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Research paper

Short-chain carnitines in adolescent major depressive disorder: Associations and biomarker potential

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ABSTRACT

Background: A growing body of evidence suggests altered short-chain carnitine biology in mental health, notably significantly reduced serum levels of L-Acetyl-carnitine (LAC) in adults with major depressive disorder (MDD). Building on these findings, our biomarker study aimed to investigate potential MDD-associated disruptions in short-chain carnitines within juvenile cohorts.

Methods: In a cross-sectional analysis with between-group comparison we contrasted a cohort of adolescents with major depressive disorder ($n = 38$) with healthy controls ($n = 38$) by assessing peripheral blood levels of free L-carnitine (FC), LAC as well as the LAC/FC ratio and studied associations with psychometrically assessed clinical characteristics. To validate our cohort allocation and thus to corroborate the MDD related specification of our carnitine associated findings by replicating existing evidence towards specific altered endocrine parameters in adolescent MDD, we furthermore obtained morning cortisol, adrenocorticotropin (ACTH) and the cortisol/ACTH ratio.

Results: No significant inter-cohort differences were observed in FC or LAC-levels, but the LAC/FC ratio was significantly elevated in adolescents with depressive disorder (M(HC) = 0.39, SE = 0.02; M(MDD) = 0.47, SE = 0.02; Cohen's d = 0.80; $p < 0.001$). After controlling for confounders, in linear regression models, MDD cohort assignment emerged as a significant predictor of an elevated LAC/FC ratio ($\beta = 0.432$; $p = 0.046$; corr. $R^2 = 0.108$), as well as reduced FC levels ($\beta = -0.225$; $p = 0.049$; corr. $R^2 = 0.120$). Binominal regression analyses further identified the LAC/FC ratio (B = 7.432; $p = 0.010$; corr. $R^2 = 0.381$) as a predictive marker for MDD cohort assignment. Overall, the significant alteration in the LAC/FC ratio reinforces the overarching hypothesis of altered carnitine biology in both adolescent and adult individuals with MDD. Whether adolescent MDD represents a partially aberrant molecular pathophysiology compared to adults, or instead reflects an underdeveloped molecular phenotype differentiation, remains an open question for further research.

1. Introduction

With a point prevalence of 8 % in adolescents (Shorey et al., 2022), major depressive disorder (MDD) is one of the most frequently occurring disorders in both outpatient and inpatient child and adolescent psychiatric care. A significant increase in case numbers over the past two decades, particularly concerning inpatient treatment needs (Statistisches Bundesamt, 2019), as well as a high comorbidity burden of depressive disorders (Mudra and Schulte-Markwort, 2020), contrasts with a considerable degree of - to some extent contradictory - heterogeneity within the evidence-based research on adolescent depression. This heterogeneity is particularly evident regarding etiopathogenesis, subtype differentiation, diagnostic classification and the implementation of reliable therapeutic concepts (Dolle et al., 2013; Maughan et al., 2013; Stringaris, 2017). Building on a growing body of molecular research on depressive disorders in adults, current child and adolescent psychiatric research is increasingly focusing on neuro-endocrine and metabolomic factors, as well as their complex interaction patterns.

1.1. MDD and carnitine-biology alterations

In view of the specific metabolome-related scientific efforts, carnitine biology — in particular the role of the acetylated component *L*-acetyl-carnitine (LAC) — is increasingly coming into focus, as it plays a potential preventive role in the pathogenesis of depressive disorders. Unlike long-chain lipids, which cannot cross the blood brain barrier and

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thus remain inaccessible to intramitochondrial metabolism inside the central nervous system (CNS), LAC is an amphiphilic molecule, that can freely pass both the inner mitochondrial membrane and the blood-brain-barrier (Ferreira and McKenna, 2017). This unique property enables LAC to function as an intramitochondrial acetyl-moiety donor, playing a pivotal role in intracellular carbon trafficking between CNS mitochondria and cytosol (Ferreira and McKenna, 2017).

In adult CNS tissue, the LAC-bound acetyl groups are preferentially utilized for the synthesis of structural lipids rather than for energy metabolism (Kawamura, 1988; Nałecz and Nałecz, 1996). In the developing brain, however, these acetyl groups serve a more diverse set of functions, including energy metabolism, and neurotransmitter synthesis besides the aforementioned formation of structural lipids (Scafidi et al., 2010). Furthermore, intramitochondrial de-acetylation of LAC provides free L-carnitine (FC), which aids in the formation of excretable acyl-CoA-derivates. This process facilitates the removal of oxidized, cytotoxic membrane lipids, highlighting the neuroprotective properties of LAC (Ferreira and McKenna, 2017).

In addition to these potential neuroprotective functions, LAC has also demonstrated potential antidepressant effects through epigenetic modulation of the glutamatergic system and hippocampal dendritic plasticity via histone acetylation in animal models as well as cell - based models (Madiraju et al., 2009; Smeland et al., 2012; Cuccurazzu et al., 2013; Nasca et al., 2018; Bigio et al., 2024). Rodents with depressive behavioral traits or exposed to stress induction showed a rapid onset and sustained reduction in depression-like or stress-induced behaviors following exogenous LAC supplementation (Cuccurazzu et al., 2013). Consistent with this, LAC-supplementation led to an increase in overall serotonin and norepinephrine levels in healthy mice. (Smeland et al., 2012).

Translating these findings to human studies, exploratory randomized controlled trials (RCTs) investigating the potential effects of LAC supplementation on reducing depressive symptom load have reported a direct antidepressant effect of exogenous LAC administration in depressed adults (Kriston et al., 2014; Wang et al., 2014; Veronese et al., 2018). In a comparative evaluation, efficacy and effectiveness were reported to be comparable to well-established antidepressants as LAC consistently showed a significantly better side-effect profile compared to standard treatments (Kriston et al., 2014; Wang et al., 2014; Veronese et al., 2018).

Crucially, evidence supports a positive correlation between peripheral and central nervous LAC-levels (Pettegrew et al., 2000), alongside a homeostatic stability of carnitine-associated markers under steady-state conditions (Alberty and Albertyová, 1997). These findings are consistent with several studies in depressed adult cohorts, that independently reported significantly reduced peripheral blood levels of LAC in comparison to healthy controls (Nasca et al., 2018; Nie et al., 2021; Liu et al., 2022). In addition to decreased LAC concentrations, another study with adult cohorts demonstrated significantly increased FC concentrations and a significantly increased FC/LAC ratio in the case of MDD (Ait Tayeb et al., 2023). This suggests that the neuroprotective and epigenetic functions of LAC may be impaired in adult depression, further emphasizing its potential as a biomarker and therapeutic target in depressive disorders.

Regarding endocrine signaling pathways, a growing number of RCTs indicate a link between depressive symptoms and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. This dysregulation is characterized by a significant disinhibition of the pituitary and, in the case of prolonged illness, also the hypothalamic cortisol feedback loop (Ford et al., 2019; Lee et al., 2021; Zajkowska et al., 2022). A potential underlying pathomechanism involves increased resistance of the glucocorticoid receptors in central nervous system, due to an interplay of genetic polymorphisms, epigenetic changes, and dysregulation in response to chronic or acute (psycho)traumatic stress (Lee et al., 2021; Zwolińska et al., 2023; Agorastos and Chrousos, 2022).

1.2. The present study

The findings above provide strong evidence that altered short-chain carnitine biology is associated with depression in adulthood, with LAC emerging as a promising target for both diagnosis and treatment. However, to our knowledge, there are no previous studies that have investigated possible associations between disrupted short-chain carnitines and adolescent depression, which has been hypothesized to differ from adult depressive disorders in both pathophysiological and symptom-related aspects (Wight et al., 2004; Bylund and Reed, 2007; Rice et al., 2019). Our research is the first to examine potential alterations in carnitine biology in adolescents with MDD. Our primary hypothesis posits that, from a macro perspective, short-chain carnitine biology is similarly disrupted in adolescents with MDD as in adults with MDD, but that, from a micro perspective, there are distinct deviations in the disruption profiles between adults and adolescents with MDD. To test this, we assessed peripheral blood levels of FC and LAC in adolescents with and without MDD to determine the extent to which adolescent findings replicate those observed in adults. In addition to analysis of blood levels, we calculated a ratio between the two carnitine metabolites to investigate potential dynamic interdependencies between these biomarkers that may indicate impaired mitochondrial biology (Ait Tayeb et al., 2023). This ratio may serve as a more accurate indicator of depression-related alterations, particularly in LAC and FC — the two most important carnitine-compounds for CNS metabolism during adolescence. In contrast to the previous study by Ait Tayeb et al. (2023), we did not implement an FC/LAC ratio, but rather the reciprocal LAC/FC ratio, to parallel the conceptualization of our ratio with studies from pediatric metabolic research, where the acylcarnitine/free carnitine ratio is used as a surrogate marker for altered carnitine biology (Ezgu, 2016).

Given the substantial evidence for highlighting the central role of oxidative stress in the pathogenesis of MDD (Bhatt et al., 2020; Chen et al., 2024; Shao et al., 2024), we expected a tendency towards depletion of FC in adolescent individuals with MDD, driven by carnitine-mediated processing of oxidized lipids under stressful conditions. In contrast, we expected minimal alterations in LAC levels, as its role in maintaining intracerebral metabolic homeostasis is more pronounced during brain development than in adulthood (Scafidi et al., 2010). Therefore, we hypothesized that the LAC/FC ratio would serve as a relevant marker in adolescents with MDD.

To further validate our cohort allocation and to substantiate the MDD-specific nature of our carnitine-associated findings, we furthermore examined whether scientifically pre-described alterations in the HPA axis, which are frequently observed in adolescent MDD patients, could be reproduced in our MDD cohort. Morning cortisol and adrenocorticotropin (ACTH) levels were measured, and intra-individual cortisol/ACTH ratios were calculated to assess possible dysregulation within these endocrine pathways. By incorporating these measures and correlating them with pre-existing evidence of altered HPA-axis characteristics in adolescent MDD, we aimed to corroborate our findings on the carnitine biology while identifying interrelated biological markers that could further elucidate the underlying mechanisms of adolescent depression.

Overall, the present study aims to investigate the association between short-chain carnitine levels and major depressive disorder in adolescents using dried blood spot (DBS), serum, and plasma measurements. We also evaluate the diagnostic potential of the FC/LAC ratio as a biomarker for MDD in this sample.

2. Materials, methods

2.1. Ethics and consent

Written informed consent was obtained from all participants and their legal guardians, with the option to withdraw from the study at any

time. All procedures were approved by the Ethics Committee of the University of Regensburg (22–3057-101) and pre-registered in the German Clinical Trials Registry (DRKS; DRKS00030637). As compensation, all participants received a gift voucher worth €50.

2.2. Sample and recruitment

Using a cross-sectional design with between-group comparison, an MDD-cohort was matched with a healthy control cohort (HC). Both cohorts underwent a single-timepoint assessment of psychometric and biological variables.

The required minimum sample size was determined using a power analysis with the G-power software (Faul et al., 2007) for both a two-tailed *t*-test and a two tailed bivariate correlation model. The significance level was set at $p < 0.05$. Based on the study by Nasca et al. (2018) with an effect size of $d = 0.8$ for the t-test-related comparison of LAC in adults and an effect size of $\rho = 0.35$ for the correlation of LAC with the psychometrically assessed depressive symptom burden, we set the effect sizes for our study in adolescents slightly lower at $d = 0.75$ and $\rho = 0.31$. This resulted in a minimum sample size of $n = 52$ for the t-test and a minimum sample size of $n = 79$ for the correlation analyzes, whereby the latter sample size was chosen.

After the initial recruitment of 80 participants with 41 candidates from the index cohort and 39 candidates from the healthy control cohort, four candidates were excluded before final enrollment: one healthy participant due to reporting a depressive symptom burden and three participants from the index cohort due to meeting at least one exclusion criterion at the time of final enrollment.

Data assessment and analysis was conducted at the Clinic for Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy at the University of Regensburg, Germany, over a period of 14 months from February 2023 to April 2024. The age range of the candidates was set at 11 to 18 years and was selected based on previous findings indicating a slight increase in the LAC/FC ratio under standardized conditions during the course of childhood, which stabilizes at a level typical of adults by the onset of puberty (Alberty and Albertyová, 1997; Osorio and Pourfarzam, 2002).

The MDD-cohort comprised 38 participants, who had all been pre-diagnosed with major depressive disorder as the primary psychiatric illness and were currently undergoing inpatient, day-clinic or outpatient treatment at the Clinic for Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy at the University of Regensburg, Germany. Prior to enrollment, diagnoses had been assigned through a multi-expert assessment and were based on the ICD-10 diagnostic criteria for major depressive disorder, including F32.1, F32.2, F33.1 and F33.2 (see Table 1) as eligible current psychiatric diagnoses (World Health Organization, 2021). All participants in the MDD cohort suffered from fluid symptomatology at the time of enrollment in terms of meeting the diagnostic criteria of MDD, which was psychometrically confirmed using the German version of the structured Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-KID) (Sheehan et al., 1998), which aligns with the diagnostic criteria for major depressive disorder according to DSM-IV (American Psychiatric Association, 2000) and ICD-10 (World Health Organization, 2021).

The MDD cohort was compared with a control cohort of 38 physically and mentally healthy participants (Healthy control cohort, HC), who had no history of psychiatric or significant somatic medical conditions and were recruited via social media and print media.

An additional inclusion criterion for both cohorts was the absence of daily intake of psychopharmacological medications for at least the past three months to minimize potential confounding effects of psychoactive drugs on carnitine metabolism. Furthermore, participants with comorbid psychiatric disorders, that could significantly mask or override depressive symptomatology - such as schizophrenia, autism spectrum disorders, borderline personality disorder, eating disorders with fluid symptomatology, or chronic substance abuse – were excluded from the study.

The general exclusion criteria for both cohorts were based on the framework outlined by Nasca et al. (2018) and comprised the presence of acute or chronic neurological or physical illness within the past six months as well as recent use of medications with the potential to significantly disrupt endocrine, inflammatory or metabolic homeostasis

Table 1
Sociodemographic and clinical characteristics.

	MDD	HC	cohort differences
Sample size	38	38	
Sex			$X^2(1) = 3.46$, $p = 0.104$[1]
Female	26 (68.4 %)	18 (47.4 %)	
Male	12 (31.6 %)	20 (52.6 %)	
Mean age in years (SEM)	15.32 (0.25)	14.47 (0.39)	$t(62) = 1.81$; $p = 0.075$[2]
Median weight in kg (IQR)	61.60 (54.00–70.25)	55.00 (46.18–68.43)	$Z = 1.47$; $p = 0.141$[3]
Median BMI in kg/m^2 (IQR)	21.50 (19.23–22.90)	19.70 (17.73–22.63)	$Z = 1.65$; $p = 0.100$[3]
Mean BMI-Percentiles (SEM)[4]	51.18 (4.58)	45.89 (4.23)	$t(74) = 0.85$; $p = 0.399$[2]
Depression diagnosis (ICD-10)			
F32.1	28 (73.7 %)	–	
F32.2	6 (15.8 %)	–	
F33.1	4 (10.5 %)	–	
F33.2	–	–	
Median IPAQ athletic activity in min. (IQR)[5]	105.00 (27.50–555.00)	220.00 (87.50–383.75)	$Z = 0.69$; $p = 0.489$[3]
Median BDI (IQR)	26.00 (20.75–40.25)	2.00 (0.00–5.00)	$Z = 7.39$; $p < 0.001$[3]
Suicidality[6]			
Acute (<4 weeks ago) suicidal thoughts	25 (65.8 %)	–	
Acute (<4 weeks ago) suicidal plans	7 (18.4 %)	–	
Positive history of suicide attempts	9 (23.7 %)	–	
NSSI[6]			
Acute (<4 weeks ago) thoughts of NSSI	22 (57.9 %)	–	
Acute (<4 weeks ago) executed NSSI	15 (39.5 %)	–	
Mean PSS (SEM)	34.58 (0.88)	19.84 (0.77)	$t(74) = 12.62$; $p < 0.001$[2]
Median CTQ (IQR)	39.50 (43.50–47.25)	27.00 (25.75–29.00)	$Z = 6.34$; $p < 0.001$[3]
Median PHQ-15 (IQR)	10.00 (7.00–12.25)	3.00 (1.00–4.25)	$Z = 5.47$; $p < 0.001$[3]
Mean PSQI (SEM)	8.47 (0.56)	2.89 (0.25)	$t(51) = 9.11$; $p < 0.001$[2]
Median GAF in % (IQR)	51.00 (47.25–62.25)	96.00 (94.00–98.00)	$Z = 7.51$; $p < 0.001$[3]

Note. MDD: MDD cohort; HC: healthy controls cohort; SEM: standard error of mean; IQR: interquartile range; BMI: body mass index; NSSI: nonsuicidal self-injury; F32.1: MDD, moderate depressive episode; F32.2: MDD, severe depressive episode without psychotic symptoms; F33.1: MDD, recurrent depressive disorder, current episode moderate; F33.2: MDD, recurrent depressive disorder, current episode severe; IPAQ: International Physical Activity Questionnaire; BDI: Beck Depression Inventory II; PSS: Perceived Stress Scale; CTQ: Childhood Trauma Questionnaire; PHQ-15: Patient Health Questionnaire (somatic symptom load); PSQI: Pittsburgh Sleep Quality Index; GAF: Global Assessment of Functioning; SITBI: Self-Injurious Thoughts and Behavior Interview.
[1] Chi-Squared test; [2] Independent *t*-Test; [3] Mann-Whitney *U* Test; [4] according to „Studie für die Gesundheit der Kinder und Jugendlichen in Deutschland“transl. German health survey for children and adolescents (KiGGS) (Neuhauser et al., 2013); [5] past 7-day extensive (anaerobic and high-intensity aerobic) and moderate (medium-intensity aerobic) activity; [6] as assessed by SITBI-SF.

within the past four weeks. Likewise, long-term treatment with pharmaceuticals known to affect these systems was required to have been discontinued at least one month prior to study participation. In addition, participants with current use of potentially abusable psychotropic substances or active infections or systemic diseases, as determined by a medical examination and clinical history, were excluded from the study.

To control for possible outlier effects due to altered metabolic configurations associated with extreme underweight or overweight, the body mass index (BMI)-percentiles of all participants had to be within +/− 2 *Z*-scores (Gatti et al., 1998).

Further details on the sample characteristics can be found in Table 1.

2.3. Psychometric and anthropometric data assessment

The clinical assessment was performed by a qualified clinical physician and included a brief physical examination, body measurements, and sociodemographic assessment (see Table 1) including age and biological sex. Particular attention was paid to the measurement of participants' body mass and height, from which the body mass index (BMI) and BMI percentiles were calculated based on the German reference cohort (Neuhauser et al., 2013). Age, biological sex, and BMI (−percentiles) were considered as important potential confounders related to carnitine metabolism and were therefore systematically documented and included in the subsequent analyzes.

This focus was based on evidence suggesting sex-specific differences in plasma carnitine levels (Alberty and Albertyová, 1997; Gatti et al., 1998), with free L-carnitine (FC) being slightly increased and *L*-acetylcarnitine (LAC) being slightly decreased in men compared to women due to sex-specific differences in tissue composition, body mass, and iron metabolism (Chiu et al., 1999).

Participants' current physical activity in the past 7 days was assessed using the short version of the International Physical Activity Questionnaire (IPAQ-SF) (Craig et al., 2003) to control for potential confounding effects on carnitine biology, in particular the transient increase in the ratio of short-chain acyl-carnitine to carnitine caused by physical exercise (Borum, 1994). The IPAQ-SF, administered as a structured clinical interview, differentiates the cumulative time spent in different activity domains in the past seven days, including vigorous (anaerobic and high-intensity aerobic), moderate (moderate-intensity aerobic), and walking activities (all exceeding 10 min duration), as well as time spent in sedentary activities. The instrument is supported by evidence-based acceptable reliability and good validity metrics for an adolescent cohort (Todorović et al., 2024). In light of the previously reported alterations in carnitine metabolism with exercise, we included a composite variable, *physical activity*, representing the combined duration of vigorous and moderate exercise in subsequent data analyzes.

The MINI-KID interview, conducted under the supervision of a board-certified child and adolescent psychiatrist, was used to assess each participant's current depressive symptom burden and to confirm the presence of Major Depressive Disorder (MDD) according to the MINI-KID diagnostic criteria. It shows high inter-rater reliability (0.89 – 0.94), and test–retest reliability (0.87–1.00) (Sheehan et al., 2010). A trained clinical physician then administered a comprehensive set of psychometric questionnaires. This included the Childhood Trauma Questionnaire (CTQ) (Klinitzke et al., 2012) with the five subscales physical, sexual, and emotional abuse as well as physical and emotional neglect to assess the quantity and quality of adverse childhood events. Klinitzke et al. reported sufficient construct validity, internal consistencies and model fit of the five-factor structure, emphasizing the importance of critically interpreting the physical neglect subscale. The Perceived Stress Scale (PSS) with the two subscales Perceived Helplessness and Lack of Self-Efficacy was used to evaluate the current subjective perception of stress with sufficient internal consistency and construct validity (Klein et al., 2016). The Patient Health Questionnaire (PHQ-15) (Kroenke et al., 2002) with good evidence-based overall reliability (Cronbach $\alpha = 0.79$) (Gräfe et al., 2004) was implemented to depict subjective somatic symptom burden, while the sum score of the Pittsburgh Sleep Quality Index (PSQI) (Backhaus et al., 2002) with the subscales overall sleep quality, sleep latency, sleep duration, sleep efficiency, sleep medication usage and daytime dysfunction was applied to assess sleep quality with adequate reliability and validity reported both in German adult cohorts (Hinz et al., 2017) and Spanish adolescent cohorts (La Vega et al., 2015).

The subjective severity of depressive symptoms was measured using the Beck's Depression Inventory II (BDI-II) (Kuehner et al., 2023) with high internal consistency (Cronbachs $\alpha \geq 0.90$) being reported (Besier et al., 2008; Dolle et al., 2012). Concomitant suicidal and non-suicidal self-injurious behaviors were assessed quantitatively and qualitatively using the short form of the Self-Injurious Thoughts and Behavior Interview (SITBI-SF), a structured diagnostic interview. The stratification included suicidal ideation, suicidal plans, suicidal gestures, suicidal attempts, thoughts of non-suicidal self-injury (NSSI), and NSSI. The SITBI-SF is based on the long form, which has demonstrated sufficient test-retest reliability, inter-rater reliability and construct validity (Fischer et al., 2014). Finally, the Global Assessment of Functioning (GAF) (Aas, 2011) was used to obtain a holistic overview of illness-related distress burden and psychosocial functioning with sufficient inter-rater reliability in adolescent cohorts (Schorre and Vandvik, 2004).

2.4. Biomarker assessment

Blood samples were collected by antecubital venipuncture using standardized techniques, scheduled for the morning hours after at least six hours of fasting and at least six hours of rest. Sample collection was synchronized with the specified individual wake-up times by setting the time for blood collection at 90 min after the individual awakening. The rationale behind this approach was the evidence-based determination of a single time point during the day when the assessment of cortisol and ACTH levels between individuals should be as reproducible as possible between individuals. The cortisol awakening response (CAR) with an interindividually varying rapid increase in cortisol levels approx. 30–45 min after awakening had to be taken into account (Clow et al., 2010), whereby special attention also had to be paid to the asynchronous wake-up times of the subjects and their effects on the CAR (van de Werken et al., 2014). In this context, existing evidence shows that a blood sampling time of 90 min after waking up generates conditions with maximum interindividual reproducibility (Clow et al., 2010; van de Werken et al., 2014; Stalder et al., 2016).

The different blood matrices of the individual subjects were obtained simultaneously and processed according to a defined protocol determining standardized conditions. Carnitine analyzes were performed using dried blood spot (DBS) assays. In the research-based use of DBS as a matrix compared to plasma for carnitine quantification, it must be taken into account that acylcarnitines as well as LAC tend to adhere to the erythrocyte surface (Sain-van der Velden et al., 2013) with a significant correlation between DBS- and plasma bound concentrations (Lu et al., 2021). At the same time the same authors report lower concentrations of FC in DBS compared to plasma with a significant correlation between DBS and plasma FC-concentrations, leading to a potentially higher specificity in the case of studies using FC as possible biomarker. Alongside, Al-Thihli et al. (2014) reported a higher specificity, especially with regard to the ratios of long-chain carnitine / short-chain carnitine, in the context of diagnosis of fatty acid ß-oxidation defects, with at the same time reduced sensitivity for DBS-based carnitine quantification in comparison to plasma-based assessment. Taking into account that our study focuses at least in part on hypothesized alterations in mitochondrial energy metabolism in the case of our adolescent depressive cohort, as well as taking into account our small study samples, the DBS method was on the one hand chosen for its clinical feasibility and on the other hand with the intention of reducing type I error (false positive) in the calculation of LAC/FC ratio. Since carnitine derivatives in DBS remain quantitatively stable at room temperature for at

least 14 days (Fingerhut et al., 2009), the analysis in our case was performed no later than two days after collection.

Morning cortisol was measured from blood serum, while morning ACTH was determined from plasma with a single pre-analytical dropout in the determination of solitary ACTH. Given that serum-cortisol and plasma-cortisol values can be used interchangeably in clinical practice and MDD-related research (World Health Organization, n.d.; Sahu et al., 2022), we chose the different matrices for the cortisol and ACTH measurements for pragmatic reasons. This decision was made in collaboration with our partner laboratory, considering improved preanalytical handling. Accordingly, the cortisol /ACTH ratio in our study was calculated using serum cortisol and plasma ACTH. This approach is further supported by previous evidence confirming the scientific basis for calculating the ratio from different matrices in the context of biomarker research (Li et al., 2018; Selek et al., 2018; Banu et al., 2023).

Quantitative analyzes were carried out by an external commercial laboratory (*SYNLAB Medizinisches Versorgungszentrum Weiden GmbH*) using electrospray ionization tandem mass spectrometry (ESI-TMS) for carnitines, enzyme immunoassay (EIA) for cortisol and luminescence immunoassay (LIA) for ACTH.

2.5. Statistical analyses

The statistical analyzes were performed with SPSS Statistics 29 (IBM Corp, 2023). To compare continuous and categorical demographic and clinical aspects between the healthy control cohort (HC) and major depressive disorder (MDD) cohort, two-tailed *t*-tests, Mann-Whitney *U* tests, and χ^2 analyzes were performed.

Given our study design with single time point measurements of potentially fluctuating variables, we opted for non-parametric tests with rank-based analysis. This approach was chosen to examine biomarker-related variables that are not normally distributed or skewed, rather than applying transformations to achieve normality.

Between-cohort comparisons of biomarker-related variables were analyzed using t-tests or their non-parametric equivalents.

To control for potential confounding effects of age, weight, BMI percentiles and physical activity on biomarker-related variables, as well as to examine associations between biomarkers and psychometrically assessed symptom components, we implemented a multilevel procedure. The selection of specific test procedures was derived from the study by Nasca et al. (2018) on carnitine quantification in adult depression:

First, we performed correlation analyzes to assess the relationships between each biomarker-related variable and intra-subject control variables (age, weight, BMI percentile, IPAQ-measured physical activity in the past 7 days), as well as subjective symptom measures (CTQ, PSS, BDI, PHQ, PSQI). In addition, biomarker concentrations were compared between biological sexes using t-tests or their non-parametric equivalents, as appropriate.

Next, we grouped the biomarkers into four separate clusters: cluster I included FC and LAC, cluster II consisted of the LAC/FC ratio, cluster III included cortisol and ACTH and cluster IV comprised the cortisol/ACTH ratio.

For biomarkers showing significant associations with control variables or symptom-related questionnaire scores, standard multiple linear regression analyzes were performed. In each model, the respective biomarker served as the dependent variable (criterion), and the predictor variables were standardized within their respective clusters (for example for cluster I cohort affiliation, age, weight and BMI-percentile as predictor variables) to ensure comparability.

These regression models served two primary purposes: (1) to examine the relationship between psychometrically assessed symptom characteristics and biomarker scores, and (2) to assess the predictive influence of depressive status (i.e., cohort affiliation) while controlling for potential intra-subject confounding factors. Therefore, in addition to the relevant symptom-related and control variables, each model included cohort affiliation (MDD vs. HC) as an additional predictor.

To assess whether biomarkers could predict cohort affiliation, we performed binary logistic regression analyzes, using cohort (MDD vs. HC) as the dependent variable. Due to multicollinearity between the raw biomarker values (e.g., FC, LAC, cortisol, ACTH) and their respective ratios (LAC/FC, cortisol/ACTH), two separate logistic models were specified—one including the raw values, and one including the ratios—along with potential sociodemographic and anthropometric covariates.

In all regression analyzes, we assessed the influence of potential outliers using centered leverage, Cook's distance, and standardized residuals. Data points were excluded only if they had shown isolated and substantially disproportionate violations across multiple different analytical approaches.

To assess the diagnostic performance of the LAC/FC ratio in relation to assignment to the MDD cohort, we furthermore performed an exploratory receiver-operating-characteristic (ROC)-analysis.

Statistical significance was set at a threshold of 0.05, and results are reported as mean (SEM) or median (IQR) unless otherwise stated.

3. Results

Body dimensions, sociodemographic and psychometric measurements.

In our study sample of 76 participants, no significant differences were found between the MDD cohort ($n = 38$) and the HC cohort (n = 38) in terms of demographic characteristics or body measurements (see Table 1).

Participants in the MDD cohort showed significantly higher subjective depressive symptom severity, somatic symptom burden, subjective stress perception, as well as significantly poorer subjective sleep quality and impaired psychosocial functioning (see Table 1). In addition, the reported rates and intensity of adverse childhood experiences, assessed with the CTQ, were significantly higher in the MDD cohort.

3.1. Biomarker assessment

3.1.1. Free L- carnitine (FC) and L-Acetyl-carnitine (LAC)

In the cross-cohort analyzes of FC and LAC, no significant differences in concentration were found between the MDD and HC cohorts (see Table 2, see Fig. 1).

Correlation analyzes of FC and LAC with control variables (age, weight, BMI percentiles, IPAQ-measured physical activity in the past 7 days), as well as with subjective symptom affection (CTQ, PSS, BDI,

Table 2
Biomarker-associated inter-cohort differences.

	MDD Mean (SEM) / Median (IQR)	HC Mean (SEM) / Median (IQR)	cohort differences
Mean Free L-carnitine (FC) in µmol/l	28.25 (1.19)	31.03 (1.28)	t(74) = 1.59; $p = 0.117$[1]
Median L-Acetyl-carnitine (LAC) in µmol/l	12.02 (10.88–13.88)	11.77 (9.17–14.79)	Z = 1.14; $p = 0.253$[2]
Mean LAC/FC ratio	0.47 (0.02)	0.39 (0.02)	t(74) = 3.50; $p < 0.001$[1]
Median Morning cortisol in nmol/l	141.50 (86.18–177.25)	85.00 (71.75–110.00)	Z = 3.29; $p = 0.001$[2]
Median Morning ACTH in pg/ml	23.60 (18.45–36.10)	14.10 (10.00–18.68)	Z = 4.56; $p < 0.001$[2]
Median cortisol/ ACTH ratio in nmol/1000 pg	4.89 (3.98–5.87)	6.19 (5.12–8.26)	Z = 2.76; $p = 0.006$[2]

Note. MDD: MDD cohort; HC: healthy controls cohort; SEM: standard error of mean; IQR: interquartile range; ACTH: adrenocorticotropin.
[1] Independent t-Test; [2] Mann-Whitney U Test.

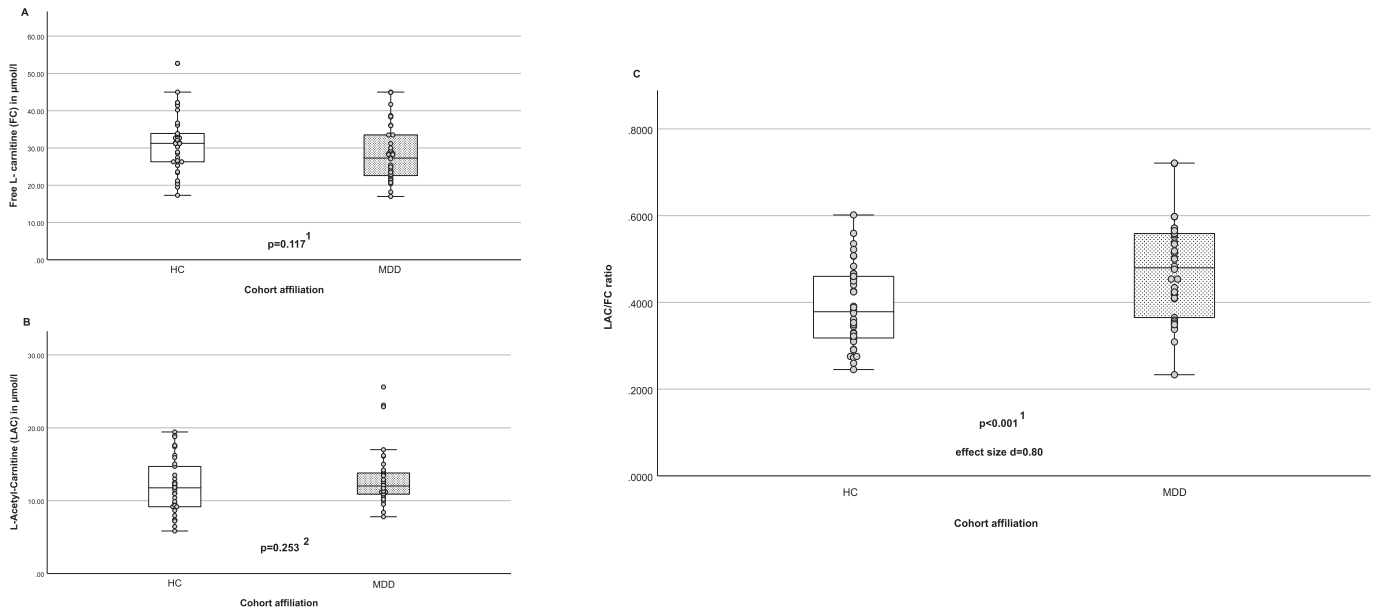

Fig. 1. Inter-cohort comparison of free carnitine (FC), L-Acetyl-Carnitine (LAC), LAC/FC ratio A-C: FC (A), LAC (B) and LAC/FC (C) concentrations in healthy controls (HC, $n = 38$) and in patients with major depressive disorder (MDD, n = 38) as assessed by ElectroSpray-Ionization-Tandem-Mass-Spectrometry (ESI-TMS). *Note.* [1] Student's two-tailed *t*-test; [2] Mann-Whitney U Test.

PHQ, PSQI) revealed weight and BMI percentiles as significantly positively correlated variables with both FC and LAC. Furthermore, LAC correlated significantly positively with age (see Supplementary Table 1). Psychometrically assessed symptom distress did not correlate with FC or LAC. Both FC levels (Z = -1.88; $p = 0.060$; Mann-Whitney-*U* test) and LAC levels (Z = -0.66; $p = 0.507$; Mann-Whitney-U test) did not differ between male and female participants.

Parallel linear regression analyzes were performed with cohort affiliation (depressive state), age, weight and BMI percentiles as predictors, and FC and LAC as criterion variables. The results showed that weight was significantly associated with increased levels of both FC and LAC. Depressive state (cohort affiliation) was significantly associated with decreased FC levels but showed no significant effect on LAC levels. Age and BMI percentiles had no significant effect on FC or LAC (see Table 3).

3.1.2. LAC/FC ratio

Regarding the calculated LAC/FC ratio, the cross-cohort analysis revealed a significantly increased mean ratio (d = 0.80) in the MDD cohort compared to the HC cohort (see Table 2, see Fig. 1).

Correlation analyzes of LAC/FC with within-subject control variables (age, weight, BMI percentile, IPAQ-measured physical activity in the last 7 days), as well as with subjective symptom distress (CTQ, PSS, BDI, PHQ, PSQI) showed significant positive correlations of LAC/FC with the quantitatively assessed history of adverse childhood experiences (CTQ), subjective stress perception (PSS), subjective depressive symptom burden (BDI) and subjective sleep quality (PSQI). Subjective somatic symptom burden (PHQ) as well as age, weight and BMI percentiles did not correlate with LAC/FC (see Supplementary Table 1), nor was there a mean difference in the comparison between the sexes ($t(74) = 1.17$; $p = 0.247$; two-tailed Student's *t*-test).

Table 3
Linear regression with carnitine related variables as criterion.

	Criterion							
	FC		LAC		LAC/FC[1]			
Predictors	β	p	β	p	β	p	β	p
Cohort affiliation[2]	-0.225	**0.049**	0.074	0.522	0.432	**0.046**	0.364	**0.047**
Age	−0.176	0.324	−0.156	0.393	–	–	–	–
Weight	0.474	**0.040**	0.524	**0.028**	–	–	–	–
BMI percentile[3]	0.005	0.978	−0.119	0.478	–	–	–	–
PSS					0.019	0.758	–	–
BDI	–	–	–	–	−0.075	0.938	–	–
CTQ	–	–	–	–	–	–	0.096	0.520
PSQI	–	–	–	–	–	–	−0.067	0.678
Corr. R-squared	0.120; p = 0.011[4]		0.082; p = 0.042[5]		0.108; $p = 0.011$		0.115; p = 0.008[6]	

Note. FC: free L-carnitine; LAC: L-Acetyl-carnitine; MDD: major depressive disorder cohort; HC: healthy controls. CTQ: Childhood Trauma Questionnaire; BDI: Beck Depression Inventory II; PSQI: Pittsburgh Sleep Quality Index;

[1] split models due to high multicollinearity of the predictors;

[2] 0➔healthy controls (HC); 1➔MDD;

[3] according to „Studie für die Gesundheit der Kinder und Jugendlichen in Deutschland"transl. German health survey for children and adolescents (KiGGS) (Neuhauser et al., 2013);

[4] exclusion of 1 participant (HC) due to isolated disproportionally elevated centered leverage;

[5] exclusion of 2 participants due to isolated disproportionally elevated centered leverage (HC) and due to isolated disproportionally elevated outlier regarding standardized residuum (MDD);

[6] exclusion of 1 participant (MDD) due to isolated disproportionally elevated centered leverage.

In the linear regression analysis to predict the LAC/FC ratio, psychometrically assessed symptom characteristics (CTQ, PSS, BDI, PSQI) and cohort affiliation were included as predictors.

Due to the high multicollinearity with high intercorrelations between the questionnaire-based variables, we had to split the regression model resulting in a single model with BDI, PSS, and cohort affiliation as well as a single model with CTQ, PSQI and cohort affiliation as predictors.

The results indicated that depressive state (via cohort affiliation) was the only parameter significantly associated with an elevated LAC/FC ratio in both models. Trauma history (CTQ), subjective stress perception (PSS), depressive symptom burden (BDI) and subjective sleep quality (PSQI) did not exert a significant predictive influence on the ratio (see Table 3).

3.1.3. Cortisol, ACTH

Both morning cortisol and ACTH levels were significantly elevated in the MDD-cohort (see Table 2).

Correlation analyzes of cortisol and ACTH with within-subject control variables (age, weight, BMI percentiles, IPAQ-rated physical activity in the past 7 days) and with subjective symptom measures (CTQ, PSS, BDI, PHQ, PSQI) revealed significant positive correlations between both endocrine parameters and the quantitatively assessed individual childhood trauma history (CTQ), subjective stress (PSS), depressive symptom burden (BDI), somatic symptom burden (PHQ), and subjective sleep quality (PSQI). Among the within-subject control variables, age showed a significant positive correlation with both cortisol and ACTH, while physical activity in the past 7 days — especially extensive anaerobic, high-intensity aerobic, and moderate-intensity aerobic activity — was significantly negatively correlated with cortisol levels. Weight and BMI percentiles showed no significant correlations with any of the endocrine parameter (see Supplementary Table 1). Comparisons between the sexes revealed no significant differences in cortisol ($Z = 0.94$, $p = 0.349$) or ACTH levels ($Z = 0.53$, $p = 0.600$).

In the linear regression analyzes for the prediction of cortisol and ACTH levels, the predictors included CTQ, PSS, PHQ, PSQI, cohort affiliation, age, and physical activity. Due to the high multicollinearity between BDI, PSS, and cohort affiliation, BDI was excluded from the models. The regression analysis for cortisol identified age as the only significant predictor, while the model for ACTH identified depressive state (via cohort affiliation) as the only significant predictor (see Supplementary Table 2).

3.1.4. Cortisol/ACTH ratio

The cortisol/ACTH ratio showed a significant decrease in participants of the MDD cohort compared to the HC cohort (see Table 2).

Correlation analyzes of cortisol/ACTH with control variables (age, weight, BMI percentile, IPAQ-rated physical activity in the past 7 days) and with subjective symptom measures (CTQ, PSS, BDI, PHQ, PSQI) revealed that only trauma history (CTQ) and depressive symptom burden (BDI) were significantly negatively correlated with cortisol/ACTH levels (see Supplementary Table 1). Furthermore, no significant differences in cortisol/ACTH levels were found between female and male participants ($Z = 0.375$, $p = 0.708$).

A linear regression analysis with CTQ, BDI, and cohort affiliation as predictors for the cortisol/ACTH ratio did not show any significant results (see Supplementary Table 2).

3.1.5. Predicting cohort affiliation

To assess the predictive influence of biomarker levels on depressive symptoms, a binomial logistic regression analysis was performed with cohort allocation as the outcome variable. Biomarker-related predictors included FC, LAC, cortisol, and ACTH, as well as, due to collinearity between the raw biomarkers and their calculated ratios, an alternative model with the ratios of LAC/FC and cortisol/ACTH. In both models, age, sex, and BMI percentiles were included as additional predictors to control for potential confounding factors. Weight was excluded from the models to achieve the required minimum sample size-to-predictor ratio (Moons et al., 2014), as the exploratory inclusion of weight did not affect the significance of the other predictors, and weight itself did not show any significant predictive power. Both models showed significant predictive power, with ACTH, the LAC/FC ratio, and the cortisol/ACTH ratio as significant predictors (see Table 4).

3.1.6. Exploratory ROC analysis

An exploratory ROC analysis of the LAC/FC ratio yielded an AUC of 0.713, indicating modest but significant ($p = 0.001$) discriminatory ability. However, no clinically useful threshold with acceptable sensitivity and specificity could be derived from the present sample.

4. Discussion

As there is evidence of altered short-chain carnitine biology in adult major depressive disorder (MDD) but not in adolescent MDD, the aim of the present study was to exploratively examine potential disturbances in short-chain carnitine biology in adolescent MDD for the first time. In addition to the crude values of free L-carnitine (FC) and L-Acetyl-carnitine (LAC), a derived ratio of LAC/FC was also determined. Endocrine parameters, such as cortisol, ACTH, and cortisol/ACTH ratio were also measured to replicate the results of various studies with depressed adolescents and hereby to validate group assignment as well as our potential findings on the carnitine biology.

In the case of FC, no significant differences in peripheral blood levels between depressed adolescents and healthy controls were observed in the initial inter-cohort comparison. However, after controlling for weight as an important confounding factor, a significant association was found between reduced FC levels and the presence of depressive

Table 4
Binomial logistic regression models with depressive state as outcome variable.

	Depressive state via cohort assignment (MDD vs HC)			
Predictors	B	Wald	p	Exp(B) (95 % CI)
Sex[1]	1.214	3.258	0.071	3.37 (0.90–12.59)
Age	0.050	0.073	0.787	1.05 (0.73–1.51)
BMI percentiles[2]	0.005	0.177	0.674	1.01 (0.98–1.03)
Free L-carnitine (FC)	−0.084	2.213	0.137	0.92 (0.82–1.03)
L-Acetyl-carnitine (LAC)	0.148	2.265	0.132	1.16 (0.96–1.41)
cortisol	−0.005	0.459	0.498	1.00 (0.98–1.01)
ACTH	0.192	10.090	**0.001**	1.21 (1.08–1.36)
R-squared (Nagelkerkes)	0.527 ($p < 0.001$)[3]			
Quality of classification (%)	77.0			
Sex	0.972	2.886	0.089	2.65 (0.86–8.12)
Age	0.271	3.089	0.079	1.31 (0.97–1.78)
BMI percentiles[1]	0.000	0.000	0.996	1.00 (0.98–1.02)
LAC/FC ratio	7.432	6.626	**0.010**	1689.72 (5.89–484,723.08)
cortisol/ACTH ratio	−0.325	7.469	**0.006**	0.72 (0.57–0.91)
R-squared (Nagelkerkes)	0.381 ($p < 0.001$)[4]			
Quality of classification (%)	70.7			

Note. BMI: body mass index; ACTH: adrenocorticotropin; MDD: depressive disorder; MDD: major depressive disorder cohort; HC: healthy controls cohort.

[1] 0➔male, 1➔female.

[2] according to „Studie für die Gesundheit der Kinder und Jugendlichen in Deutschland“transl. German health survey for children and adolescents (KiGGS) (Neuhauser et al., 2013);

[3] $n = 74$, exclusion of 1 participant from MDD due to pre-analytic dropout in ACTH-assessment as well as exclusion of 1 participant from HC due to isolated disproportionally elevated Cook's distance;

[4] $n = 75$, exclusion of 1 participant from MDD due to pre-analytic dropout in ACTH-assessment.

symptoms. These findings are partially consistent with those reported by Nasca et al. (2018) in adults but contrast with the data of Ait Tayeb et al. (2023), who observed significantly elevated FC levels in adults with MDD.

Likewise, in contrast to the repetitive congruent findings in adults (Nasca et al., 2018; Nie et al., 2021; Liu et al., 2022; Ait Tayeb et al., 2023), where assignment to an MDD cohort is associated with significantly reduced *L*-acetyl-carnitine (LAC) levels, LAC levels in our adolescent sample did not differ significantly in cross-cohort comparison, even when controlling for confounding variables via regression analysis.

The main finding of the present study was a significant increase in the LAC/FC ratio in depressed adolescents compared to healthy controls. This was further supported by statistical analyzes showing that MDD cohort affiliation contributed to an increased LAC/FC ratio in a linear regression model after controlling for relevant confounders, and that an increased LAC/FC ratio was significantly related to MDD cohort affiliation in a binomial regression model. The MDD-related increase in the LAC/FC ratio in our adolescent cohorts was also found to be incongruent with existing evidence in adult cohorts, in which the reciprocal FC/LAC ratio is increased in depression (Ait Tayeb et al., 2023).

These aforementioned incongruities—specifically, the differing patterns of short-chain carnitine alterations between adolescent and adult MDD patients compared to their respective healthy control groups—can be understood as physiologically plausible when considered in light of the following factors:

Building on the current evidence linking chronic stress exposure (Nielsen et al., 2020; Thomas-Odenthal et al., 2024) and oxidative stress (Bhatt et al., 2020; Chen et al., 2024; Shao et al., 2024) to the onset of depressive symptoms in both adolescents and adults — assuming a polygenic depressive diathesis—we propose that specific carnitine alterations arise as part of the diathesis-stress interaction that drives the pathogenesis. It is known that in both adolescents and adults affected by chronic or recurrent stress and a concomitant lack of sufficient physiological coping mechanisms due to depressive diathesis, anabolic processes—partly driven by LAC-mediated acetyl-Co-Enzyme A (CoA) utilization—are downregulated. This is in contrast to an upregulation of catabolic metabolism, primarily reliant on glucose utilization, under stress conditions (Theorell, 2008; Vasunilashorn and Cohen, 2014; Herbet et al., 2019). This shift from anabolism to catabolism is postulated to be much more pronounced in adults, as there is evidence of an endocrine-mediated genuine dominance of anabolic metabolic processes in the adolescent body (Mauras, 2006). Apart from this, it is assumed that chronic stress exposure in adolescents, as in adults, not only induces metabolic alterations but also increases intracellular oxidative stress, which leads, among other consequences, to increased membrane lipid oxidation. The degradation of these cytotoxic oxidized lipids is facilitated by increased peroxisomal and mitochondrial activity (Jo et al., 2020; Kim and Bai, 2022).

In adolescent brain tissue, a substantial portion of carnitine-mediated intramitochondrial acetyl-CoA is utilized at rest by β-oxidation (Kawamura, 1988; Nałecz and Nałecz, 1996; Scafidi et al., 2010). A stress-induced metabolic shift towards catabolic pathways other than β-oxidation, but with basally maintained ß-oxidation rates, may result in only slightly reduced deconjugation of LAC-bound acetyl groups whereas the stress-induced degradation of oxidized lipids may lead to a transient accumulation of acetyl moieties. This process may ultimately lead to a new steady-state concentration of LAC and acetyl-CoA that is largely comparable to baseline values.

Coincidentally, the disposal of degraded long-chained carbon-derivatives is assumed to be exerted to a considerable extent by carnitine-mediated transport through the carnitine shuttle, which leads to a reduction in the free L-carnitine concentrations within the mitochondria as well as intracellularly and extracellularly alongside an increase in carnitine-ac(*et*)ylation. With this in mind, it is hypothesized that the LAC/FC ratio increases in response to iterative stress in adolescents with an MDD-related diathesis: a hypothesis that is supported by the findings of the present study. The fact that none of the demographic or physical variables showed significant interactions with the LAC/FC ratio further strengthens the notion that an increased LAC/FC ratio is a distinctive feature of MDD.

In adult brain tissue, where, in contrast to the utilization profile in adolescents, a substantial portion of carnitine-mediated intramitochondrial acetyl-CoA is utilized for the maintenance of structural lipid and transmitter homeostasis at resting state (Kawamura, 1988; Nałecz and Nałecz, 1996), it is postulated that the intramitochondrial alterations induced by the diathesis-stress-interaction differ to some degree differ from those in adolescents. In this context, a significant reduction in acetyl-CoA processing as a result of profound metabolic alterations associated with prolonged stress exposure in adults may play a pivotal role. The latter aspect seems to be particularly evident in cholinergic neuronal networks, as acetyl-CoA is a crucial substrate for acetylcholine synthesis (Jankowska-Kulawy et al., 2022). The significant reduction in acetyl-CoA processing may lead to consecutive reduction in LAC levels. As a direct consequence, the quantitative equilibrium between LAC and FC could shift in favor of an overall increase in FC, especially since FC loses importance in its function as an excretory modulator for oxidized lipids, as the degraded lipids are to be increasingly utilized as part of altered maintenance metabolism (Yang et al., 2022). A simplified illustration of these postulated different disturbances of short-chain-carnitine metabolism with more discrete alterations in adolescent MDD and profoundly disturbed mitochondrial metabolism in adult MDD is displayed in Fig. 2.

Another notable finding is that, our results regarding ACTH and the cortisol/ACTH ratio are consistent with established evidence of activating HPA axis dysregulation associated with depressive states or traits (Ford et al., 2019; Zwolińska et al., 2023; Zajkowska et al., 2022), despite the lack of repeated pooled assessments. This suggests that our two cohorts are validly contrasted in terms of distinct depression-related molecular profiles, thus validating our findings regarding altered carnitine biology in adolescents with MDD.

The significant MDD-related decrease in cortisol/ACTH ratio is probably primarily linked to a downregulated cortisol feedback loop with hyposensitivity of the hypothalamic cortisol receptor, as described in the literature (Ford et al., 2019; Lee et al., 2021; Zwolińska et al., 2023; Zajkowska et al., 2022), and not linked to a potential hyporeactivity of the adrenal cortex.

One of the key strengths of the present study lies in the cohort composition, which contrasts two multidimensionally characterized cohorts with high internal consistency and clear cross-cohort differences regarding psychiatric symptom burden. The fact that even singular biomarker assessment in our adolescent cohorts was able to replicate existing evidence of MDD-specific endocrine alterations supports this assumption. Furthermore, the inclusion of the LAC/FC ratio in addition to the raw short-chain carnitine values, allows for more distinctive detection of dynamic contrasts within the carnitine cycle and its alterations in MDD.

The results of the current study should be interpreted in light of its limitations, which primarily arise from the pilot nature of the study and the limited sample size. This also affects the finding that, although the LAC/FC ratio in the ROC analysis showed significant discriminatory ability, a clinically useful threshold with acceptable sensitivity and specificity could not be established in the present sample. Further studies with larger sample sizes are needed to determine clinically useful cut-off thresholds for diagnostic purposes.

The quantitative analysis of endocrine parameters must be considered in light of the constraints that in our study only a singular assessment of known physiologically fluctuating endocrine biomarkers was conducted. The rationale behind this approach was to focus on carnitine biology with high stability in relation to blood concentrations (Alberty and Albertyová, 1997) and to concomitantly assess endocrine parameters with the intention of exploratively replicating known evidence in

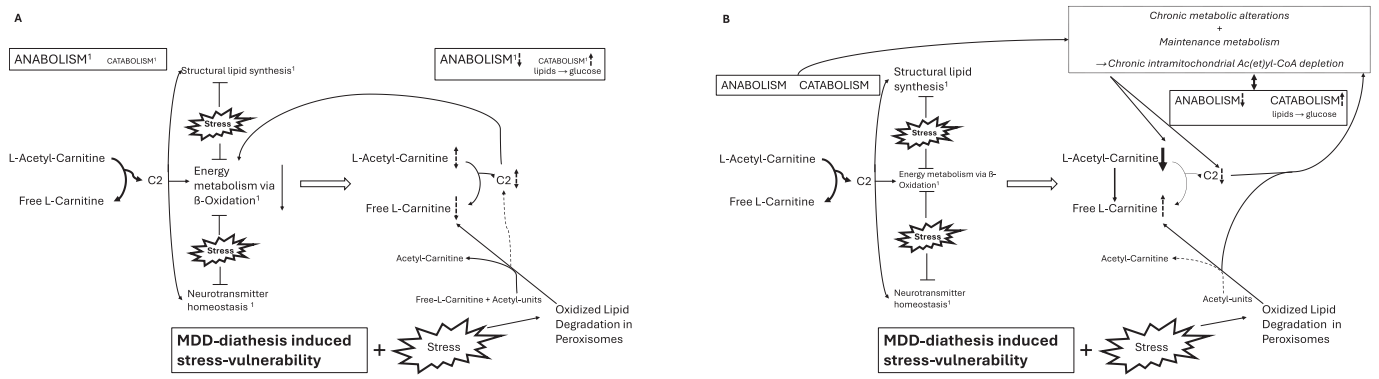

Fig. 2. Evidence-derived hypothesis of differing stress-induced perturbation patterns in short-chain carnitine metabolism between adolescents and adults with Major Depressive Disorder (MDD). *A: Simplified illustration of short-chain carnitine associated intramitochondrial alterations in adolescent brain tissue due to interaction of stress and MDD-diathesis.* In the case of adolescents, evidence states a relative dominance of anabolic growth processes in comparison to catabolic processes in resting state despite a partial shift towards catabolic processes under strain conditions. The stress-diathesis interaction leads to a downregulation of acetyl-Coenzyme A utiliziation mainly via ß-oxidation, while ß-oxidation rates persist at basal levels. The stress-diathesis interaction also leads to an upregulation of carnitine-mediated lipid disposal.

➔ Result is a merely slight downregulation of intramitochondrial acetyl-deconjugation and a short-term accumulation of Ac(*et*)yl-moieties.

➔ The new steady state concentration of intramitochondrial acetyl-Coenzyme A and intramitochondrial as well as - consecutively due to free membrane passing – extracellular L-Acetyl-Carnitine may be relatively unaltered compared to the initial situation.

➔ As a result of free L-Carnitine (FC) -mediated lipid disposal there may be a decrease of intramitochondrial and - consecutively due to free membrane passing - extracellular FC.

B: *Simplified illustration of short-chain carnitine associated intramitochondrial alterations in adult brain tissue due to interaction of stress and MDD-diathesis*. In the case of adults, evidence states a relative balance of anabolic processes and catabolic processes in resting state with pronounced shift towards catabolic processes under strain conditions. The chronic Stress-diathesis interaction leads to a generalized acetyl-Coenzyme A depletion and consecutively to a depletion of intramitochondrial and - consecutively due to free membrane passing - extracellular L-Acetyl-Carnitine. The dominance of maintenance metabolism under strain conditions also hypothetically requires rapid intra-compartmental utilization of ac(*et*)yl-moieties, also the oxidized lipid components. ➔Postulated consequence is an increase of intramitochondrial and consecutively - due to free membrane passing - extracellular FC as a result of profoundly perturbed intramitochondrial short-chain carnitine metabolism. *Note.* [1] Font size reflects relative proportions of the influencing variables to each other as well as changes line width reflect stress-induced alterations. MDD: Major Depressive Disorder; C2: Acetyl-moieties, mostly Acetyl-Coenzyme A.

terms of an altered HPA axis in our cohorts, while experimentally measuring these endocrine parameters in a clinical feasible practice via one-time blood analysis.

Another aspect, that needs to be taken into account with regard to our LAC/FC ratio-related findings is the fact of slightly different correlation coefficients between DBS- and plasma-based analyzes of FC vs. LAC in the published literature. (Sain-van der Velden et al., 2013; Lu et al., 2021). However, it should be noted that both studies included relatively small sample sizes and participants with somatic diseases, which likely contributed to a broader fluctuation range in hematocrit values — an important confounding factor in DBS testing.

The lack of association between biomarker-related variables and psychometrically assessed subjective symptom burden in our sample may be due, at least in part, to a marked divergence between subjective self-perception and expert opinion-based clinical judgement in our adolescent cohort. Of note, nearly one-fifth of participants with MDD in our study self-reported only mild depressive symptomatology (BDI sum score < 20) in our study despite being diagnosed with moderate to severe depression by experienced, board-certified clinical physicians, supporting this interpretation. Future studies with larger sample sizes should focus on intra-cohort analyzes to investigate these biomarker-symptom associations. In particular, the molecular and phenotypic differentiation of subtypes in adolescent depression should be emphasized, as this holds crucial implications for tailored therapy and prognosis.

An important conclusion for future research, is the need to distinguish whether these alterations in carnitine biology in depressed adolescents represent state-dependent indicators of depressive symptoms – as hypothesized in our derivation above – or whether they represent stable, persistent trait markers of an endogenous predisposition to MDD. In their cross-sectional approach, Nasca et al. observed a direct correlation between reduced LAC scores and depressive symptom burden, independent of pharmacotherapeutic interventions. Similarly, Nie et al. (2021) and Ait Tayeb et al. (2023) reported a significant tendency for reversal of significant MDD-associated short-chain carnitine alterations along with regression of depressive symptoms following effective treatment, suggesting that altered carnitine biology in depression may be more state-dependent. Longitudinal studies focusing on the course of symptoms and their association with pharmacologic or therapeutic interventions are needed in both adults and adolescents to investigate the differentiation of carnitine biology in MDD between state and trait. The possibly less pronounced alterations in short-chain carnitine levels observed in adolescent MDD could also be linked to the comparatively lower response rates to pharmacologic interventions in adolescents compared to adults with MDD (Bylund and Reed, 2007).

Further research should also focus on whether short-chain carnitines, similar to findings in adults (Kriston et al., 2014; Wang et al., 2014; Veronese et al., 2018), could serve as potential treatment options for affective disorders in adolescents, although current evidence suggests greater efficacy in older adults (Veronese et al., 2018). In addition, there is evidence of an association between disturbances in intracerebral carnitine biology and neurodegenerative disorders (Pettegrew et al., 2000; Pennisi et al., 2020), suggesting possible cross-links between MDD-related alterations in carnitine metabolism and cognitive dysfunction. Future studies should investigate whether intraindividual profiles of carnitine derivatives and their reciprocal ratios could serve as predictive biomarkers for the severity and quality of affective state-related cognitive dysfunction in adolescents with MDD.

Overall, our results support the hypothesis that carnitine biology is altered not only in MDD in adulthood but also in adolescence. The discrepancies in LAC-related results between the adolescent cohort and adult participants suggest different carnitine utilization profiles in adolescence and higher exposure to exogenous factors contributing to more pronounced metabolic alterations in adulthood. In this context, the increased LAC/FC ratio could serve as a distinctive marker for MDD in adolescence. Thus, the present study contributes to the evaluation of novel biomarkers, treatment options, and prevention strategies for MDD

in adolescents, a disorder characterized by high treatment resistance (Strawn et al., 2020) and poorer prognosis for stable remission (Zisook et al., 2007).

Authorship statement

All authors confirm to have made substantial contributions to all of the following:

1. The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
2. Drafting the article or revising it critically for important intellectual content.
3. Final approval of the version to be submitted.

All authors agree to be accountable for all aspects of the work to ensure that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

We confirm that this manuscript is original, has not been published elsewhere, and is not under consideration for publication elsewhere. The article's publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.

If accepted, the article will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder. All authors have no conflicts of interest to disclose.

Availability of data

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Maximilian Niebler: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Irina Jarvers:** Writing – review & editing, Formal analysis. **Romuald Brunner:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Stephanie Kandsperger:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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Declaration of competing interest

The authors 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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Appendix A. Supplementary data

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