., 2022; Schijven et al., 2023), yet their underlying pathophysiological mechanisms are unclear. Proton magnetic resonance spectroscopy (^{1}H MRS) is a non-invasive technique that enables in vivo quantification of brain metabolite levels, which are thought to provide an indirect measure of the biological processes in which they participate (Rae, 2014). Brain ^{1}H MRS is used clinically in a

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range of medical conditions (Blüml et al., 2022). In the field of psychiatry, its use is still limited to the research domain, where a growing body of studies has found alterations in levels of brain metabolites in severe mental health disorders (Ino et al., 2023; Lázaro et al., 2012; Merritt et al., 2023; Moriguchi et al., 2019). Brain MRI studies, supported by preclinical, cellular and molecular evidence (Silbereis et al., 2016), have described neuromaturational changes from birth to early adulthood (Bethlehem et al., 2022). Therefore, the study of brain structure and function during this age period needs to take developmental effects into consideration (Vijayakumar et al., 2018). The onset of most psychiatric disorders coincides with this time frame, ranging between 8 and 35 years of age, with a peak during adolescence (Solmi et al., 2021). Earlier onset of psychiatric disorders is associated with poorer outcomes (Molina-García et al., 2021), likely due to the potentially greater impact of pathophysiological processes on brain development (Marín, 2016; Uhlhaas et al., 2023). Research examining age effects on levels of brain metabolites in samples spanning from childhood to adulthood is an essential step in the path towards potential use of ^{1}H MRS-based biomarkers in psychiatry (Cichocka and Bereś, 2018). However, even though research quantifying brain metabolites in humans dates back over three decades (Kreis et al., 1993), ^{1}H MRS studies in healthy subjects (reviewed in Table 1) have primarily focused on adults.

In the last decades, studies in psychiatry have mainly employed ^{1}H MRS in brain areas selected due to their relevance to the pathophysiology of mental health disorders. The frontal and temporal lobes have been selected in numerous studies due to their role in processes relevant across psychiatric conditions, such as emotion regulation and memory (Chabert et al., 2022a; Kaminski et al., 2021; Moriguchi et al., 2019). Research in this field has focused on metabolites that are most accurately measured using conventional ^{1}H MRS techniques (e.g. the PRESS sequence): glutamate (Glu), Glu + glutamine (Glx), N-acetyl-aspartate + *N*-acetyl-aspartyl-Glu (NAA+NAAG, tNAA), myo-Inositol (mIns), glycerophosphocholine and phosphocholine (tCho) and creatine + phosphocreatine (tCr). These metabolites play diverse roles in the brain. Glu and Glx are associated with energy metabolism and excitatory neurotransmission (Basu et al., 2021; Rae, 2014). mIns, a precursor of membrane phospholipids which is primarily stored in astrocytes, plays a key role in numerous signal transduction pathways and functions as an osmolyte (Blüml et al., 2013). Both constituents of the tNAA signal are synthesized in neurons, NAA acts as an osmolyte and provides oligodendrocytes with acetyl groups, while NAAG triggers astrocytes to increase blood flow, and hence provide neurons with oxygen and glucose in response to increased neuronal demand (Baslow, 2010). tCho signal constituents, glycerophosphocholine and phosphocholine, are involved in the degradation and synthesis of cellular membranes, respectively. Hence, they are both linked to cell density, even though individually they reflect either membrane degradation or proliferation and repair (Stovell et al., 2017). tCr is thought to play a critical role in maintaining brain energy balance through its involvement in the reversible transfer of a phosphate group from ATP to creatine or from phosphocreatine to ADP, facilitating the rapid transfer of high energy phosphates to maintain brain ATP levels according to energy demands (Rackayova et al., 2017). Therefore, studies quantifying these metabolites in psychiatric conditions have the potential to deepen understanding of biological processes underpinning mechanisms of disease (Ino et al., 2023; Moriguchi et al., 2019).

Studies assessing the effects of age on metabolite levels in the frontal lobe in young adults (aged 18 to 35 years) have generally reported reductions in Glu (Grachev et al., 2001; Grachev and Apkarian, 2001; Hädel et al., 2013; Marsman et al., 2013), Glx (Ding et al., 2016; Gao et al., 2013) and N-acetyl-aspartate (NAA) (Brooks et al., 2001; Maudsley et al., 2009, 2012; Schubert et al., 2004), and increases in mIns (L. Chang et al., 1996; Lind et al., 2020), tCho (Chiu et al., 2014; Lind et al., 2020; Maudsley et al., 2012) and tCr (L. Chang et al., 1996; Chiu et al., 2014; Harada et al., 2001; O'Gorman et al., 2011) over time. The few studies documenting age effects on metabolites measured in the temporal lobe have found negative relationships between age and Glu, Glx, tNAA (Angelie et al., 2001; R. Chang et al., 2016; Driscoll et al., 2003; King et al., 2008; Maudsley et al., 2009, 2012; Sporn et al., 2019; Szentkuti et al., 2004) and tCho (King et al., 2008; Maudsley et al., 2012; Sporn et al., 2019). Fewer studies have examined age-related effects in brain metabolites in frontal and temporal regions during late childhood or adolescence (aged 7 to 17 years) (Ghisleni et al., 2015; Hashimoto et al., 1994; Holmes et al., 2017; Perdue et al., 2023; Shimizu et al., 2017; Yang et al., 2015; Zacharopoulos et al., 2021), revealing a pattern of decreases in frontal Glu (Shimizu et al., 2017; Zacharopoulos et al., 2021) and increases in frontal and temporal NAA (Holmes et al., 2017; Perdue et al., 2023; Zacharopoulos et al., 2021) and tCr (Holmes et al., 2017; Zacharopoulos et al., 2021). Table 1 lists studies examining age effects in levels of brain metabolites in healthy individuals. Importantly, most evidence to date relies on small studies focusing on a narrow age range, and no study so far has provided a continuous assessment from childhood extending into mid adulthood. Examination of sex influences on measures of brain metabolites is even more limited. A small number of studies have reported sex effects in frontal and temporal lobes (see Table 1 and Supplementary Table 1). These effects include decreased Glu and Glx in the dorsolateral prefrontal cortex (O'Gorman et al., 2011) and increased Glu in the hippocampus (Hädel et al., 2013) in females relative to males, increased NAA levels in both the frontal (Cichocka et al., 2018) and temporal lobes in females relative to males (García Santos et al., 2010; Maudsley et al., 2012) and increased levels of choline in the frontal lobe in females relative to males (García Santos et al., 2010). Both increased (Maudsley et al., 2012) and decreased (Hädel et al., 2013) levels of tCho have been documented in the temporal lobe in females relative to males. None of these studies has tested the interaction between age and sex during youth (Supplementary Table 1).

The aim of the current study was to identify, for the first time, normative age and sex -related effects on the levels of the main brain metabolites measured using a standard in vivo ^{1}H MRS, within an age range relevant to the onset of most psychiatric disorders. We thus examined the effect of age and sex, and their interaction, on levels of Glu, Glx, mIns, tNAA, tCho and tCr measured using ^{1}H MRS in healthy individuals aged 7 to 34 years old.

In light of previous findings, in the dorsomedial prefrontal region (dmPF), we expected to observe a negative association between age and Glu, Glx and tNAA, as well as a positive association between age and mIns, tCho and tCr during adulthood. We also set out to test non-linear relationships, as the age range of the sample comprised children and adolescents, who could exhibit a different pattern of age-association compared to adults. In the medial temporal lobe (mTL), we expected to observe a negative association between age and tNAA during adulthood, which could be different during childhood and adolescence. In terms of other metabolites in the mTL, the effects of sex, and the interaction between age and sex in each VOI, we did not formulate a specific hypothesis due to the paucity of previous evidence.

2. Methods

2.1. Participants

A total of 135 individuals (93 females and 42 males) aged between 7 and 34 years were initially included in this study. Participants were recruited from schools and other community settings through advertisements and word of mouth, by staff working in research projects at the Department of Child and Adolescent Psychiatry and Psychology, by the Imaging in Mood- and Anxiety-related Disorders and by the Pathogenesis of Autoimmune Neuronal Disorders, at the Hospital Clinic of Barcelona and Fundació Clínic Recerca Biomèdica - Institut d'Investigacions Biomèdiques August Pi i Sunyer. Participants were classified as male or female according to the sex assigned at birth. All participants underwent a clinical evaluation employing the Kiddie Schedule for Affective Disorders and Schizophrenia - Present and

Table 1
Synthesis of evidence on the effect of sex and age in metabolite concentrations in frontal and temporal areas in samples of child, adolescent and adult healthy participants (Komoroski et al., 1999; Lally et al., 2016; Tuovinen et al., 2022).

	Year	n	Age	Glu	Glx	Ins	NAA	Cho	Cr	Quantification method
Frontal areas										
Studies including children/adolescents										
Hashimoto et al.	1994	11	0-15				-	-	-	Cr-referenced
Perdue et al.*	2023	124	2-7	-	-	-	↑	↓	-	Absolute
Holmes et al.*	2017	64	5-10	-	↑	-	↑	-	↑	Absolute
Studies including children/adolescents and adults										
Shimizu et al.	2017	22	4-13; 18-33	↓			-			Cr-referenced
Zacharopoulos et al.	2021	293	6-20	↓		-	↑	-	↑	Absolute
Ghisleni et al.	2015	56	13-53	-						Absolute
Studies including adults										
Tuovinen et al.*	2022	57	18-30					**		Cr-referenced
Lind et al.	2020	60	18-26; 39-50; 69-79	-		↑	-	↑	-	Absolute
Marsman et al.*	2013	33	18-31	↓			-	-	↓	Absolute
Maudsley et al.*	2009	88	18-59				↓	-	-	Both
Maudsley et al.*	2012	140	18-84				↓**	↑	↑	Both
García-Santos et al.*	2010	20	19-29			-	-	-		Cr-referenced
Grachev et al.*	2000	19	19-31	-		-	-	-		Cr-referenced
Grachev et al.	2001	52	19-31; 40-52	↓		-	-	-	-	Cr-referenced
Grachev & Apkarian	2001	35	19-31; 40-52	↓		-	-	-		Cr-referenced
Hädel et al.*	2013	118	19-55	↓			-	-**	-	Absolute
(Komoroski et al., 1999)*	1999	21	19-67							Cr-referenced
Chang et al.	1996	36	19-78		-	↑	-	↑	↑	Absolute
(Lally et al., 2016)*	2016	13	20-54	-						Absolute
Schubert et al.				-			-			Cr-referenced
	2004	40	20-60	-			↓			Absolute

(*continued on next page*)

Table 1 (*continued*)

Brooks et al.	2001	50 (males)	20-70				↓	-	-	Absolute
Harada et al.	2001	50	20-70				-	-	↑	Absolute
Ding et al.*	2016	81	20-70		↓	-	-	-	-	Absolute
Gao et al.*	2013	100	20-76		↓					Cr-referenced
Sailasuta et al.*	2008	50	21-71	-			-	-	-	Absolute
Chang et al.*	2009	62	21-71	-			-	-	-	Absolute
Chiu et al.*	2014	30	22-82			-	↑	↑	↑	Absolute
Fukuzako et al.*	1997	36	24-78				-	-		Cr-referenced
O'Gorman et al.*	2011	14	25-38	-**	-**	-	-	-	↑	Absolute
Temporal areas										
Studies including children/adolescents										
Cichocka et al.*	2018	49	6-15				**			Both
Studies including children/adolescents and adults										
Yang et al.	2015	80	0-18; 19-39; 40+	-	-	↑	-	-	-	Both
Studies including adults										
Lind et al.	2020	60	18-26; 39-50; 69-79	-		↑	-	-	-	Absolute
Maudsley et al.*	2009	88	18-59				↓	-	-	Both
Reyngoudt et al.*	2012	90	18-76			↑	-	-	-	Cr-referenced
Maudsley et al.*	2012	140	18-84				↓	↓	↑	Both
García-Santos et al.*	2010	20	19-29			↓	-**	-**		Cr-referenced
Hadel et al.*	2013	118	19-55	↓**			-	-	-	Absolute
Komoroski et al.*	1999	39	19-67							Cr-referenced

(*continued on next page*)

Table 1 (*continued*)

				-			↓			Cr-referenced
Schubert et al.	2004	40	20-60	-			-			Absolute
Driscoll et al.	2003	32	20-39; 60-85				↓	-		Cr-referenced
Ding et al.*	2016	81	20-70		-	-	-	-	-	Absolute
Chang et al.*	2016	121	20-79				↓	-	-	Absolute
Angelie et al.*	2001	32	21-61				↓			Cr-referenced
Szentuki et al.	2004	35	22-27; 60-75				↓			Cr-referenced
Chiu et al.*	2014	30	22-82			-	↑	-	-	Absolute
Fukuzako et al.*	1997	36	24-78				-	-	-	Cr-referenced
King et al.*	2008	12	25-35; 68-72				↓	↓	↓	Absolute
Gruber et al.	2008	22	18-59			-	-	-	-	Absolute
Sporn et al.	2019	38	18-63		-	↓	↓	↓	↓	Absolute

Arrows facing upwards (in green) illustrate age-positive associations. Arrows facing downwards (in blue) illustrate age-negative associations. A hyphen on a gray background represents a metabolite which was examined but did not yield significant effects. *The study tested either sex or sex by age effects. **The study found significant sex effects on this metabolite. Gray background represents a tested sex effect on this metabolite which was not significant. Except for Perdue et al., 2023 and Holmes et al., 2017, which contain longitudinal data, all studies are cross-sectional. Glu: glutamate; Glx: Glu + glutamine; mIns: myo-Inositol; Cho: tCho (glycerophosphocholine + phosphocoline) or choline; NAA: tNAA (N-acetyl-aspartate + *N*-acetyl-aspartyl-glutamate) or N-acetyl-aspartate; Cr: tCr (creatine + phosphocreatine) or creatine. Note that some studies reported single-metabolite labels, despite the fact that associated spectral peaks at these chemical shifts inherently reflect contributions from multiple metabolites. For a more detailed depiction of sex and sex by age interactions, see Supplementary Table 1.

Lifetime version (KSADS-PL) (Kaufman et al., 1997) or the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) in order to assess current and lifetime history of psychiatric disorders. The exclusion criteria were as follows: personal history of any severe mental health disorder diagnosis (psychosis, schizophrenia and related disorders, bipolar disorders, autism spectrum disorders, major depressive disorder and obsessive-compulsive disorder), current diagnosis of any DSM axis I disorder, current psychotropic medication, substance use disorder diagnosis, intellectual disability, neurological disorders, head injury with loss of consciousness, and pregnancy. Educational attainment was assessed on a scale of 1 to 7 following Hollingshead-Redlich criteria (Hollingshead and Redlich, 2007), where 1 equals not finishing elementary school and 7 the highest level of educational attainment (having graduate or professional training). Participants under the age of 18 were assigned the educational attainment of their parents; if both parents provided this information, the highest of the two was assigned. All participants, and/or their parents or legal guardians in the case of participants under 18 years, provided written informed consent/assent for a protocol approved by the ethics committee of the Hospital Clinic Barcelona.

2.2. MRI data acquisition and processing

Neuroimaging data were collected on a 32-channel phased-array head coil on a 3 Tesla Siemens MAGNETOM TIM Trio or Prisma Fit scanner (Siemens Healthcare, Erlangen, Germany) at the Magnetic Resonance Image Core Facility of IDIBAPS. A high-resolution brain structural image was obtained from each subject employing a T1-weighted 3D Magnetization-Prepared Rapid Acquisition Gradient Echo (MP-RAGE) sequence (TR = 2300 ms; TE = 3.01 ms; flip angle = 9°; matrix size = 256×256; FOV = 240×240 mm^2; slice thickness = 1 mm; number of slices = 240; voxel size = 0.93×0.93×1 mm^3). An axial T2 sequence was also acquired. A neuroradiologist reviewed all scans to rule out qualitative radiological abnormalities.

^{1}H MRS spectra were acquired employing a PRESS (Point RESolved Spectroscopy) sequence with standard chemical shift-selective water suppression and shimming method (TE = 30 ms, TR = 3000 ms). For this purpose, two VOIs were selected and manually centered, one located in the left dmPF (20×30×15 mm^3, number of averages = 96, single-voxel runtime = 5′, Fig. 1) and another located in the left mTL (20×20×30 mm^3, number of averages = 96, single-voxel runtime = 5′, Fig. 1). For each VOI, a spectrum with the same TE/TR parameters but no water suppression was acquired, to be used as a reference for absolute quantification (single-voxel runtime = 1′). The anatomical landmarks for consistently placing the MRS voxels in these two brain areas across participants were as follows, for the dmPF: 'left frontal lobe superior to the medial frontal gyrus and anterior to the superior frontal gyrus' and for the mTL: 'left parahippocampal gyrus posterior to the uncus of the temporal lobe and below the lateral sulcus'.

Metabolite concentration quantification was performed using LCModel version 6.3 software (Provencher, 2001), using water scaling to obtain absolute concentrations. Water scaling was performed considering the reference unsuppressed water spectra, to which we applied a correction factor that accounts for voxel tissue composition. Specifically, we used the equation: wconc = (43,300 fGM + 35,880 fWM + 55,556 fCSF)/(1-fCSF), where fWM, fGM and fCSF are the fractions of

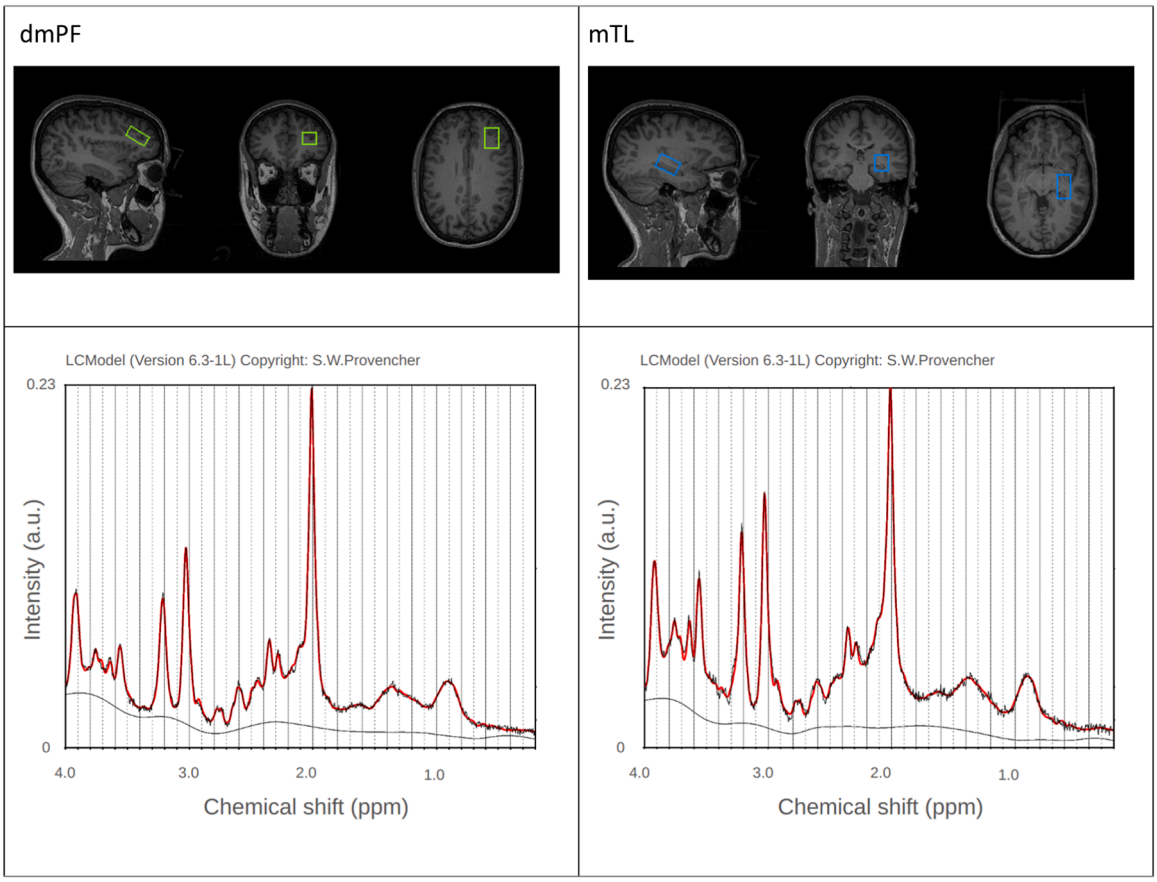

Fig. 1. Examples of VOI location in native space and retrieved spectra.

white matter (WM), gray matter (GM) and cerebro-spinal fluid (CSF) in the voxel, respectively (LCModel Manual, http://s-provencher.com/pub/LCModel/manual/manual.pdf, page 131, Provencher, 2001). The fractions of GM, WM and CSF in each VOI were estimated by segmenting the T1-weighted image with the unified segmentation algorithm implemented in SPM12 (Ashburner and Friston, 2005), and then computing the percentage of each tissue in the VOI (Near et al., 2021). Metabolites were linearly fitted with a basis-set tailored to process PRESS 3T sequences with TE=30 s (http://s-provencher.com/lcm-basis.shtml). This basis set included 17 metabolites (alanine, aspartate, creatine, parphosphocreatine, gamma aminobutyric acid, glucose, glutamine, glutamate, glutathione, phosphocholine and glycerophosphocholine, myo-inositol, lactate, N-acetylaspartate, N-acetylaspartyilglutamate, scylloinositol and taurine), and the following lipid (lip) and macromolecules (MM): lip13a, lip13b, lip13a+lip13b, lip09, lip20, MM9, MM20, MM12, MM14, MM17, MM14+lip13a+lip13b+MM12, MM09+lip09, MM20+lip20. The metabolites of interest that were further included in the analysis were: Glu, Glx, mIns, tNAA, tCho and tCr. The concentration of Glu, Glx, mIns, tNAA, tCho and tCr were considered for further analysis since these are the most commonly evaluated metabolites when PRESS sequences are employed (Near et al., 2021) To ensure data quality, VOI location and spectral profile were visually inspected, and spectra were further excluded from analysis based on the following criteria: FWHM (full-width at half-maximum) > 12 Hz, SNR (signal-to-noise ratio) < 10. At the metabolite level, we further excluded individual metabolite measurements whose relative Cramér-Rao Lower Bound (CRLB) exceeded 15 %. This additional criterion led to the exclusion of four measures of Glu and two measures of Glx in the mTL VOI. Finally, to obtain molal concentrations (mmol metabolite/kg tissue water), we applied a voxel-specific two-step correction for relaxation and partial volume effects, following the consensus recommendations of Near et al. (2021) and adapting the processing scripts provided by DeMayo et al. (2023), as implemented previously by Perdue et al. (2023). First, tissue-water mole fractions were calculated from previously determined tissue fractions combined with tissue-specific water densities (Gasparovic et al., 2006). Second, relaxation correction factors for water and metabolites were computed using literature-derived T_1 and T_2 values (for water, from Gasparovic et al., 2006; for metabolites, from Posse et al., 2007). Subsequently, raw LCModel-derived water-scaled metabolite concentrations in each VOI were corrected by applying these compartment-specific water relaxation corrections and metabolite relaxation factors, explicitly excluding cerebrospinal fluid (CSF). The resulting metabolite concentrations are thus expressed in molal units.

2.3. Statistical analyses

To describe the sample and the variables of interest, the mean, standard deviation and range were computed for age, educational attainment and metabolite levels. To rule out outlier values in metabolite concentrations, the Grubbs test was performed for each metabolite in each VOI employing the 'outliers' package in R (v 0.15). In order to study the effect of age and its interaction with sex on metabolite concentrations, linear, quadratic and cubic models were tested. The quadratic and cubic terms were added hierarchically to the model. Further, to determine which model provided a more suitable fit to the data, when appropriate, a likelihood ratio test (LRT) was used to assess the final model. If the result of the LRT was not significant, the more parsimonious model was selected. MRI scanner model, GM tissue fraction in the VOI (computed as the proportion of GM relative to the total tissue fraction excluding CSF, Perdue et al., 2023) and sex were included as covariates. To test the effect of sex on metabolite levels, we employed

linear models with age, GM tissue fraction and MRI scanner model as covariates. For the sake of completeness, we tested associations between metabolites within each VOI by performing Spearman correlations, which we depicted in a correlation matrix for each VOI. We then tested the effect of age on the relationship between metabolite levels for each VOI employing moderation analyses adjusted for the aforementioned covariates. The formulae of each model can be found in Supplementary Section 1. Linearity, independence, homoscedasticity, and normality of the residuals were inspected. False discovery rate (FDR) was employed to correct for multiple comparisons. Both FDR corrected and uncorrected results are reported. All statistical analyses were performed in R version 4.3.1. (Core Team, 2023).

Distribution of cases by scanner model is depicted in Table 2. In order to rule out potential scanner effects, the scanner model was included as a covariate in all models, and sensitivity analyses were conducted with data from the scanner with the larger number of acquisitions. The effect of another potential confounding factor (occasional cannabis use) was also tested.

3. Results

3.1. Demographic characteristics of the sample

From the initial sample of 135 participants, 7 complete cases were excluded after not having data that complied with the quality control criteria in either VOI (n = 4, mean age = 25.82; sd = 8.49; range = 15.753–35.41, 50 % females) or due to outlier removal (n = 3, mean age = 21.85; sd = 7.56; range = 14.18–28.69, 100 % females). Among the 128 participants included in the analysis, 123 had data surviving both quality control criteria and outlier removal for the dmPF, 89 for the mTL, and 84 for both VOIs. There were no significant differences in age or sex according to inclusion/exclusion following quality control/outlier removal (dmPF: $t = 0.85$; $p = 0.4$; $\chi^2 = 0.05$; $p = 0.8$; mTL: $t = 0.5$; $p = 0.6$; $\chi^2 = 0.23$; $p = 0.6$).

Demographic and clinical characteristics of the sample after excluding acquisitions that did not comply with the quality assessment are depicted in Table 2.

Table 2
Demographic characteristics of the final sample.

		Total $N = 128$	Dorsomedial prefrontal region $N = 123$	Medial temporal lobe $N = 89$
Age (n: mean [sd; range])		128: 20.0 [6.4; 7.6–34.1]	123: 20.0 [6.4; 7.6–34.1]	89: 17.7 [5.1; 7.6–31.8]
Sex (% females)		68.8 %	69.1 %	65.2 %
Educational attainment (mean [sd; range])		6.3 [1.2; 3–7]	6.3 [1.2; 3–7]	6.1[1.3; 3–7]
Ethnicity	Latinx	6 (4.7 %)	6 (4.9 %)	2 (2.2 %)
	Other ethnic categories	9 (7.0 %)	9 (7.3 %)	9 (10.1 %)
	White	113 (88.3 %)	108 (87.8 %)	78 (87.7 %)
Occasional cannabis use		15 (11.70 %)	13 (10.6 %)	12 (13.5 %)
MRI scanner model	Trio	10 (7.8 %)	9 (7.3 %)	7 (7.9 %)
	Prisma	118 (92.2 %)	114 (92.7 %)	82 (92.1 %)

dmPF: dorsomedial prefrontal region; mTL: medial temporal lobe.

3.2. Overall metabolite concentration

The mean concentration of metabolites for each VOI are displayed in Table 3. Comparisons of metabolite levels between the two VOIs are reported in Supplementary Table 2.

3.3. Effects of age on brain metabolite concentrations

3.3.1. Glutamate and glutamate + glutamine

In the dmPF VOI there was a significant main, negative effect of age on the concentration of Glu ($\beta = -0.07$, $p_{fdr} < 0.01$) and Glx ($\beta = -0.07$, $p_{fdr} < 0.01$), which adjusted to a linear model (Table 4, Fig. 2). No effect of age was observed on the concentration of Glu or Glx in the mTL (Table 4).

3.3.2. Myo-Inositol

There was no effect of age on mIns in the dmPF or the mTL (Table 4).

3.3.3. N-acetyl-aspartate + N-acetyl-aspartyl-glutamate

In the dmPF VOI, there was a significant quadratic association between age and tNAA ($\beta_1 = 0.20$; $\beta_2 = -0.004$; p_{fdr} 0.039, Table 4, Fig. 2), whereby age was positively associated with levels of this metabolite up to age 20.58 years and negatively associated from then onwards, until the age of 34.14 years. This model was a better fit than the linear model ($F(1,1145) = 5.57$, $p = 0.020$ Details on the increased explained variance by the quadratic term can be found in Supplementary Table 3). No effect of age was found for the concentration of tNAA in the mTL (Table 4).

3.3.4. Glycerophosphocholine + phosphocholine

tCho levels showed a positive linear age-association ($\beta = 0.01$, p_{fdr} 0.040, Table 4, Fig. 2) in the dmPF. No age effects were found for tCho levels in the mTL (Table 4).

3.3.5. Creatine + phosphocreatine

A quadratic association was observed between tCr and age ($\beta_1 = 0.10$; $\beta_2 = -0.002$, $p_{fdr} = 0.039$, Table 4, Fig. 2), consisting of a positive relationship between tCr and age until the age of 21.69, and a negative relationship from there onwards up to the age of 34.14. This model was a better fit than the linear model ($F(1117) = 4.93$., $p = 0.028$. For details on the increased explained variance by the quadratic term, see Supplementary Table 3). There was no effect of the age on tCr levels in the mTL (Table 4).

3.4. Effects of sex on brain metabolite concentrations

No effect of sex was observed on metabolite concentration in the dmPF (Table 5, Supplementary Figure 3). In the mTL VOI, there was a significant effect of sex on Glu, Gl, mIns and tCho levels, whereby levels of these metabolites were higher in males compared to females (adjusted mean Glu females = 7.26 (6.53–8.00), adjusted mean Glu males = 8.35 (7.47–9.24); adjusted mean Glx females = 9.57 (8.66–10.47), adjusted mean Glx males = 11.01 (9.92–12.10); adjusted mean mIns females = 4.97 (4.55 – 5.38), adjusted mean mIns males = 5.74 (5.25 - 6.23); adjusted mean tCho females = 1.93 (1.8 - 2.06), adjusted mean tCho males = 2.13 (1.97 - 2.28); Table 5, Fig. 3). No age by sex interaction effect was observed in any of the VOIs (Supplementary Table 4).

3.5. Relationship between metabolite levels

In the dmPF VOI, there were significant positive correlations between all metabolites except for tCho, which was only correlated with tNAA and mIns, which was exclusively correlated with Glu and tCr (Supplementary Figure 4). Within the mTL all tested metabolites were inter-correlated (Supplementary Figure 4). No effect of age was found on the between-metabolite relationships.

Table 3
Metabolite concentrations in the dmPF and the mTL.

	Dorsomedial prefrontal region				Medial temporal lobe			
	Sample size [1]	Mean	SD	Range	Sample size [1]	Mean	SD	Range
Signal to noise ratio	123	42.33	7.41	27–58	89	24.73	6.19	11–39
Linewidth (Hz)	123	5.89	1.36	3.83–11.5	89	8.7	2.03	5.75–12.65
Water concentration (mmol/kg)	123	42,595.99	10,605.18	36,798.79–116,919.76	89	54,917.16	59,753.62	36,700.57–549,138.83
Gray matter tissue fraction	123	0.4	0.11	0.06–0.65	89	0.5	0.12	0.06–0.66
White matter tissue fraction	123	0.56	0.15	0.05–0.93	89	0.4	0.14	0.01–0.93
CSF tissue fraction	123	0.05	0.09	0–0.57	89	0.09	0.17	0.01–0.9
Glu (mmol/kg)	123	8.84	1.09	5.63–11.23	83	7.09	1.7	2.75–11.73
Glu CRLB	123	5.04	0.76	3–9	83	7.12	2.11	4–14
Glx (mmol/kg)	123	10.17	1.48	6.58–14.26	81	8.88	2.15	4.99–15.19
Glx CRLB	123	5.94	1.03	4–11	81	7.64	1.93	4–13
mIns (mmol/kg)	123	4.98	0.53	3.33–6.11	89	5.16	1.03	2.8–8.33
mIns CRLB	123	3.7	0.56	3–5	89	4.37	1.27	3–9
tNAA (mmol/kg)	119	11.37	0.96	8.38–13.39	88	7.24	1.55	3.57–10.83
tNAA CRLB	119	2.13	0.4	1–4	88	4.28	1.39	2–9
tCho (mmol/kg)	123	1.76	0.21	1.36–2.38	89	1.91	0.32	1.18–2.91
tCho CRLB	123	2.59	0.49	2–3	89	3.31	1.07	2–7
tCr (mmol/kg)	122	7.11	0.53	5.61–8.26	87	6.23	1.02	3.47–8.26
tCr CRLB	122	2.04	0.2	2–3	87	2.99	0.97	2–7

dmPF: dorsomedial prefrontal region; mTL: medial temporal lobe; CSF: cerebrospinal fluid; Glu: glutamate; Glx: Glu + glutamine; mIns: myo-Inositol; tCho: glycerophosphocholine + phosphocholine; tNAA: N-acetyl-aspartate + *N*-acetyl-aspartyl-Glu; tCr: creatine + phosphocreatine. Water concentration and metabolite concentrations are expressed in mmol/kg of wet weight.[1]The sample size columns indicate the number of participants with available data after quality control and outlier removal.

Table 4
Associations between metabolite concentration and age.

	Metabolite		β	SE	t-value	R^2	p-value uncorrected	p-value corrected
Dorsomedial prefrontal region	Glu (mmol/kg)		−0.07	0.01	−4.98	0.28	<0.01**	<0.01**
	Glx (mmol/kg)		−0.07	0.02	−3.54	0.25	<0.01**	<0.01**
	mIns (mmol/kg)		0.01	0.01	0.67	−0.01	.503	.503
	tNAA (mmol/kg)	Age	0.20	0.08	2.24	0.04	.026*	.039*
		Age^2	−0.004	0.002	−2.36		.019*	.039*
	tCho (mmol/kg)		0.01	0.003	2.13	0.15	.035*	.040*
	tCr (mmol/kg)	Age	0.10	0.05	2.21	0.12	.029*	.039*
		Age^2	0.002	0.001	−2.22		.028*	.039*
Medial temporal lobe	Glu (mmol/kg)		−0.06	0.04	−1.69	0.10	.0952	.277
	Glx (mmol/kg)		−0.04	0.04	−0.94	0.15	.350	.350
	mIns (mmol/kg)		−0.02	0.02	−1.13	0.11	.260	.312
	tNAA (mmol/kg)		−0.01	0.01	−1.26	0.11	.138	.277
	tCho (mmol/kg)		−0.05	0.03	−1.50	0.04	.213	.312
	tCr (mmol/kg)		−0.05	0.02	−2.39	0.05	.019*	.115

** indicates p-values below 0.01 and * reflects p-values under 0.05 and above 0.01. Linear, quadratic and cubic models were tested for all metabolites. For the sake of clarity, only the model with the best fit is provided. dmPF: dorsomedial prefrontal region; mTL: medial temporal lobe; t: absolute concentration; tGlu: glutamate; Glx: Glu + glutamine; mIns: myo-Inositol; tCho: glycerophosphocholine + phosphocholine; tNAA: N-acetyl-aspartate + *N*-acetyl-aspartyl-Glu; tCr: creatine + phosphocreatine. Metabolite concentrations are expressed in mmol/kg of wet weight.

3.6. Sensitivity analyses

Sensitivity analyses excluding subjects with current occasional cannabis use (Supplementary Table 5) and excluding data acquired in the Siemens Trio 3 Tesla scanner (Supplementary Table 6), yielded very similar results as the main analyses, meeting the same level of statistical significance throughout. An exception to this was the effect of age on tNAA and tCr levels in the dmPF, which, after excluding acquisitions performed in the Siemens Trio 3 Tesla scanner, showed trend-level effects (p_{FDR}=0.062 for both metabolites), yet comparable effect sizes to the analyses with all datapoints. Both the R^2 of the model and the explained variance of the age term were similar to those obtained in the main analyses and increased in the quadratic model for both tNAA (R^2 linear model = −0.02, partial R^2 for the age term = 0.0003; R^2 quadratic model= 0.01; partial R^2 for the quadratic age term = 0.04) and tCr (R^2 linear model = 0.11, partial R^2 for the age term = 0.001; R^2 quadratic model = 0.13; partial R^2 for the quadratic age term = 0.03).

4. Discussion

To our knowledge, this is the first study to examine the effect of age and sex on levels of brain metabolites measured using ^{1}H MRS in the dmPF and the mTL, in a continuous fashion from late childhood through to early adulthood. This coincides with the period of peak onset of most psychiatric disorders (Solmi et al., 2021) and has enabled the examination of non-linear relationships between age and brain metabolite concentrations. Our results suggest that the association between age and levels of brain metabolites follows both linear and quadratic relationships, dependent on metabolite and brain region, during this timeframe. While we found an effect of sex on levels of Glu, Glx, tCho and mIns in the mTL, we did not observe any age by sex interaction in either brain region.

Glu is the primary excitatory neurotransmitter in the brain and plays a key role in multiple neural processes, such as those involved in synaptic plasticity during neurodevelopment (Parellada and Gassó, 2021). The quantification of Glu levels using ^{1}H MRS is often reported alongside the one of glutamine, due to their overlapping spectral peak. Glutamine is the main precursor of neuronal Glu through the glutamate-glutamine

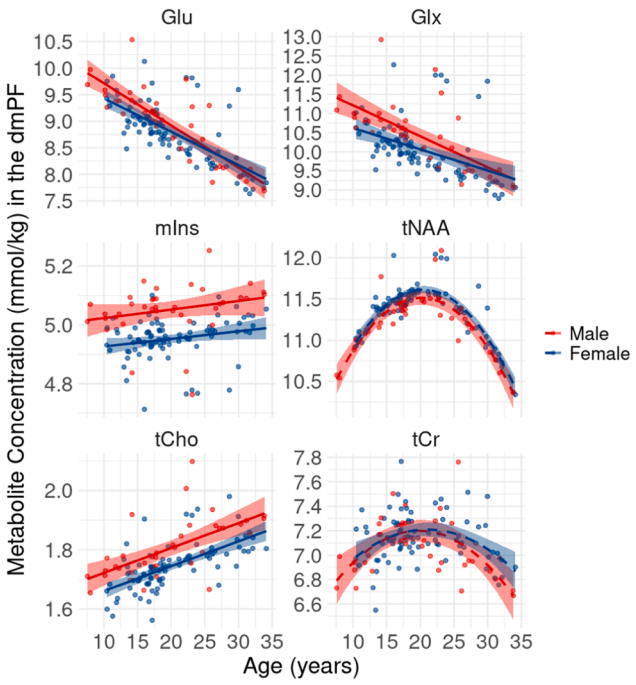

Fig. 2. Age-related effects on brain metabolite levels in the dorsomedial prefrontal volume of interest.
Upper-left graph: negative linear relationship between Glu and age in the dmPF; upper-right graph: negative linear relationship between tGlx and age in the dmPF; middle-left graph: no significant relationship between mIns and age in the dmPF; middle-right graph: significant quadratic relationship between age and tNAA levels in the dmPF; lower-left graph: positive linear relationship between tCho and age levels in the dmPF; lower-right graph: significant quadratic effect of age on tCr levels in the dmPF. For an unscaled version of this figure please see Supplementary Figure 1, and for a graphical representation of the relationship between age and absolute mTL metabolite levels see Supplementary Figure 2. dmPF: dorsomedial prefrontal region; mTL: medial temporal lobe. tGlu: glutamate; Glx: glutamate + glutamine; mIns: myo-Inositol; tCho: glycerophosphocholine + phosphocholine; tNAA: N-acetyl-aspartate + *N*-acetyl-aspartyl-glutamate; tCr: creatine + phosphocreatine. Metabolite concentrations are expressed in mmol/kg of wet weight.

cycle, whereby astrocytes prevent excitotoxicity by reuptaking glutamate from the synaptic cleft and synthesizing glutamine to further release it for neuronal uptake (Ramadan et al., 2013). Both Glu and glutamine also play a role in the Krebs cycle and in GABA (gamma-aminobutyric acid) and aspartate synthesis (Rae, 2014). A previous investigation in a sample aged 6 to 20 years reported a decline in Glu levels with age in the frontal lobe (Zacharopoulos et al., 2021), and some studies conducted in adult samples have also documented a decrease in frontal Glu and Glx with age (Grachev et al., 2001; Grachev and Apkarian, 2001; Hädel et al., 2013; Marsman et al., 2013; Shimizu et al., 2017). Our findings extend this evidence by illustrating a linear decline in levels of Glu and Glx in the dmPF from childhood to adulthood. In the context of brain development, this decrease in Glu and Glx with age could reflect a progressive reduction of glutamatergic excitatory synapses that are no longer needed in the adult brain (Marín, 2016; Parellada and Gassó, 2021). However, it must be noted that several previous studies analyzing Glx levels in the frontal lobe have reported conflicting findings in the relationship between this metabolite and age (L. Chang et al., 1996; Ding et al., 2016; Gao et al., 2013; Holmes et al., 2017; O'Gorman et al., 2011; Perdue et al., 2023). In the mTL, we did not observe a significant relationship between age and Glu or Glx. This is consistent with previous studies conducted in adults (Ding et al., 2016; Hädel et al., 2013; Lind et al., 2020; Sporn et al., 2019; Yang et al., 2015), which did not report significant age effects (Hädel et al., 2013).

We observed that mIns levels did not show an association with age during this time frame in the dmPF nor the mTL. Previous reports from adult samples showed either increases (L. Chang et al., 1996; Lind et al., 2020) or no age effects (Chiu et al., 2014; Ding et al., 2016; García Santos et al., 2010; Grachev et al., 2001; Grachev and Apkarian, 2000, 2001; O'Gorman et al., 2011) in mIns levels in the prefrontal region, and inconsistent results in the mTL (Chiu et al., 2014; Ding et al., 2016; García Santos et al., 2010; Gruber et al., 2008; Lind et al., 2020; Reyngoudt et al., 2012; Sporn et al., 2019). mIns participates as a second messenger in intracellular signaling processes, functions as an osmolyte and is integrated in the lipidic moiety of cell membranes (Blüml et al., 2013; Rae, 2014). Given the wide range of roles of mIns in the brain, our results do not rule out potential age effects in a specific mIns pool, although they suggest there are no overall age effects during this timeframe. tNAA is synthesized in neurons. While NAA contributes to myelin synthesis, it also assimilates a glutamate molecule to form NAAG. Both compounds are essential in maintaining brain osmotic balance, moreover, the latter stimulates astrocytes to induce a focal increase in blood flow in response to neural activity (Baslow and Guilfoyle, 2016). Hence the tNAA signal is a result of complex neuron-glia metabolic interactions (Rae, 2014; Saccaro et al., 2024). Previous studies in children and adolescents have reported increases in tNAA with age in prefrontal regions (Holmes et al., 2017; Perdue et al., 2023; Zacharopoulos et al., 2021), while reports in adults have shown inconsistent findings (Brooks et al., 2001; L. Chang et al., 1996, 2009; Ding et al., 2016; Fukuzako et al., 1997; García Santos et al., 2010; Grachev et al., 2001; Grachev and Apkarian, 2000, 2001; Hädel et al., 2013; Harada et al., 2001; Lind et al., 2020; Maudsley et al., 2009, 2012; Sailasuta et al., 2008). In the present study, we found a quadratic association between age and tNAA in the

Table 5
Effects of sex on brain metabolite concentrations.

	Metabolite	Females (adjusted mean and 95 % CI)	Males (adjusted mean and95 % CI)	p-value uncorrected	p-value corrected
Dorsomedial prefrontal region	tGlu (mmol/kg)	9.35 (9.01 - 9.68)	9.44 (9.04 - 9.84)	0.604	–
	tGlx (mmol/kg)	11.03 (10.56 - 11.5)	11.33 (10.78 - 11.88)	0.229	–
	mIns (mmol/kg)	4.86 (4.67 - 5.06)	4.96 (4.73 - 5.19)	0.344	–
	tNAA (mmol/kg)	11.61 (11.26 – 11.97)	11.57 (11.15 - 11.99)	0.821	–
	tCho (mmol/kg)	1.82 (1.75 - 1.89)	1.88 (1.8 - 1.97)	0.103	–
	tCr (mmol/kg)	7.25 (7.06 - 7.44)	7.16 (6.94 - 7.38)	0.363	–
Medial temporal lobe	tGlu (mmol/kg)	7.26 (6.53 - 8.00)	8.35 (7.47 - 9.24)	<0.01**	<0.01**
	tGlx (mmol/kg)	9.57 (8.66 - 10.47)	11.01 (9.92 - 12.10)	<0.01**	<0.01**
	mIns (mmol/kg)	4.97 (4.55 - 5.38)	5.74 (5.25 - 6.23)	<0.01**	<0.01**
	tNAA (mmol/kg)	7.7 (7.04 - 8.35)	8.08 (7.32 - 8.85)	.264	.264
	tCho (mmol/kg)	1.93 (1.8 - 2.06)	2.13 (1.97 - 2.28)	<0.01**	<0.01**
	tCr (mmol/kg)	6.41 (5.96 - 6.86)	6.76 (6.21 - 7.31)	.125	.150

** indicates p-values below 0.01 and * reflects p-values under 0.05 and above 0.01. dmPF: dorsomedial prefrontal region; mTL: medial temporal lobe; t: absolute concentration; Glu: glutamate; Glx: Glu + glutamine; mIns: myo-Inositol; tCho: glycerophosphocholine + phosphocholine; tNAA: N-acetyl-aspartate + *N*-acetyl-aspartyl-Glu; tCr: creatine + phosphocreatine. Metabolite concentrations are expressed in mmol/kg of wet weight.

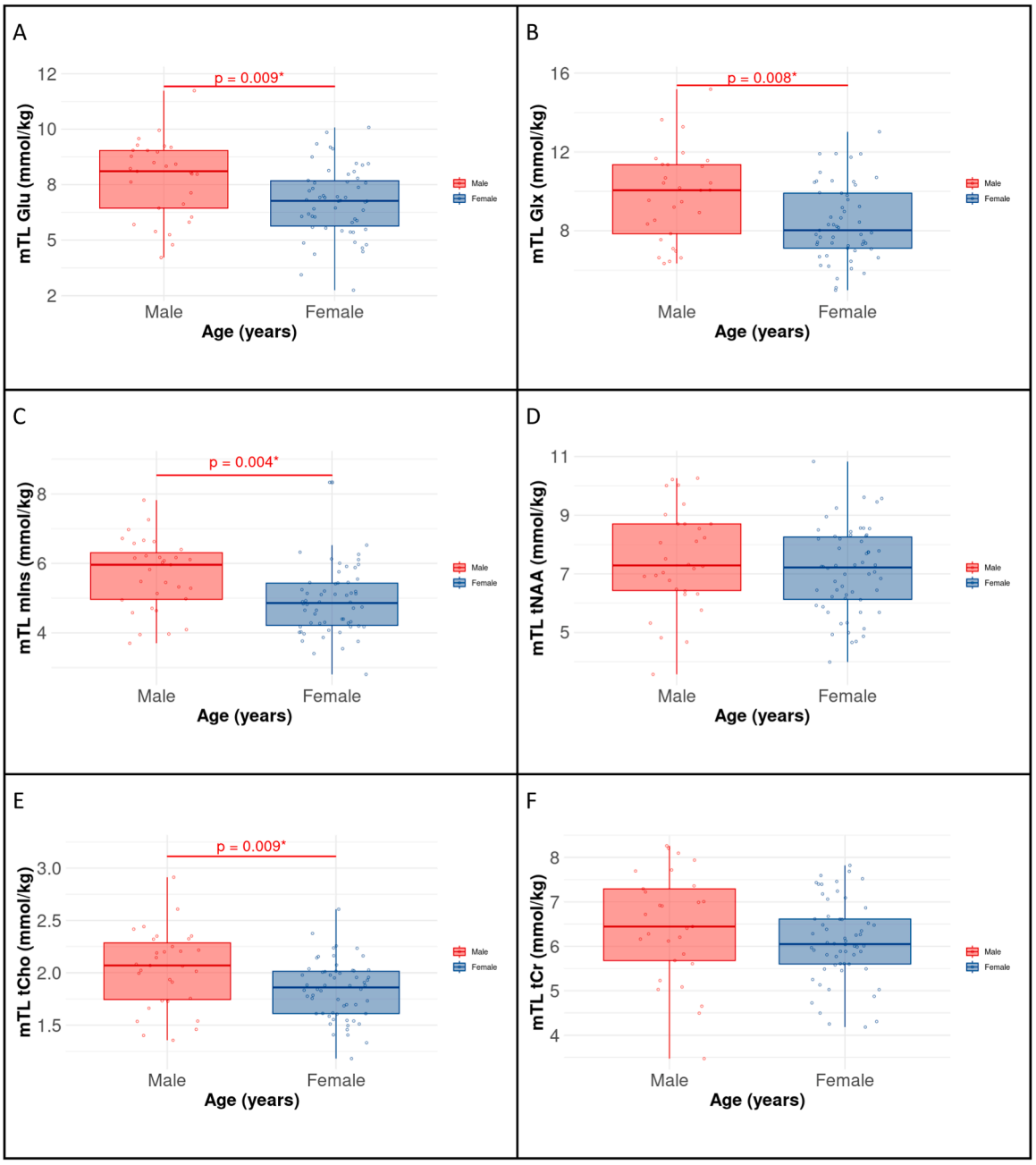

Fig. 3. Sex effects on metabolite concentrations in the mTL.
A, B: Higher levels of Glu and Glx in males than females; C: Higher levels of mIns in males than in females; E: Higher levels of tCho in males than in females. mTL: medial temporal lobe; t: absolute concentration; Glu: glutamate; Glx: Glu + glutamine; mIns: myo-Inositol; tCho: glycerophosphocholine + phosphocholine; tNAA: N-acetyl-aspartate + *N*-acetyl-aspartyl-Glu; tCr: creatine + phosphocreatine. Metabolite concentrations are expressed in mmol/kg of wet weight.

dmPF, whereby tNAA was positively associated with age until approximately age 22 and negatively associated from then on. tNAA provides acetate for synthesis of myelin fatty acids in oligodendrocytes (Moffett et al., 2007; Perdue et al., 2023). Gray matter volume progressively decreases from late childhood onwards, while brain myelination increases into young adulthood (Bethlehem et al., 2022). In this developmental context, the positive association between tNAA and age up until 22 years may correspond to ongoing myelin synthesis during childhood and adolescence, which subsequently decreases in prominence after early adulthood (Grydeland et al., 2019). tNAA showed a significant positive correlation with Glu and Glx, which is consistent with their metabolic relationship, whereby NAAG, which is considered as a non-excitotoxic reservoir of glutamate (Baslow, 2010), is catabolized into NAA and Glu (Moffett et al., 2007; Rae, 2014). tCho has been associated with cell density and cell membrane phospholipids (Rae, 2014; Stovell et al., 2017). Previous studies have reported an age-related decline in tCho levels in young children (Perdue et al., 2023), and both age-related increases (Chang et al., 1996; Chiu et al., 2014; Lind et al., 2020; Maudsley et al., 2012) or no effect of age (Brooks et al., 2001; Chang et al., 2009; Ding et al., 2016; Fukuzako et al., 1997; García Santos et al., 2010; Grachev et al., 2001; Grachev and Apkarian, 2000, 2001; Hädel et al., 2013; Harada et al., 2001; Marsman et al., 2013; Maudsley et al., 2009; Sailasuta et al., 2008) have been documented in adults in the dmPF. We observed an age-related increase in the concentration of tCho in this region, starting in late childhood and continuing into adulthood. Both main constituents of the tCho signal,

PCh and GPC, are linked through the CDP-choline pathway. This metabolic route is key for the synthesis of phosphatidylcholine, which, together with phosphatidylethanolamine, is the most abundant constituent of human cell membranes (Fagone and Jackowski, 2013). PCho is a precursor for phosphatidylcholine synthesis, while GPC is a result of the breakdown of phosphatidylcholine. Thus, this positive association between age and tCho could be indicative of changes in cell membrane present during "late" neurodevelopment. These changes could either reflect synthesis of membrane phospholipids, such as those involved in synaptic proliferation or myelination, or breakdown of membrane constituents, essential to synaptic pruning (Rae, 2014). In contrast, no age-related association was observed for tCho measured in the mTL, which is largely consistent with previous findings (Chiu et al., 2014; Ding et al., 2016; Driscoll et al., 2003; Fukuzako et al., 1997; García Santos et al., 2010; Gruber et al., 2008; Lind et al., 2020; Maudsley et al., 2009; Reyngoudt et al., 2012). tCr plays a key role in the energetic homeostasis of the brain, and creatine participates as an osmolyte (Stein et al., 2023) and modulates glutamatergic neurotransmission through NMDA receptor mediated calcium response (Rae and Broer, 2015). tCr was significantly positively correlated with every other metabolite measured in the dmPF (except for tCho) and the mTL, which is consistent with its broad role in cellular energy dynamics, as tCr is abundant in tissues with high energy demand. The use of creatine-referencing is widespread in studies assessing brain metabolites (Chabert et al., 2022a), however previous reports cautioned against creatine-referencing, due to varying concentrations of creatine across brain regions and neurodevelopmental periods, and the impact of other factors such as brain activity or diet (Rae, 2014). In the frontal lobe, some studies reported age-related increases in creatine-containing compounds in both children and adults, while studies in adults only yielded mixed, positive and negative age-associations (Table 1). In our sample, we have observed a quadratic association between age and tCr in the dmPF, whereby there was a positive relationship between this metabolite and age until 25 years, followed by a negative age-association. Therefore, our results add to the suggested caution in using creatine referencing, especially in the dmPF, in samples spanning from youth to early adulthood, given the non-linear relationship with age over this life period.

Overall, we found no significant age effects on levels of any studied metabolite in the mTL. This may be due to potential differences in the timing of maturation of this region compared to the prefrontal region, as suggested by longitudinal structural imaging studies (Bethlehem et al., 2022), meaning that the developmental processes in which these metabolites participate may occur prior to the investigated age range. An additional factor to consider is the poorer quality of spectra inherent to this region (Gajdošík et al., 2021), which led to a smaller sample size following quality control procedures, which may have impacted on the ability to detect significant effects within those who were included.

When examining sex effects alone, we observed differences between males and females in Glu, Glx, tCho and mIns in the mTL. This finding aligns with the fact that structures within this brain region, such as the hippocampus, are directly involved in sexual hormone regulation, which could in turn impact regional metabolite concentrations (Kight et al., 2020). Focusing on larger samples centered around puberty, using more biologically meaningful measures such as Tanner stage (Emmanuel and Bokor, 2022), serum hormone levels or menstrual cycle stage, will contribute to better understand sex-effects on metabolite levels in this brain region (Heller et al., 2024). In contrast, we observed no sex or age by sex interactions in the dmPF, which could potentially be due to smaller effects of sex in this region. It is also plausible that the metabolites under investigation are not directly involved in sex-influenced processes in this area of the brain, or that sex effects on the development of the dmPF are subtle or heterogeneous, in accordance with the notion that sexual influences on MRI-based measures have been found to be rarely dimorphic (Eliot et al., 2021).

Finally, it is noteworthy that we found significant differences in the concentration of all metabolites between the two VOIs. This is likely related to the regional specialization of the brain, with differences in function and cytoarchitecture between the two brain regions (Cichocka et al., 2016).

In this study, there are several methodological considerations that need to be acknowledged. First, although our focus was on understanding age effects over a wide age range, this is a cross-sectional study using one acquisition per participant. This design enables shorter recruitment periods and reduces methodological confounds. Nevertheless, future studies with longitudinal designs examining age-related trajectories or changes will be crucial towards further elucidating the dynamic changes in metabolite levels over time. Likewise, the aim of the study was to cover the age range of onset of most severe psychiatric disorders, however, some severe neuropsychiatric conditions, such as autism spectrum disorders, have an onset earlier on (Solmi et al., 2021). Next, due to our ^{1}H MRS acquisition protocol, glutamine was analyzed together with Glu as Glx, hence we were unable to examine age-related effects specific to glutamine, which could be characterized by an opposite trend compared to Glu according to some studies (Cichocka and Bereś, 2018). In addition, we were unable to examine other metabolites that have also been implicated in psychiatric disorders, such as GABA (Simmonite et al., 2023). Furthermore, the use of single-voxel ^{1}H MRS limits investigations to pre-specified brain regions, omitting other potentially informative brain areas. In addition, this approach focuses on relatively large volumes, which can complicate the analysis of highly specialized and compartmentalized structures within the brain, such as the amygdala and hippocampus in the mTL, which may have different metabolic profiles across their nuclei. Therefore, our findings may not fully capture the metabolite-age associations within these specific structures, and further research employing multi-voxel techniques, which allow for a higher spatial resolution, is needed to address this limitation. It is also important to note that our study did not encompass a comprehensive analysis of all brain areas implicated in psychiatric disorders. Notably, regions such as the basal ganglia and the thalamus, which are known to play significant roles in psychiatric conditions (Chabert et al., 2022b; Merritt et al., 2023; Moriguchi et al., 2019), were not included in our analysis due to feasibility. Nevertheless, future investigations should consider incorporating a more comprehensive evaluation of relevant brain regions to provide a more holistic understanding of the age-related metabolic trajectories in the brain. T1, T2 and proton density constants are known to change subtly with age, including progressive T2 shortening from early childhood through adulthood (Gräfe et al., 2021). While our study used standard constants appropriate for the age range spanning from childhood to young adulthood — consistent with current practice (Ghisleni et al., 2015; Holmes et al., 2017; Perdue et al., 2023)— this approach may introduce quantification bias, particularly in developmental comparisons. The potential for T2-related misestimation of metabolite levels represents a limitation of this kind of studies. Future studies that incorporate subject- or age-specific T2 measurements will be essential to improve accuracy in metabolite quantification. Likewise, the reported correlations between metabolite levels should be interpreted with caution, given the closeness of the spectral peaks of these metabolites, which can challenge the precise quantification of individual metabolites (Hong and Shen, 2023). Importantly, future studies that include a higher proportion of diverse ethnic groups are essential to study the generalizability of these findings. Finally, this study has employed the category of sex assigned at birth as a proxy for chromosomal dotation, biological sex-related physiological characteristics and hormonal status, which is an oversimplification of such. Moreover, our sample had a higher proportion of females than males. However, both sexes were represented across the age range included in the study, and there was sufficient statistical power to compare males and females. Nevertheless, this may have somewhat limited our capacity to detect age by sex interaction effects.

5. Conclusion

Our findings demonstrate that the most common brain metabolites assessed using ^{1}H MRS exhibit both linear and non-linear relationships with age, spanning from late childhood to early adulthood, and that these associations are region-specific. Additionally, we observed significant sex effects on metabolite levels within the mTL. These results emphasize the importance of considering non-linear age-related effects, sex differences, and avoiding creatine referencing when designing and interpreting ^{1}H MRS studies in psychiatry within this age range.

Data code availability statement

The data for this project is confidential, but anonymized data may be shared once Data Transfer Agreements with the Institut d'Investigacions Biomèdiques (IDIBAPS) - Fundació Clínic per a la Recerca Biomèdica (FCRB) are in place between institutions. Researchers interested in access to the data may contact Gisela Sugranyes at gernest@clinic.cat. Data processing and analysis code are also available upon request.

Data availability

Data will be made available upon reasonable request and subject to approval by the institutional ethics committee.

CRediT authorship contribution statement

María Ortuño: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adriana Fortea:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation. **Isabel Valli:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **Emma Muñoz-Moreno:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Formal analysis. **Roger Borràs:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Irene Martínez-Serrano:** Writing – original draft, Validation, Resources, Data curation. **Mireia Masias Bruns:** Software, Methodology, Formal analysis, Data curation. **Patricia Camprodon-Boadas:** Writing – original draft, Validation, Supervision, Conceptualization. **Enric Vilajosana:** Resources, Project administration, Data curation. **Lydia Fortea:** Resources, Project administration, Data curation. **Joaquim Raduà:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation. **Elena de la Serna:** Writing – original draft, Resources, Funding acquisition, Data curation. **Inmaculada Baeza:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. **Josefina Castro-Fornieles:** Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Gisela Sugranyes:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Gisela Sugranyes has received travel support from Angelini Pharma and consulting fees from Fenix Group. Inmaculada Baeza has received honoraria and travel support from Angelini and Otsuka-Lundbeck. These sources of support are unrelated to the topic of the current article. Joaquim Raduà has received CME honoraria from Inspira Networks for a machine learning course promoted by Adamed, outside the submitted work. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2025.121353.

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