Immunometabolic Blood Biomarkers of Developmental Trajectories of Depressive Symptoms: Findings From the ALSPAC Birth Cohort

Running title: Immunometabolic markers of depression trajectories

Authors:

Ruby S. M. Tsang[1,2,3]	PhD	ruby.tsang@bristol.ac.uk	0000-0002-2520-526X
Daniel Stow[4]	PhD	d.stow@qmul.ac.uk	0000-0002-9534-4521
Alex S. F. Kwong[1,5]	PhD	akwong@ed.ac.uk	0000-0003-1953-2771
Nicholas A. Donnelly[1,2,6]	PhD, MRCPsych	nick.donnelly@bristol.ac.uk	0000-0003-2234-8545
Holly Fraser[1,2,3]	MSc	holly.fraser@bristol.ac.uk	0000-0001-9752-0042
Inês A. Barroso[7]	PhD	ines.barroso@exeter.ac.uk	0000-0001-5800-4520
Peter A. Holmans[8]	PhD	holmanspa@cardiff.ac.uk	0000-0003-0870-9412
Michael J. Owen[8,9]	PhD, FRCPsych	owenmj@cardiff.ac.uk	0000-0003-4798-0862
Megan L. Wood[10]	PhD	m.l.wood@leeds.ac.uk	0000-0003-1882-2355
LINC Consortium*			
Marianne B. M. van den Bree[8,9]	PhD	vandenbreemb@cardiff.ac.uk	0000-0002-4426-3254
Nicholas J. Timpson[1,2]	PhD	n.j.timpson@bristol.ac.uk	0000-0002-7141-9189
Golam M. Khandaker[1,2,3,11]	PhD, FRCPsych	golam.khandaker@bristol.ac.uk	0000-0002-4935-9220

Affiliations:

[1] MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK

[2] Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

[3] Centre for Academic Mental Health, Population Health Sciences, University of Bristol, Bristol, UK

[4] Wolfson Institute of Population Health, Queen Mary University of London, London, UK

[5] Division of Psychiatry, University of Edinburgh, Edinburgh, UK

[6] Avon and Wiltshire NHS Mental Health Partnership NHS Trust, Bristol, UK

[7] Exeter Centre of Excellence for Diabetes Research, University of Exeter, UK

[8] Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, UK

[9] Neuroscience and Mental Health Innovation Institute, Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, UK

[10] School of Psychology, University of Leeds, Leeds, UK

[11] NIHR Bristol Biomedical Research Centre, Bristol, UK

* A list of members and their affiliations appear at the end of the paper.

Corresponding author:

Dr Ruby Tsang

Population Health Sciences, Bristol Medical School

Oakfield House, Oakfield Grove

Bristol BS8 2BN

United Kingdom

ruby.tsang@bristol.ac.uk

Abstract

Studies of longitudinal trends of depressive symptoms in young people could provide insight into aetiologic mechanism, heterogeneity and origin of common cardiometabolic comorbidities for depression. Depression is associated with immunological and metabolic alterations, but immunometabolic characteristics of developmental trajectories of depressive symptoms remain unclear. Using depressive symptoms scores measured on 10 occasions between ages 10 and 25 years in the Avon Longitudinal Study of Parents and Children (n=7302), we identified four distinct trajectories: low-stable (70% of the sample), adolescent-limited (13%), adulthood-onset (10%) and adolescent-persistent (7%). We examined associations of these trajectories with: i) anthropometric, cardiometabolic and psychiatric phenotypes using multivariable regression (n=1709-3410); ii) 67 blood immunological proteins and 57 metabolomic features using empirical Bayes moderated linear models (n=2059 and n=2240 respectively); and iii) 28 blood cell counts and biochemical measures using multivariable regression (n=2256). Relative to the low-stable group, risk of depression and anxiety in adulthood was higher for all other groups, especially in the adolescent-persistent ($OR_{depression}$=22.80, 95% CI 15.25-34.37; OR_{GAD}=19.32, 95% CI 12.86-29.22) and adulthood-onset ($OR_{depression}$=7.68, 95% CI 5.31-11.17; OR_{GAD}=5.39, 95% CI 3.65-7.94) groups. The three depression-related trajectories vary in their immunometabolic profile, with evidence of little or no alterations in the adolescent-limited group. The adulthood-onset group shows widespread classical immunometabolic changes (e.g., increased immune cell counts and insulin resistance), while the adolescent-persistent group is characterised by higher BMI both in childhood and adulthood with few other immunometabolic changes. These findings point to distinct mechanisms and intervention opportunities for adverse cardiometabolic profile in different groups of young people with depression.

Introduction

The first two decades of life represent a critical epoch for human neurodevelopment when most serious mental illnesses of adult life first emerge.[1] Half of all lifetime cases of common mental disorders including depression and anxiety start by 14 years and 75% by 24 years.[2] The first onset of clinically recognised depressive episodes typically occurs between the ages of 12 and 15 years[3] and the increase in new onset of depression peaks between the ages of 15 and 18 years.[4] Depressive symptoms in childhood and adolescence, including those below diagnostic thresholds, are associated with an elevated risk of depression and other psychiatric diagnoses subsequently in adulthood.[2, 5-7] These findings highlight the need for studying depressive symptoms during early life.

Characterisation of longitudinal profiles of depressive symptoms during development could help understand the pathogenesis and heterogeneity of later depression, as different individuals may arrive at the same destination via different routes. There is growing evidence to suggest characteristic depression trajectories in childhood and adolescence are differentially associated with risk factors and outcomes. Existing studies have reported associations of a 'high' or 'increasing' depression trajectory with female sex, lower socioeconomic status, stressful life events, conduct issues, substance use, and parental psychopathology.[3, 8, 9] Trajectories with higher symptom burden have been associated with subsequent depression and other psychiatric diagnoses, lower educational attainment, income and poorer psychosocial adjustment.[8-10] However, less is known about underlying biological correlates of depression trajectories, including blood-based biomarker signatures. A better understanding of the biological correlates may help uncover mechanistic insights and identify accessible predictive markers for depression.

Existing literature suggests that depression and specific symptoms or symptom dimensions of depression are associated with immunometabolic dysfunction, but there is limited work on immunometabolic correlates of depression trajectories. Depression is associated with immunometabolic alterations such as chronic low-grade inflammation,[11, 12] neuroendocrine dysregulations,[13] as well as less favourable metabolic and lipid profiles.[12, 14] Overall effect sizes

for some of these associations are inconsistent, which could be partly due to clinical or phenotypic heterogeneity within cross-sectional studies.[12] For instance, immunometabolic alterations appear to be more pronounced or common in individuals endorsing atypical energy-related symptoms of depression (e.g., hyperphagia, weight gain, hypersomnia, or leaden paralysis) as opposed to melancholic symptoms.[12, 15] At the symptom level, inflammatory markers are particularly associated with somatic and neurovegetative symptoms of depression (e.g., fatigue, altered sleep and appetite) as opposed to psychological symptoms (e.g., hopelessness, excessive/inappropriate guilt).[16, 17] Some of these findings are supported by Mendelian randomization analyses reporting a potentially causal link between inflammatory markers (e.g., C-reactive protein (CRP) or interleukin 6 (IL-6)) and fatigue, anhedonia, sleep problems, appetite and psychomotor changes.[18, 19]

The accumulation of risk model for chronic diseases posits that cumulative exposures across the life course result in diverging health trajectories and widening health inequalities as people age.[20] By characterising depression trajectories, developmental windows when trajectories begin to diverge can be identified, and we can then examine potential factors driving such divergence and biological dysregulations linked to subsequent disease risk. By studying the biomarker signatures of depression trajectories, we may also gain further insight into the origins of higher levels of cardiometabolic multimorbidity in individuals with depression.[21, 22]

The aims of the current study were threefold: (i) to model depression trajectories from childhood to early adulthood to classify individuals into more homogeneous subgroups, (ii) to examine associations between these subgroups and risk of psychiatric and cardiometabolic outcomes in early adulthood, and (iii) to examine associations of these subgroups with clinical and blood immunometabolic markers including proteomic, metabolomic and biochemical measures in early adulthood. By examining the broader biomarker signature across different domains including the immune proteome, metabolome, and clinical biochemistry, we aim to provide more comprehensive insights into biological pathways and systems possibly involved in the development and persistence of depressive symptoms in young people.

Materials and Methods

Description of cohort

This study uses data from the Avon Longitudinal Study of Parents and Children (ALSPAC). Pregnant women resident in the former county of Avon, United Kingdom (UK) with expected dates of delivery between 1st April 1991 and 31st December 1992 were invited to take part in the ALSPAC study. The initial recruitment enrolled 14541 pregnancies, which resulted in 14062 live births and 13988 infants still alive at 12 months. Further recruitment of eligible participants took place when the oldest children were approximately seven years of age; the total sample size for analyses using any data collected after the age of seven is therefore 15447 pregnancies; of these, 14901 children were alive at 12 months of age.[23, 24]

The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/

Data

Sociodemographic and health variables

Sociodemographic characteristics used to characterise the identified depressive symptom trajectories include sex, ethnicity, maternal education, maternal occupational social class, socioeconomic deprivation, and family adversity during pregnancy. Health characteristics examined include smoking, at-risk drinking, carotid intima-media thickness, carotid-femoral pulse wave velocity, metabolic syndrome and its components, obesity, and psychiatric outcomes and medications. Detailed description of these variables as well as those included as covariables in the biomarker analyses are presented in **Methods S1**.

Depressive symptoms

Self-reported depressive symptoms were assessed using the 13-item Short Mood and Feelings Questionnaire (SMFQ).[25] We used data collected on 10 occasions between the ages of 10 and 25 years (ages 10, 12, 13, 16, 17, 18, 21, 22, 23, 25), ending with the last questionnaire

146 administered in 2017-2018, prior to the start of the COVID-19 pandemic (see **Table S1**).
147 Questions were answered based on the two-weeks prior to completing the questionnaire. Each
148 SMFQ item is scored as 0 = “not true”, 1 = “sometimes true” and 2 = “always true”, resulting in a
149 total SMFQ sum score 0-26 (higher score reflects more symptoms). For individuals who had
150 missing data on fewer than three questions, score was imputed to the median value for missing
151 items. For each time-point, those with missing data on more than three questions had their total
152 score recoded as missing.
153
154 Circulating blood biomarkers
155 For this analysis, blood biomarkers were assayed in blood samples collected at the face-to-face
156 research clinic undertaken at 24 years. A total of 92 circulating inflammatory proteins were
157 measured using the Olink Target 96 Inflammation panel (Olink Analysis Service, Uppsala,
158 Sweden); proteins with ≥50% values below the limit of detection (LOD) were excluded leaving 67
159 proteins to be included (**Table S2**). Over 220 metabolomic features (148 metabolites and 77
160 ratios) were quantified using a high-throughput ^{1}H-NMR spectroscopy-based platform
161 (Nightingale Health, Helsinki, Finland) using a standardised protocol and parameters described
162 elsewhere.[26-28] Lipoprotein subclasses were excluded from the analysis to minimise redundancy
163 of information, leaving a subset of 57 metabolomic features (9 cholesterol measures, 12
164 apolipoproteins and lipids measures, 3 lipoprotein particle sizes, 16 fatty acids and saturation
165 measures, 3 glycolysis-related metabolites, 8 amino acids, 3 ketone bodies, 2 fluid balance-
166 related measures and 1 inflammation-related measure) to be included in the analysis (**Table S3**).
167 All 26 blood count and chemistry measures collected at the same clinic interaction were included
168 (**Table S4**). Additionally, we computed the aspartate aminotransferase/alanine aminotransferase
169 (AST/ALT) ratio and the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).
170 Further information on data collection and processing of all blood biomarkers are presented in
171 **Methods S1**.
172
173 Covariables

Prior to statistical analysis, we plotted a directed acyclic graph (DAG) showing theoretical relationships between depression trajectories (independent variable), immunometabolic markers (dependent variables) and important covariables based on the literature (**Figure S1**). The minimum adjustment set of confounders included in models was sex at birth, maternal education, maternal occupational social class, and body mass index (BMI) at age 10.

Statistical analysis

Characterisation of depression trajectories

Latent class trajectory modelling was performed using the *lcmm* R package[29] to identify subgroups with distinct SMFQ trajectories. This type of modelling seeks to identify homogenous groups of individuals with similar trajectories within a heterogeneous population by combining a latent class model and a mixed model. Models are estimated within the maximum likelihood framework.[29] The *lcmm* package distinguishes time of measurement and occasion, so individuals with missing data can still be included; we included those with at least three measurements for better modelling of non-linear trajectories. A multi-step approach adapted from the model selection framework suggested by Lennon et al.[30] and van der Nest et al.[31] was used, with the order of steps changed to address potential overextraction of latent classes from model under-specification as reported in the simulation literature[32, 33]. The steps followed:

1. Scope literature and inspect plots to inform polynomial order and potential number of classes. We modelled smfq ~ age + age^2 and estimated models up to six latent classes.
2. Estimate growth mixture models (GMM) with random intercepts and class-specific proportional random-effect variance-covariance matrix with increasing number of classes. Select the most appropriate number of classes *k* based on model convergence, model fit (Bayesian information criterion (BIC), Integrated Completed Likelihood (ICL), and relative entropy), smallest class size ≥5% and visual inspection of the trajectories.
3. Test alternative model structures with *k* classes – GMM with random intercepts and common random-effect variance-covariance matrix, and group-based trajectory models (GBTM); compare model fit indices, smallest class size and visually inspect trajectories as above, and

assess model adequacy (average posterior probabilities ≥0.7 and odds of correct classification ≥5 for all classes).

4. Refine trajectory shape by testing up to second-degree fractional polynomials including (-2, -1, -0.5, 0, 0.5, 1, 2, 3) where 0 refers to log X and repeated polynomials refer to (X^i + X^i * log X). Select final model based on model convergence and model fit.

Age (in years) was used as the time variable in all models. No covariables were included in these latent class mixed models as the aim is to describe the trajectories; covariables were accounted for in the next step when testing for associations with phenotypes of interest and biomarkers. For each model, an automatic grid search with 50 sets of random initial values and up to 10 iterations was run to reduce the odds of the model converging towards a local maximum and then up to 500 iterations were allowed for the final estimation. Using the selected model, posterior probabilities for class membership were then estimated and individuals were assigned to the class of highest posterior probability in the entire sample using the *predictClass* function. We first performed the latent class mixed modelling on the subsample with three or more data points and then predicted class membership in the entire sample to reduce uncertainty in the modelling stage and to maximise sample sizes in the subsequent analyses. Additional information on the modelling is presented in **Supplementary Methods S1,** R scripts are provided in **Supplementary Methods S2**. The reporting of this study adheres to the Guidelines for Reporting on Latent Trajectory Studies (GRoLTS)[34] (**Table S5**).

Associations with clinical and sociodemographic variables

Sociodemographic characteristics are stratified by trajectory class and summarised using mean (SD), median [interquartile range] or count (%) as appropriate, with differences between trajectories tested with chi-square or Kruskal-Wallis tests. Associations of trajectory membership with psychiatric or cardiometabolic outcomes of interest at age 24 or 28 years were tested using multivariable linear or logistic regressions, using the largest trajectory class as the reference group and adjusting for sex, maternal education, occupational social class and BMI at age 10. These variables are described in detailed in **Supplementary Methods S1**.

231

232 Associations with immunometabolic biomarkers

233 For both proteomic and metabolomic data, associations between depression trajectories and
234 markers were evaluated using multiple linear models fitted in the *limma* R package.[35] *Limma*
235 uses an empirical Bayes method to moderate the standard errors of the estimated log-fold
236 changes by borrowing strength from linear models of the other analytes and allowing for different
237 variability between analytes and between samples. Planned contrasts of each of the intermediary
238 trajectories against the trajectory with the most individuals were conducted. With the blood count
239 and clinical chemistry data, linear regressions were fitted with blood markers as dependent
240 variables and SMFQ trajectory class as the independent variable.

241

242 For each of these markers, the basic model included sex, maternal education and maternal
243 occupational social class as covariables and the adjusted model further included BMI at age 10
244 as a covariable. Correction for multiple testing was performed for each set of models using the
245 Benjamini-Hochberg procedure, using a false discovery rate (FDR) q-value threshold of <0.1.
246 This threshold was chosen due to the large number of biomarkers tested, a relatively small
247 sample size and the exploratory nature of this work. R scripts for these analyses are provided in
248 **Supplementary Methods S2**.

249

250 Sensitivity analyses

251 To address potential error carried over from the probabilistic latent class assignment into the
252 association analyses, we performed two sets of sensitivity analyses, the first set by restricting the
253 sample to individuals who had a modal posterior probability ≥0.7, and the second set by using
254 the individuals' posterior probabilities for each latent class as separate terms in the models.

255

256 Data extraction and initial data cleaning was performed in StataMP version 17.[36] Further data
257 preparation and statistical analyses were conducted in R versions 4.1.1 and 4.2.1,[37] using
258 packages *tidyverse* (v2.0.0), *lcmm* (v2.0.2), *LCTMtools* (v0.1.3), *tableone* (version 0.13.2), *knitr*

(v1.43), *kableExtra* (v1.3.4), *limma* (v3.54.2), and *broom* (v1.0.5). Plots were generated using *ggplot2* (v3.4.2), *ggpubr* (v0.6.0), and *ggrepel* (v0.9.3).

Ethical approval

Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Results

Sample

Latent class trajectory modelling was performed on data from 7302 participants who had SMFQ scores available from at least three time-points between ages 10 and 25 years. Once the best-fitting model was identified, posterior probabilities and class membership were estimated in the entire sample, and 9595 individuals were assigned class membership. Of these 9595 individuals, 2256 had sufficient biomarker and complete covariable data to be included in the biomarker analyses (**Figure S2**).

Depressive symptom trajectories from childhood to early adulthood

Following comparison of model fit and adequacy statistics and visual inspection of trajectory plots (**Table S6 and Figures S2-S3**), a four-class group-based trajectory model was identified as best describing the data. As shown in **Figure 1**, the four identified depressive symptom trajectories from childhood to early adulthood can be described as follows: low-stable – those who consistently had no or low levels of depressive symptoms (69.6%, n=6680), adolescent-limited – those who had elevated depressive symptoms in childhood/adolescence that decreased over time (13.3%, n=1280), adolescent-persistent – those who had elevated depressive symptoms in childhood/adolescence that remained high into adulthood (7.0%, n=672) and adulthood-onset –

287 those who started with low levels of depressive symptoms that increased in late
288 adolescence/early adulthood (10.0%, n=973).
289
290 *Characteristics of depression trajectories*
291 Descriptive statistics for characteristics of these individuals, stratified by trajectory, are presented
292 in **Table 1** below. There were more women in all three depression-related trajectories:
293 adolescent-limited (66.3%), adolescent-persistent (76.5%), and adulthood-onset trajectories
294 (64.6%). Additionally, the adolescent-persistent trajectory was associated with lower maternal
295 education and greater family adversity during pregnancy. Descriptive statistics for the same
296 characteristics of the subset of individuals who were included in the biomarker analyses are
297 presented in **Table S7**.
298
299 [INSERT FIGURE 1 HERE]

Table 1. Characteristics of depressive symptom trajectories in the ALSPAC birth cohort.

	Low-stable (n=6680)	Adolescent-limited (n=1280)	Adolescent-persistent (n=672)	Adulthood-onset (n=963)	*p*
Sex: Female	3064 (45.9%)	849 (66.3%)	514 (76.5%)	622 (64.6%)	<0.001
Ethnicity: Non-white	240 (4.1%)	44 (3.9%)	36 (6.2%)	40 (4.8%)	0.097
Maternal education					0.026
CSE or none	894 (15.1%)	159 (13.9%)	107 (17.9%)	120 (14.1%)	
Vocational	560 (9.5%)	91 (8.0%)	53 (8.9%)	64 (7.5%)	
O-level	2064 (34.9%)	406 (35.6%)	231 (38.6%)	293 (34.5%)	
A-level	1483 (25.1%)	308 (27.0%)	141 (23.6%)	230 (27.1%)	
Degree	911 (15.4%)	178 (15.6%)	66 (11.0%)	142 (16.7%)	
Maternal occupational social class					0.556
I – highest	356 (7.0%)	63 (6.6%)	28 (5.5%)	45 (6.3%)	
II	1714 (33.8%)	335 (35.0%)	161 (31.8%)	250 (34.9%)	
III (non-manual)	2137 (42.2%)	392 (41.0%)	216 (42.7%)	302 (42.1%)	
III (manual)	350 (6.9%)	66 (6.9%)	34 (6.7%)	57 (7.9%)	
IV or V[1] – lowest	509 (10.0%)	100 (10.5%)	67 (13.2%)	63 (8.8%)	
English IMD 2000 quintile					0.723
1 – least deprived	1665 (32.1%)	355 (35.3%)	146 (30.7%)	242 (33.3%)	
2	1044 (20.1%)	188 (18.7%)	100 (21.0%)	143 (19.7%)	
3	956 (18.4%)	174 (17.3%)	84 (17.6%)	140 (19.3%)	
4	790 (15.2%)	153 (15.2%)	77 (16.2%)	115 (15.8%)	
5 – most deprived	739 (14.2%)	137 (13.6%)	69 (14.5%)	86 (11.8%)	
Family Adversity Index	1 [0, 2]	1 [0, 2]	1 [0, 3]	1 [0, 2]	<0.001
BMI at age 10	17.43 [15.97, 19.73]	17.63 [16.07, 19.96]	18.25 [16.24, 21.00]	17.43 [15.92, 19.60]	<0.001
Number of SMFQ measurements	4 [2, 7]	6 [3, 8]	5 [3, 7]	6 [4, 9]	<0.001

[1] Categories have been collapsed due to small cell counts in subsequent analyses.

Notes: Numbers presented as mean (SD), median [IQR], or n (%). Percentages are column percentages and computed based on the number of individuals with available data on each variable. Group comparisons were conducted using Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normal continuous variables.

306 Abbreviations: BMI – body mass index; CSE – Certificate of Secondary Education; O-level – Ordinary level; A-

307 level – Advanced level; IMD – Index of Multiple Deprivation; SMFQ – Short Mood and Feelings Questionnaire

308

Table 2. Associations of depressive symptom trajectories with anthropometric and cardiometabolic outcomes at 24 years and psychiatric outcomes at 24 and 28 years

		Adolescent-limited	Adolescent-persistent	Adulthood-onset
Outcomes assessed at age 24	n	Adjusted unstandardised regression coefficient (SE)		
cIMT (continuous)	1565	-0.0002 (0.0033)	-0.0099 (0.0050)	-0.0003 (0.0036)
cfPWV (continuous)	1708	-0.0578 (0.0708)	-0.1429 (0.1109)	0.0108 (0.0790)
		Adjusted odds ratio (95% CI)		
Smoking	2731	**1.66 (1.32-2.09)**	**2.66 (1.93-3.67)**	**1.70 (1.32-2.17)**
AUDIT-C score ≥ 5	2704	0.97 (0.79-1.21)	**0.65 (0.48-0.89)**	0.79 (0.63-1.01)
BMI ≥ 30kg/m^2	2731	1.41 (0.99-2.01)	**1.93 (1.19-3.06)**	1.34 (0.89-1.99)
Elevated waist circumference	2707	1.21 (0.93-1.56)	1.24 (0.84-1.80)	1.16 (0.86-1.54)
Triglycerides ≥1.7mmol/L	2241	1.37 (0.86-2.11)	1.14 (0.53-2.23)	1.50 (0.93-2.33)
HDL <1.0mmol/L	2241	0.86 (0.61-1.20)	1.13 (0.71-1.77)	1.34 (0.95-1.85)
SBP ≥ 130mmHg	2751	0.85 (0.58-1.22)	0.60 (0.29-1.12)	0.96 (0.65-1.38)
DBP ≥ 85mmHg	2751	1.32 (0.69-2.69)	0.73 (0.17-2.21)	1.32 (0.55-2.82)
Fasting glucose ≥5.6mmol/L	2241	0.86 (0.65-1.14)	0.92 (0.58-1.42)	0.97 (0.72-1.31)
Metabolic syndrome	2759	0.97 (0.59-1.55)	0.78 (0.35-1.54)	1.23 (0.75-1.96)
ICD-10 depressive episode	2729	**4.03 (2.73-5.95)**	**22.80 (15.25-34.37)**	**7.68 (5.31-11.17)**
ICD-10 GAD	2719	**3.59 (2.41-5.33)**	**19.32 (12.86-29.22)**	**5.39 (3.65-7.94)**
Outcomes assessed at age 28				
Prescribed antidepressants in past 5y	2792	**3.02 (2.28-3.99)**	**7.29 (5.22-10.17)**	**5.12 (3.88-6.75)**
Prescribed anxiolytics in past 5y	2828	**2.31 (1.25-4.16)**	**6.76 (3.74-12.05)**	**4.16 (2.39-7.18)**

Notes: Regression models were adjusted for sex, maternal education, maternal occupational social class, and BMI at age 10. Effect estimates presented are unstandardised regression coefficients for continuous outcomes and odds ratios (95% confidence intervals) for binary outcomes. The reference group for all analyses is the low-stable trajectory. Text in bold indicates evidence for the association after FDR correction of p-values. Metabolic syndrome was defined based on the 2009 consensus definition from the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute, i.e. the presence of any three of the following five risk factors: elevated waist circumference (≥94cm for white men, ≥90cm for non-white men, ≥80cm for women); elevated triglycerides (≥1.7mmol/L), reduced high-density lipoprotein-cholesterol

320 (<1.0mmol/L), elevated blood pressure (systolic ≥130mmHg or diastolic ≥85mmHg), and elevated fasting glucose
321 (≥5.6mmol/L).
322 Abbreviations: AUDIT-C – Alcohol Use Disorders Identification Test for Consumption; BMI – body mass index;
323 cfPWV – carotid-femoral pulse wave velocity; cIMT – carotid intima-media thickness; DBP – diastolic blood
324 pressure; GAD – generalised anxiety disorder; HDL – high-density lipoprotein; ICD-10 – International
325 Classification of Diseases 10th Revision; SBP – systolic blood pressure
326
327
328

Adulthood cardiometabolic and psychiatric outcomes associated with depression trajectories

Compared to the low-stable trajectory, after adjusting for sex, maternal education, maternal occupational class and BMI at age 10, all three depression-related trajectories were associated with ICD-10 diagnosis of depression, generalised anxiety disorder, being prescribed an antidepressant or anxiolytic at ages 24 and 28 years. However, the magnitude of association varied between the trajectories, with the risk for these outcomes being the highest for the adolescent-persistent trajectory (approximately 20-fold risk), followed by the adulthood-onset and adolescent-limited trajectories. The adolescent-persistent trajectory was additionally associated with obesity (**Table 2**). Unadjusted model results are presented in **Table S8**.

Differentially abundant proteins associated with depression trajectories

Relative to the low-stable trajectory, after adjusting for sex, maternal education, maternal occupational class and BMI at age 10, one protein (C-C motif chemokine 25 [CCL25]) was upregulated in the adolescent-limited trajectory; four proteins (fibroblast growth factor 21 [FGF-21], hepatocyte growth factor [HGF], eukaryotic translation initiation factor [4E-BP1], and eotaxin-1 [CCL11]) were upregulated in the adolescent-persistent trajectory; and five proteins (FGF-21, fibroblast growth factor 19 [FGF-19], CUB domain-containing protein 1 [CDCP1], HGF, CCL11) were upregulated in the adulthood-onset trajectory (**Figure 2**). Full model results are presented in **Tables S9-10**.

Differentially abundant metabolites associated with depressive symptom trajectories

Relative to the low-stable trajectory, after adjusting for sex, maternal education and occupational class, and BMI at age 10, creatinine was decreased in the adolescent-persistent trajectory. Three metabolite ratios (omega-3 to total fatty acids [omega-3/FA], docosahexaenoic acid to total fatty acids [DHA/FA], polyunsaturated fatty acids to total fatty acids [PUFA/FA] ratios) were decreased whereas the monosaturated to total fatty acids (MUFA/FA) and apolipoprotein B to apolipoprotein A1 (ApoB/ApoA1) ratios were increased in the adult-onset trajectory (**Figure 3**). Full model results are presented in **Tables S11-12**.

Blood count and clinical chemistry markers

Relative to the low-stable trajectory, after adjusting for sex, maternal education and occupational class, and BMI at age 10, there was evidence for decreased AST levels in the adolescent-limited trajectory, and increased HOMA-IR, insulin, neutrophil and white blood cell (WBC) counts in the adulthood-onset trajectory (**Figure 5**). Full model results are presented in **Tables S13-14**.

Sensitivity analyses

The sensitivity analyses showed patterns of associations that are largely similar to those observed in the primary analyses, with consistent associations of depression-related trajectories with anthropometric, cardiometabolic and psychiatric outcomes, and top-ranking immunometabolic biomarkers with similar effect sizes in the same directions (**Supplementary Methods S3**).

[INSERT FIGURES 2-4 HERE]

Discussion

Depression is a complex heterogeneous disorder, which poses a challenge for discovering biomarkers associated with disease onset and/or progression. We have taken a longitudinal approach to identifying blood-based biomarkers for depression by examining longitudinal patterns of depressive symptoms in the population during the critical developmental epoch of childhood, adolescence and early adulthood. Using data from a prospective birth cohort, we have identified four longitudinal population subgroups based on repeated measures of depressive symptoms over a 15-year period from ages 10 to 25 years. We show that majority of participants (approximately 70%) have little or no depressive symptoms (low-stable group). We identified three depression-related groups which comprise a group with higher symptom levels during childhood and adolescence which later decrease (adolescent limited group, 13%), a group with symptoms emerging during puberty which persist throughout adolescence through to adulthood (adolescent persistent group, 7%), and a group with symptoms emerging during late adolescence/early adulthood and increasing thereafter (adulthood onset, 10%).

We have examined health phenotypes and blood biomarkers associated with these subgroups for greater insight into the developmental course of depression and associated biomarkers. Our analyses show that compared to the low-stable group, risk of depression and anxiety in adulthood is higher for all three depression-related groups. However, such risk is particularly elevated risk for the adolescent-persistent group (19 to 23-fold risk) followed by the adulthood-onset group (five to eight-fold risk). Interestingly, the group where higher levels of symptoms are mostly limited to adolescence, they still have a three-fold increased risk of depression in adulthood.

Having examined health phenotypic and blood proteomic, immunological and metabolic biomarker associations for these three groups, we show that the adolescent-limited group is distinct from the other two depression-related groups as it showed little immunometabolic alterations. In contrast, both the adolescent-persistent and adulthood-onset groups are associated with immunometabolic changes, but the exact pattern of associations varies between the two groups. The adolescent-persistent group was associated with higher BMI in childhood and adulthood, whereas the adulthood-onset group did not show this, but rather more widespread alterations in blood-based metabolic parameters including insulin resistance, insulin levels and changes in fatty acid ratios. Blood proteomic changes were largely similar between the two groups and involved proteins that mainly act as growth factors, cytokines and chemokines. While immunometabolic associations persisted given adjustment for childhood BMI in the adolescent-persistent group, the presence or absence of this BMI adjustment had an impact on some association estimates in the adolescent-persistent and adulthood-onset groups. While this may relate to the apparent differences in BMI by trajectories, it is difficult to distinguish this as an artefact of adjustment or a true impact of BMI.

Epidemiological studies consistently report a bidirectional relationship (both cross-sectionally and longitudinally) between obesity and depression,[38] whereas Mendelian randomization studies support a causal role of BMI on major depressive disorder and depressive symptoms but not vice

versa.[39, 40] The comorbidity between obesity and depression is generally associated with poorer prognosis, with studies reporting associations with a more chronic course of depression in adulthood[41, 42] as well as poorer treatment response.[43] Our findings add to this evidence by showing an association between higher childhood BMI and persistent depressive symptoms between adolescence and early adulthood.

Many of the alterations observed in the adulthood-onset trajectory are already well studied markers of cardiometabolic disease risk. The ApoB/ApoA1 ratio is associated with cardiovascular diseases and metabolic syndrome,[44, 45] and can be used to predict longer-term cardiovascular risk when measured in early life.[46, 47] Higher values of HOMA-IR are associated with an increased risk of developing type 2 diabetes mellitus (T2DM), systemic arterial hypertension and non-fatal major adverse cardiovascular events.[48] Lower blood omega-3 fatty acid concentrations are associated with poorer cardiovascular outcomes[49, 50] and may also contribute to chronic systemic inflammation, whereas changes in MUFA, PUFA and DHA concentrations in early adulthood were associated with incident obesity, insulin resistance and elevated blood pressure 10 years later.[51] Since dietary intake and supplementation are the main predictors of blood levels of omega-3 fatty acids,[52, 53] with lifestyle factors such as BMI, smoking and alcohol consumption also playing a role,[54] this suggests that the adulthood-onset group may benefit from lifestyle intervention to prevent future cardiometabolic disease.

The overlapping proteomic signals observed between the adolescent-persistent and adulthood-onset trajectories potentially suggest shared underlying mechanisms (genetic or environmental) or shared biological responses to depression, which warrant further study for their roles in the pathophysiology of depression and cardiometabolic disease. FGF-21 is a novel regulator of glucose and lipid metabolism that mainly acts through an FGF receptor 1 (FGFR1)/β-klotho receptor complex and the Ras/Raf MAPK signalling pathway, which have been implicated in the pathophysiology of depression and therapeutic effects of antidepressants.[55-57] Elevated circulating FGF-21 concentrations have also been shown to be associated with a range of cardiometabolic markers and diseases.[58-62] HGF mediates inflammatory responses to tissue

445 injury and regulates cell growth and morphogenesis through the activation of the
446 HGF/mesenchymal-epithelial transition factor (c-Met) signalling pathway, which has downstream
447 effects on the Raf/MAPK and PI3K/Akt pathways. Altered HGF/c-Met signalling has been
448 suggested to play a role in the pathogenesis of depression in adolescents through disrupting
449 interneuron development.[63] CCL11 is a chemokine involved in the selective recruitment of
450 eosinophils into sites of inflammation and has been implicated in various allergic and
451 inflammatory conditions.[64] It can be transported across the blood-brain barrier[65] and is an age-
452 related systemic factor associated with reduced synaptic plasticity and impaired hippocampal-
453 dependent learning and memory in mice.[66] In humans, CCL11 levels increase with age,[66] and
454 there is emerging evidence to suggest that CCL11 levels are associated with psychiatric
455 disorders.[67-71] In summary, the overlapping proteomic signals between the adolescent-persistent
456 and adulthood-onset trajectories highlight potential roles of physiological stress from lifestyle or
457 environmental factors, disruptions in neurodevelopment, and neurogenesis and cellular
458 senescence in the underlying vulnerability or biological response to depression, and may be key
459 biomarkers relevant to illness pathogenesis.
460
461 An advantage of our work is that by examining depressive symptoms longitudinally using a latent
462 class trajectory approach, we can account for population heterogeneity and obtain better
463 characterisation of subgroups and their changes over time. Existing literature shows that
464 depression is associated with alterations in various immunometabolic biomarkers, including
465 increased inflammatory cytokines,[72, 73] WBC,[74] neutrophils,[74] T-lymphocytes and other immune
466 cell counts,[74] HOMA-IR,[75] insulin,[75] lipids and fatty acids.[76, 77] Using longitudinal data from young
467 people, we add to this evidence base by showing that classical immunometabolic changes are
468 particularly associated with an adulthood-onset trajectory, rather than other developmental
469 subgroups of depressive symptoms, including one with persistent symptoms since adolescence.
470
471 This study has several limitations. Firstly, the approach of treating assigned class membership as
472 discrete in assessing relationships with other variables has been shown to underestimate the
473 strength of the relationships.[78] However, as we are mainly interested in subpopulations with

different depressive symptom trajectories, this approach allows for easier interpretation and translation. Secondly, depression is episodic in nature and the use of polynomials cannot fully capture the dynamics of depressive symptom severity over time; however, this approach was chosen over other methods (e.g., splines) for model parsimony.

Furthermore, the sample size in this study is relatively small and may be underpowered to detect differences after stratifying individuals into four separate trajectories. For this reason, we did not further stratify our analyses by sex or potentially relevant variables (e.g., BMI). While we have adjusted our biomarker analyses for several potential confounders, residual confounding could still be an issue. For instance, we have not accounted for medication use or chronic disease etc., but these are likely to be uncommon in young people. As the biomarkers were measured at age 24, which is after the onset of depressive symptoms in many individuals, further research is required to assess the direction and causality of associations we have identified.

In conclusion, we identified distinct developmental trajectories of depression from childhood to early adulthood, which show differential associations with cardiometabolic and psychiatric outcomes, and are characterised by distinct immunometabolic profiles. In particular, individuals with persistent depressive symptoms from childhood through to early adulthood were more likely to have higher BMI both in childhood and in adulthood and few other immunometabolic changes, whereas individuals who develop depressive symptoms towards early adulthood show classical immunometabolic alterations in immune cell counts, insulin resistance and fatty acid profiles. These findings point to distinct mechanisms and intervention opportunities for different groups of young people with depressive symptoms.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We would also like to thank members of the LINC study public advisory group for their contribution.

This work was funded by the Tackling Multimorbidity at Scale Strategic Priorities Fund programme (MR/W014416/1) delivered by the UK Medical Research Council (MRC) and the UK National Institute for Health Research (NIHR) in partnership with the UK Economic and Social Research Council and in collaboration with the UK Engineering and Physical Sciences Research Council. RSMT, DS and MLW are supported by this grant. ASFK is supported by a Wellcome Early Career Award (227063/Z/23/Z). NAD was supported by an NIHR Clinical Lectureship in General Adult Psychiatry. MBMvdB acknowledges additional funding support from the MRC (MR/W028395/1, MR/W020297/1, MR/T033045/1 and MR/S037667/1), NIMH (U01MH119758), the Wellcome Trust (226709/Z/22/Z) and Welsh Government (HS 22 04). NJT is a director of the MRC/ESRC/UKRI supported Population Research UK Coordination Hub (ES/Y008340/1), supported by a Wellcome Trust Investigator award (202802/Z/16/Z), is the PI of the Avon Longitudinal Study of Parents and Children (MRC & WT 217065/Z/19/Z), is supported by the University of Bristol NIHR Biomedical Research Centre (BRC-1215-2001), the MRC Integrative Epidemiology Unit (MC_UU_00011/1), CRUK and works within the CRUK Integrative Cancer Epidemiology Programme (C18281/A29019) and with support from CRUK (PRCPJT-May22\100028). GMK acknowledges funding support from the MRC (MC_UU_00032/06), which forms part of the MRC Integrative Epidemiology Unit at the University of Bristol. This grant also supports HF. GMK acknowledges additional funding from the Wellcome Trust (201486/Z/16/Z and 201486/B/16/Z), the MRC (MR/W014416/1; MR/S037675/1; and MR/Z50354X/1), and the NIHR Bristol Biomedical Research Centre (NIHR 203315). The views expressed are those of the authors and not necessarily those of the UK NIHR or the Department of Health and Social Care.

The UK MRC and Wellcome (217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and RSMT, NJT and GMK will serve as guarantors for the contents of this paper. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf).

LINC Consortium members:

Marianne B. M. van den Bree, George Kirov, Michael J. Owen, James T. R. Walters, Peter A. Holmans, Jane Lynch, Ioanna K. Katzourou, Nabila Ali, Lowri O'Donovan (Cardiff University, UK)

David A. van Heel, Sarah Finer, Daniel Stow (Queen Mary University of London, UK)

Golam M. Khandaker, Nicholas J. Timpson, John A. A. Macleod, Julie P. Clayton, Ruby S. M. Tsang, Jane Sprackman, Shahid Khan (University of Bristol, UK)

Inês A. Barroso, Rupert A. Payne (University of Exeter, UK)

Mark Mon-Williams, Megan L. Wood (University of Leeds, UK)

Hilary C. Martin (Wellcome Sanger Institute, UK)

Thomas Werge, Andrés Ingason, Morteza Vaez, Lam O. Huang (Institute of Biological Psychiatry, Denmark)

Conflict of Interest

None

References

1. Solmi M, Radua J, Olivola M, Croce E, Soardo L, Salazar de Pablo G *et al.* Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular Psychiatry* 2022; **27**(1)**:** 281-295.

2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry* 2005; **62**(6)**:** 593-602.

3. Shore L, Toumbourou JW, Lewis AJ, Kremer P. Review: Longitudinal trajectories of child and adolescent depressive symptoms and their predictors – a systematic review and meta-analysis. *Child and Adolescent Mental Health* 2018; **23**(2)**:** 107-120.

4. Hankin BL, Abramson LY, Moffitt TE, Silva PA, McGee R, Angell KE. Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *J Abnorm Psychol* 1998; **107**(1)**:** 128-140.

5. Copeland WE, Adair CE, Smetanin P, Stiff D, Briante C, Colman I *et al.* Diagnostic transitions from childhood to adolescence to early adulthood. *Journal of Child Psychology and Psychiatry* 2013; **54**(7)**:** 791-799.

6. Fergusson DM, Woodward LJ. Mental Health, Educational, and Social Role Outcomes of Adolescents With Depression. *Archives of General Psychiatry* 2002; **59**(3)**:** 225-231.

7. Georgiades K, Lewinsohn PM, Monroe SM, Seeley JR. Major Depressive Disorder in Adolescence: The Role of Subthreshold Symptoms. *Journal of the American Academy of Child & Adolescent Psychiatry* 2006; **45**(8)**:** 936-944.

8. Yaroslavsky I, Pettit JW, Lewinsohn PM, Seeley JR, Roberts RE. Heterogeneous trajectories of depressive symptoms: Adolescent predictors and adult outcomes. *Journal of Affective Disorders* 2013; **148**(2)**:** 391-399.

9. Musliner KL, Munk-Olsen T, Eaton WW, Zandi PP. Heterogeneity in long-term trajectories of depressive symptoms: Patterns, predictors and outcomes. *Journal of Affective Disorders* 2016; **192:** 199-211.

10. Weavers B, Heron J, Thapar AK, Stephens A, Lennon J, Bevan Jones R *et al.* The antecedents and outcomes of persistent and remitting adolescent depressive symptom trajectories: a longitudinal, population-based English study. *The Lancet Psychiatry* 2021; **8**(12)**:** 1053-1061.

11. Khandaker GM, Dantzer R, Jones PB. Immunopsychiatry: important facts. *Psychological Medicine* 2017; **47**(13)**:** 2229-2237.

12. Milaneschi Y, Lamers F, Berk M, Penninx BWJH. Depression Heterogeneity and Its Biological Underpinnings: Toward Immunometabolic Depression. *Biological Psychiatry* 2020; **88**(5)**:** 369-380.

13. Nikkheslat N, Pariante CM, Zunszain PA. Chapter 3 - Neuroendocrine Abnormalities in Major Depression: An Insight Into Glucocorticoids, Cytokines, and the Kynurenine Pathway. In: Baune BT (ed). *Inflammation and Immunity in Depression*. Academic Press2018, pp 45-60.

14. Bot M, Milaneschi Y, Al-Shehri T, Amin N, Garmaeva S, Onderwater GLJ *et al.* Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biological Psychiatry* 2020; **87**(5)**:** 409-418.

15. Lamers F, Milaneschi Y, Vinkers CH, Schoevers RA, Giltay EJ, Penninx BWJH. Depression profilers and immuno-metabolic dysregulation: Longitudinal results from the NESDA study. *Brain, Behavior, and Immunity* 2020; **88:** 174-183.

16. Milaneschi Y, Kappelmann N, Ye Z, Lamers F, Moser S, Jones PB *et al.* Association of inflammation with depression and anxiety: evidence for symptom-specificity and potential causality from UK Biobank and NESDA cohorts. *Molecular Psychiatry* 2021; **26**(12)**:** 7393-7402.

17. Frank P, Jokela M, Batty GD, Cadar D, Steptoe A, Kivimäki M. Association Between Systemic Inflammation and Individual Symptoms of Depression: A Pooled Analysis of 15 Population-Based Cohort Studies. *American Journal of Psychiatry* 2021; **178**(12)**:** 1107-1118.

18. Kappelmann N, Arloth J, Georgakis MK, Czamara D, Rost N, Ligthart S *et al.* Dissecting the Association Between Inflammation, Metabolic Dysregulation, and Specific Depressive Symptoms: A Genetic Correlation and 2-Sample Mendelian Randomization Study. *JAMA Psychiatry* 2021; **78**(2)**:** 161-170.

19. Pistis G, Milaneschi Y, Vandeleur CL, Lasserre AM, Penninx BWJH, Lamers F *et al.* Obesity and atypical depression symptoms: findings from Mendelian randomization in two European cohorts. *Translational Psychiatry* 2021; **11**(1)**:** 96.

20. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. *International Journal of Epidemiology* 2002; **31**(2)**:** 285-293.

21. Ditmars HL, Logue MW, Toomey R, McKenzie RE, Franz CE, Panizzon MS *et al.* Associations between depression and cardiometabolic health: A 27-year longitudinal study. *Psychological Medicine* 2022; **52**(14)**:** 3007-3017.

22. Harshfield EL, Pennells L, Schwartz JE, Willeit P, Kaptoge S, Bell S *et al.* Association Between Depressive Symptoms and Incident Cardiovascular Diseases. *JAMA* 2020; **324**(23)**:** 2396-2405.

23. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J *et al.* Cohort Profile: The 'Children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *International Journal of Epidemiology* 2013; **42**(1)**:** 111-127.

24. Northstone K, Lewcock M, Groom A, Boyd A, Macleod J, Timpson N, Wells N. The Avon Longitudinal Study of Parents and Children (ALSPAC): an update on the enrolled sample of index children in 2019 [version 1; peer review: 2 approved]. *Wellcome Open Research* 2019; **4**(51).

25. Angold A, Costello EJ, Messer SC, Pickles A. Development of a short questionnaire for use in epidemiological studies of depression in children and adolescents. *International Journal of Methods in Psychiatric Research* 1995; **5**(4)**:** 237-249.

26. Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R *et al.* High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* 2009; **134**(9)**:** 1781-1785.

27. Inouye M, Kettunen J, Soininen P, Silander K, Ripatti S, Kumpula LS *et al.* Metabonomic, transcriptomic, and genomic variation of a population cohort. *Molecular Systems Biology* 2010; **6**(1)**:** 441.

28. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circulation: Cardiovascular Genetics* 2015; **8**(1)**:** 192-206.

29. Proust-Lima C, Philipps V, Liquet B. Estimation of Extended Mixed Models Using Latent Classes and Latent Processes: The R Package lcmm. *Journal of Statistical Software* 2017; **78**(2)**:** 1 - 56.

30. Lennon H, Kelly S, Sperrin M, Buchan I, Cross AJ, Leitzmann M *et al.* Framework to construct and interpret latent class trajectory modelling. *BMJ Open* 2018; **8**(7)**:** e020683.

31. van der Nest G, Lima Passos V, Candel MJJM, van Breukelen GJP. An overview of mixture modelling for latent evolutions in longitudinal data: Modelling approaches, fit statistics and software. *Advances in Life Course Research* 2020; **43:** 100323.

32. van der Nest G, Lima Passos V, Candel MJJM, van Breukelen GJP. Model fit criteria curve behaviour in class enumeration – a diagnostic tool for model (mis)specification in longitudinal mixture modelling. *Journal of Statistical Computation and Simulation* 2022; **92**(8)**:** 1640-1672.

33. Diallo TMO, Morin AJS, Lu H. Impact of Misspecifications of the Latent Variance–Covariance and Residual Matrices on the Class Enumeration Accuracy of Growth Mixture Models. *Structural Equation Modeling: A Multidisciplinary Journal* 2016; **23**(4)**:** 507-531.

34. van de Schoot R, Sijbrandij M, Winter SD, Depaoli S, Vermunt JK. The GRoLTS-Checklist: Guidelines for Reporting on Latent Trajectory Studies. *Structural Equation Modeling: A Multidisciplinary Journal* 2017; **24**(3)**:** 451-467.

35. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 2015; **43**(7)**:** e47-e47.

36. StataCorp. Stata Statistical Software: Release 17. StataCorp LLC: College Station, TX, USA, 2021.

37. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria, 2022. https://www.R-project.org/.

38. Milaneschi Y, Simmons WK, van Rossum EFC, Penninx BWJH. Depression and obesity: evidence of shared biological mechanisms. *Molecular Psychiatry* 2019; **24**(1)**:** 18-33.

39. Hartwig FP, Bowden J, Loret de Mola C, Tovo-Rodrigues L, Davey Smith G, Horta BL. Body mass index and psychiatric disorders: a Mendelian randomization study. *Scientific Reports* 2016; **6**(1)**:** 32730.

40. van den Broek N, Treur JL, Larsen JK, Verhagen M, Verweij KJH, Vink JM. Causal associations between body mass index and mental health: a Mendelian randomisation study. *Journal of Epidemiology and Community Health* 2018; **72**(8)**:** 708.

41. Vogelzangs N, Beekman AT, Boelhouwer IG, Bandinelli S, Milaneschi Y, Ferrucci L, Penninx BW. Metabolic depression: a chronic depressive subtype? Findings from the InCHIANTI study of older persons. *J Clin Psychiatry* 2011; **72**(5)**:** 598-604.

42. Nigatu YT, Bültmann U, Reijneveld SA. The prospective association between obesity and major depression in the general population: does single or recurrent episode matter? *BMC Public Health* 2015; **15**(1)**:** 350.

43. Grigolon RB, Trevizol AP, Gerchman F, Bambokian AD, Magee T, McIntyre RS *et al.* Is Obesity A Determinant Of Success With Pharmacological Treatment For Depression? A Systematic Review, Meta-Analysis And Meta-Regression. *Journal of Affective Disorders* 2021; **287:** 54-68.

44. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J *et al.* Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *The Lancet* 2008; **372**(9634)**:** 224-233.

45. Ulloque-Badaracco JR, Al-kassab-Córdova A, Hernandez-Bustamante EA, Alarcon-Braga EA, Huayta-Cortez M, Carballo-Tello XL *et al.* Association of apolipoproteins and lipoprotein(a) with metabolic syndrome: a systematic review and meta-analysis. *Lipids in Health and Disease* 2023; **22**(1)**:** 98.

46. Juonala M, Viikari JSA, Kähönen M, Solakivi T, Helenius H, Jula A *et al.* Childhood Levels of Serum Apolipoproteins B and A-I Predict Carotid Intima-Media Thickness and Brachial Endothelial Function in Adulthood: The Cardiovascular Risk in Young Finns Study. *Journal of the American College of Cardiology* 2008; **52**(4)**:** 293-299.

47. Ojanen X, Cheng R, Törmäkangas T, Rappaport N, Wilmanski T, Wu N *et al.* Towards early risk biomarkers: serum metabolic signature in childhood predicts cardio-metabolic risk in adulthood. *eBioMedicine* 2021; **72**.

48. González-González JG, Violante-Cumpa JR, Zambrano-Lucio M, Burciaga-Jimenez E, Castillo-Morales PL, Garcia-Campa M *et al.* HOMA-IR as a predictor of Health Outcomes in Patients with Metabolic Risk Factors: A Systematic Review and Meta-analysis. *High Blood Pressure & Cardiovascular Prevention* 2022; **29**(6)**:** 547-564.

49. Harris WS, von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Preventive Medicine* 2004; **39**(1)**:** 212-220.

50. Harris WS, Tintle NL, Etherton MR, Vasan RS. Erythrocyte long-chain omega-3 fatty acid levels are inversely associated with mortality and with incident cardiovascular disease: The Framingham Heart Study. *Journal of Clinical Lipidology* 2018; **12**(3)**:** 718-727.e716.

51. Kaikkonen JE, Jula A, Viikari JSA, Juonala M, Hutri-Kähönen N, Kähönen M *et al.* Associations of Serum Fatty Acid Proportions with Obesity, Insulin Resistance, Blood Pressure, and Fatty Liver: The Cardiovascular Risk in Young Finns Study. *The Journal of Nutrition* 2021; **151**(4)**:** 970-978.

52. Harris WS, Pottala JV, Lacey SM, Vasan RS, Larson MG, Robins SJ. Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study. *Atherosclerosis* 2012; **225**(2)**:** 425-431.

53. Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Progress in Lipid Research* 2016; **63:** 132-152.

54. de Groot RHM, Emmett R, Meyer BJ. Non-dietary factors associated with n-3 long-chain PUFA levels in humans – a systematic literature review. *British Journal of Nutrition* 2019; **121**(7)**:** 793-808.

55. Wang JQ, Mao L. The ERK Pathway: Molecular Mechanisms and Treatment of Depression. *Molecular Neurobiology* 2019; **56**(9)**:** 6197-6205.

56. Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS. A Role for MAP Kinase Signaling in Behavioral Models of Depression and Antidepressant Treatment. *Biological Psychiatry* 2007; **61**(5)**:** 661-670.

57. Duman RS, Voleti B. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. *Trends in Neurosciences* 2012; **35**(1)**:** 47-56.

58. Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou Z-G, Liu F *et al.* Serum FGF21 Levels Are Increased in Obesity and Are Independently Associated With the Metabolic Syndrome in Humans. *Diabetes* 2008; **57**(5)**:** 1246-1253.

59. Kralisch S, Tönjes A, Krause K, Richter J, Lossner U, Kovacs P *et al.* Fibroblast growth factor-21 serum concentrations are associated with metabolic and hepatic markers in humans. *Journal of Endocrinology* 2013; **216**(2)**:** 135-143.

60. Semba RD, Sun K, Egan JM, Crasto C, Carlson OD, Ferrucci L. Relationship of Serum Fibroblast Growth Factor 21 with Abnormal Glucose Metabolism and Insulin Resistance: The Baltimore Longitudinal Study of Aging. *The Journal of Clinical Endocrinology & Metabolism* 2012; **97**(4)**:** 1375-1382.

61. Chen C, Cheung BMY, Tso AWK, Wang Y, Law LSC, Ong KL *et al.* High Plasma Level of Fibroblast Growth Factor 21 Is an Independent Predictor of Type 2 Diabetes: A 5.4-year population-based prospective study in Chinese subjects. *Diabetes Care* 2011; **34**(9)**:** 2113-2115.

62. Li H, Fang Q, Gao F, Fan J, Zhou J, Wang X *et al.* Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *Journal of Hepatology* 2010; **53**(5)**:** 934-940.

63. Ciuculete DM, Voisin S, Kular L, Welihinda N, Jonsson J, Jagodic M *et al.* Longitudinal DNA methylation changes at MET may alter HGF/c-MET signalling in adolescents at risk for depression. *Epigenetics* 2020; **15**(6-7)**:** 646-663.

64. Teixeira AL, Gama CS, Rocha NP, Teixeira MM. Revisiting the Role of Eotaxin-1/CCL11 in Psychiatric Disorders. *Frontiers in Psychiatry* 2018; **9**.

65. Erickson MA, Morofuji Y, Owen JB, Banks WA. Rapid Transport of CCL11 across the Blood-Brain Barrier: Regional Variation and Importance of Blood Cells. *Journal of Pharmacology and Experimental Therapeutics* 2014; **349**(3)**:** 497.

66. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G *et al.* The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 2011; **477**(7362)**:** 90-94.

67. Teixeira AL, Reis HJ, Nicolato R, Brito-Melo G, Correa H, Teixeira MM, Romano-Silva MA. Increased serum levels of CCL11/eotaxin in schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2008; **32**(3)**:** 710-714.

68. Grassi-Oliveira R, Brieztke E, Teixeira A, Pezzi JC, Zanini M, Lopes RP, Bauer ME. Peripheral chemokine levels in women with recurrent major depression with suicidal ideation. *Revista Brasileira de Psiquiatria* 2012; **34**(1)**:** 71-75.

69. Panizzutti B, Gubert C, Schuh AL, Ferrari P, Bristot G, Fries GR *et al.* Increased serum levels of eotaxin/CCL11 in late-stage patients with bipolar disorder: An accelerated aging biomarker? *Journal of Affective Disorders* 2015; **182:** 64-69.

70. Simon NM, McNamara K, Chow CW, Maser RS, Papakostas GI, Pollack MH *et al.* A detailed examination of cytokine abnormalities in Major Depressive Disorder. *European Neuropsychopharmacology* 2008; **18**(3)**:** 230-233.

71. Leighton SP, Nerurkar L, Krishnadas R, Johnman C, Graham GJ, Cavanagh J. Chemokines in depression in health and in inflammatory illness: a systematic review and meta-analysis. *Molecular Psychiatry* 2018; **23**(1)**:** 48-58.

72. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Molecular Psychiatry* 2016; **21**(12)**:** 1696-1709.

73. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: A meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain, Behavior, and Immunity* 2020; **87:** 901-909.

74. Foley ÉM, Parkinson JT, Mitchell RE, Turner L, Khandaker GM. Peripheral blood cellular immunophenotype in depression: a systematic review and meta-analysis. *Molecular Psychiatry* 2023; **28**(3)**:** 1004-1019.

75. Fernandes BS, Salagre E, Enduru N, Grande I, Vieta E, Zhao Z. Insulin resistance in depression: A large meta-analysis of metabolic parameters and variation. *Neuroscience & Biobehavioral Reviews* 2022; **139:** 104758.

76. Davyson E, Shen X, Gadd DA, Bernabeu E, Hillary RF, McCartney DL *et al.* Metabolomic Investigation of Major Depressive Disorder Identifies a Potentially Causal Association With Polyunsaturated Fatty Acids. *Biological Psychiatry* 2023.

77. Perry BI, Oltean BP, Jones PB, Khandaker GM. Cardiometabolic risk in young adults with depression and evidence of inflammation: A birth cohort study. *Psychoneuroendocrinology* 2020; **116:** 104682.

78. Bolck A, Croon M, Hagenaars J. Estimating Latent Structure Models with Categorical Variables: One-Step Versus Three-Step Estimators. *Political Analysis* 2004; **12**(1)**:** 3-27.

862

Figure Legends

Figure 1. Predicted Marginal Mean Depressive Symptom Trajectories from Childhood to Early Adulthood in the ALSPAC Cohort

Lines showing predicted marginal mean depressive symptom trajectories with shaded areas representing 95% confidence intervals.

Figure 2. Volcano Plots Showing Differential Immune Protein Abundance Levels in Depressive Symptom Trajectories

Panels **a** and **b** show results from basic and adjusted models respectively. The reference group for all analyses is the low-stable trajectory.

Abbreviations: 4E-BP1 – eukaryotic translation initiation factor; CCL11 – eotaxin-1; CCL25 – C-C motif chemokine 25; CDCP1 – CUB domain-containing protein 1; FGF-19 – fibroblast growth factor 19; FGF-21 – fibroblast growth factor 21; HGF – hepatic growth factor

Figure 3. Volcano Plots Showing Differential Metabolite Abundance Levels in Depressive Symptom Trajectories

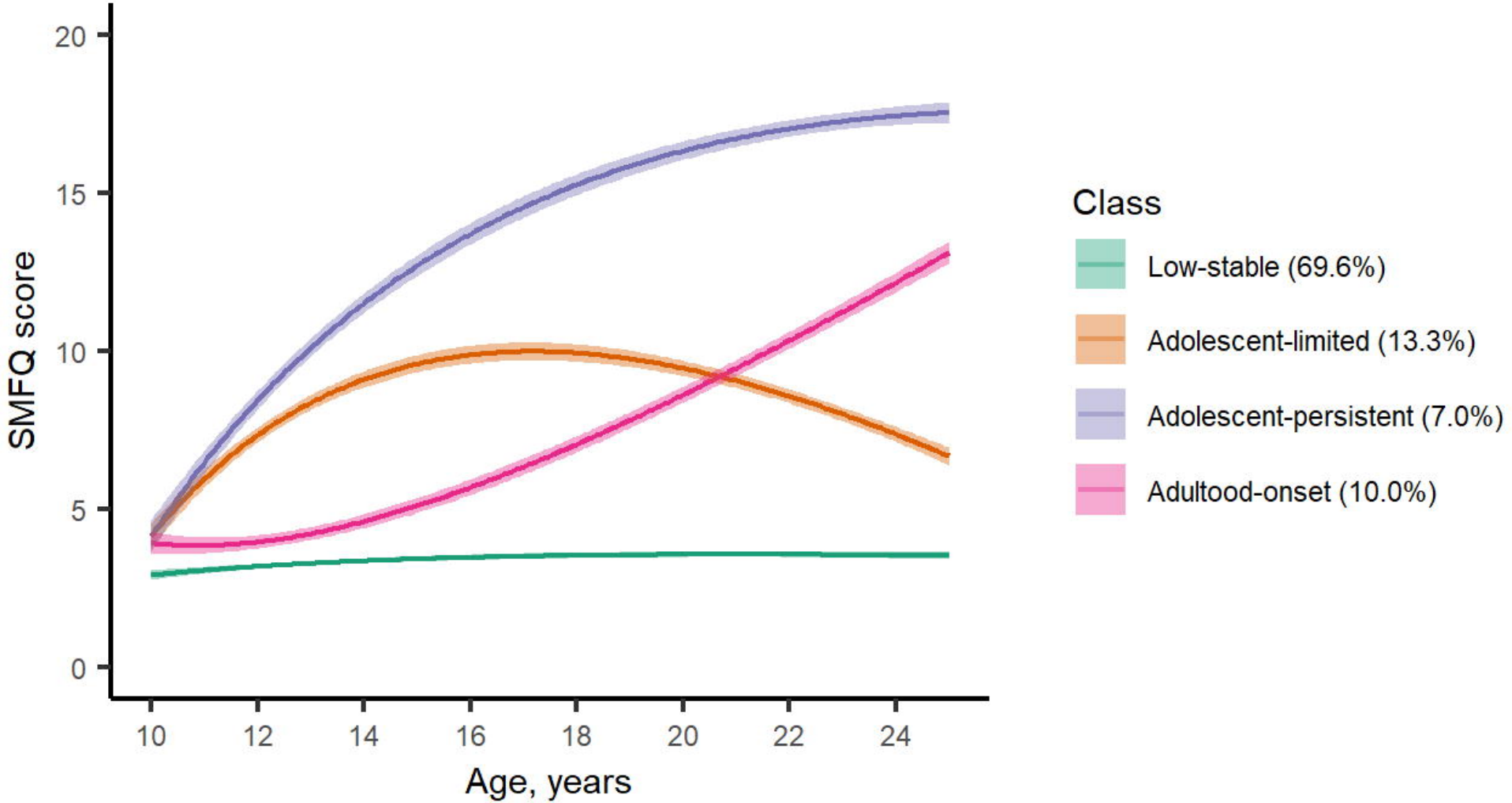
Panels **a** and **b** show results from basic and adjusted models respectively. The reference group for all analyses is the low-stable trajectory. Orange points indicate upregulation and blue points indicate downregulation.

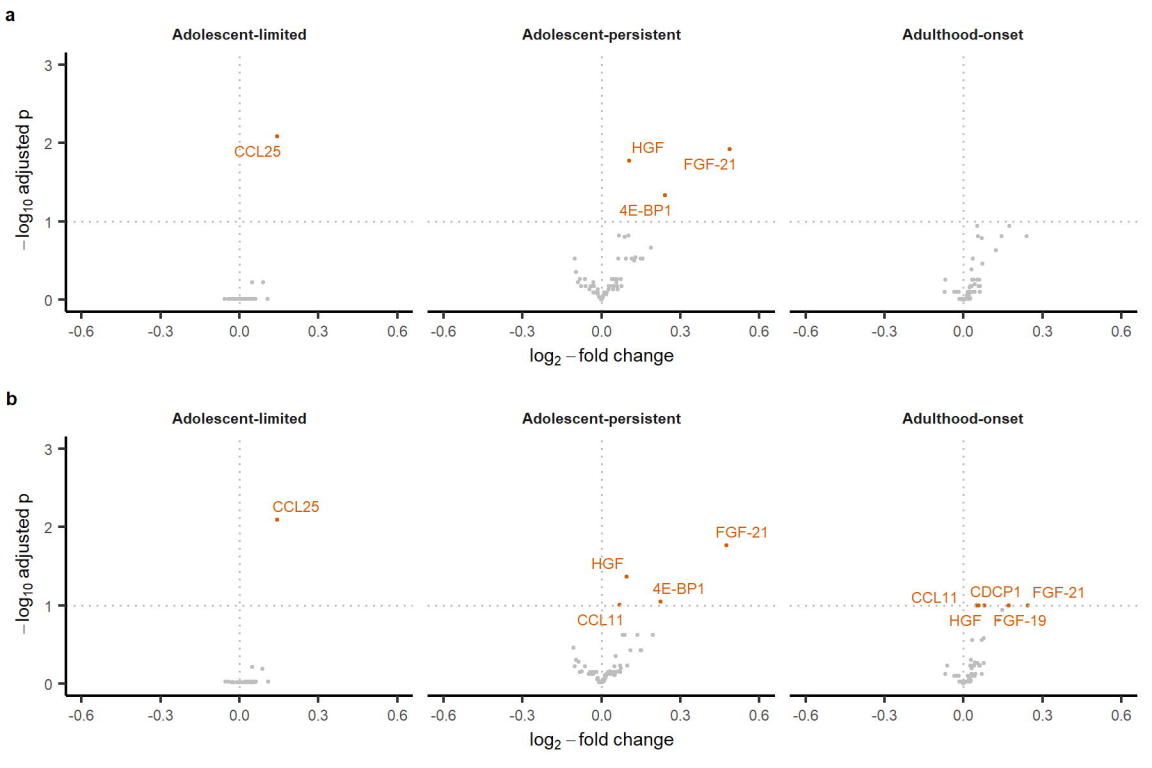
Abbreviations: ApoB/ApoA1 – apolipoprotein B to apolipoprotein A1 ratio, DHA/FA – docosahexaenoic acid to total fatty acids ratio, MUFA/FA – monounsaturated fatty acids to total fatty acids ratio, Omega-3/FA – omega-3 fatty acids to total fatty acids ratio, PUFA/FA – polyunsaturated fatty acids/total fatty acids ratio

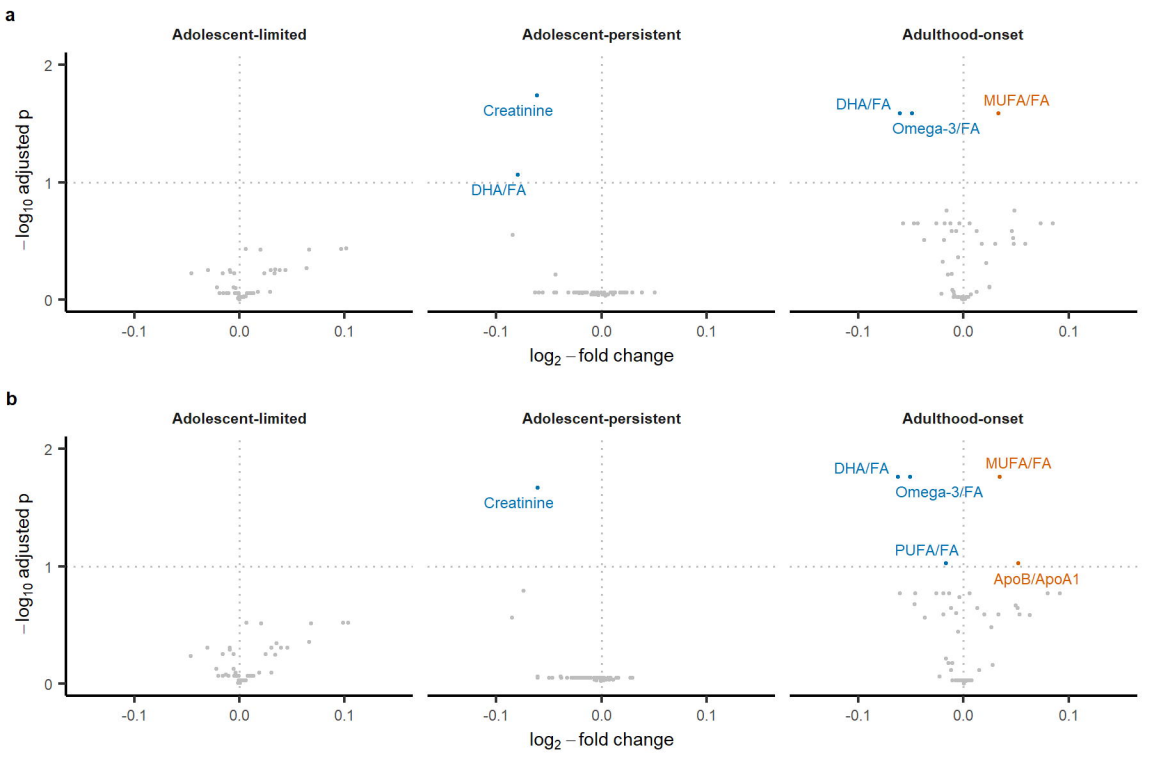
Figure 4. Differential Levels of Full Blood Count and Clinical Biochemistry Biomarkers in Different Depressive Symptom Trajectories

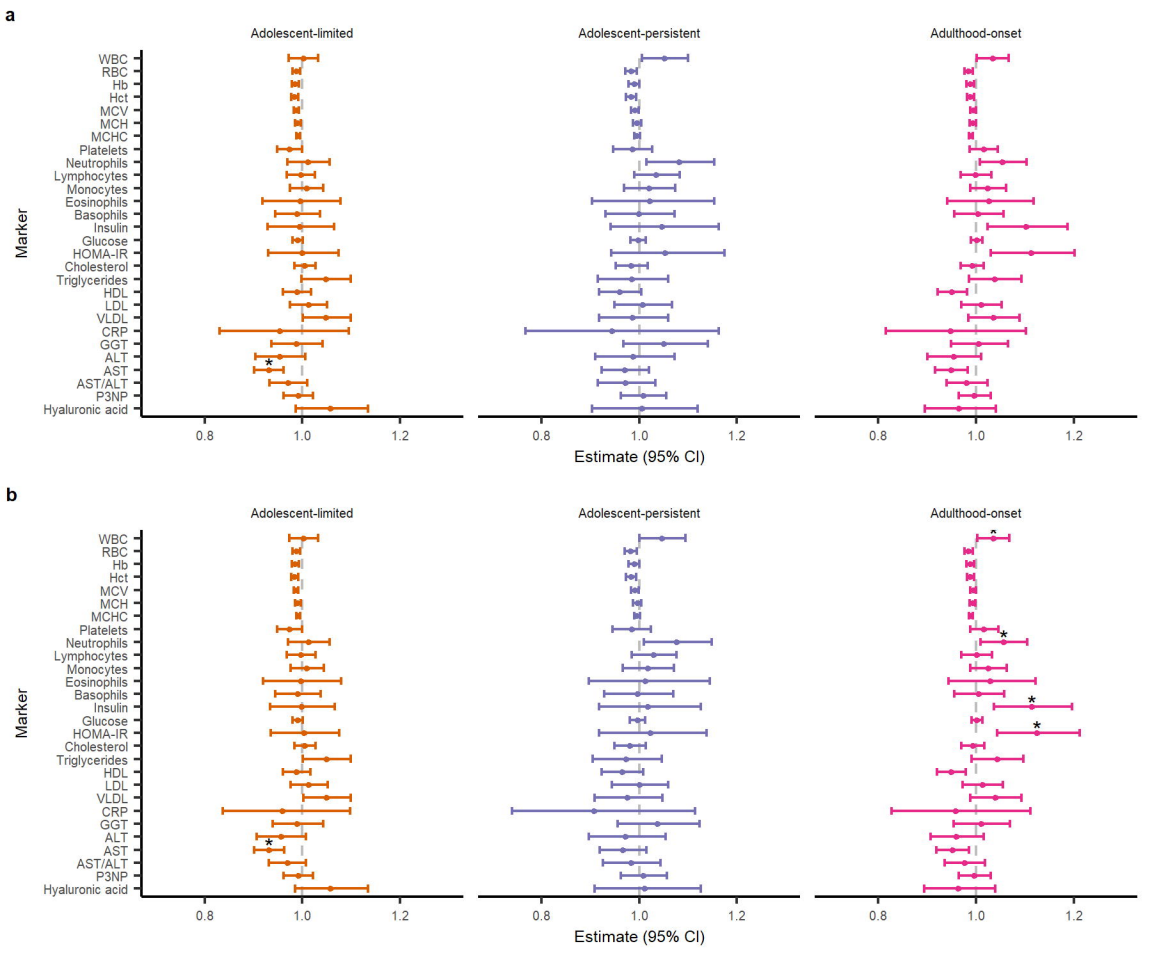
Dot-and-whisker plots showing effect estimates and 95% confidence intervals for each biomarker. The effect estimates and 95% confidence intervals from the models have been back-transformed

into their original scale for ease of interpretation; effect estimates represent the percentage difference in mean levels of each biomarker for respective trajectory, in relation to the low-stable trajectory (reference group). Panels a and b show results from basic and adjusted models respectively. Asterisks indicate evidence for the association after FDR correction of p-values. Abbreviations: WBC – white blood count, RBC – red blood count, Hb – haemoglobin, Hct – haematocrit, MCV – mean cell volume, MCH – mean cell haemoglobin, MCHC – mean corpuscular haemoglobin concentration, HOMA-IR – Homeostatic Model Assessment for Insulin Resistance, HDL – high-density lipoprotein, LDL – low-density lipoprotein, VLDL – very low-density lipoprotein, CRP – C-reactive protein, GGT – gamma-glutamyl transpeptide, ALT – alanine aminotransferase, AST – aspartate aminotransferase, P3NP – procollagen-3 N-terminal peptide


SMFQ score
20
15
10
5
0
10
12
14
16
18
20
22
24
Age, years
Class
Low-stable (69.6%)
Adolescent-limited (13.3%)
Adolescent-persistent (7.0%)
Adultood-onset (10.0%)


a
Adolescent-limited
Adolescent-persistent
Adulthood-onset
CCL25
HGF
FGF-21
4E-BP1
−log10 adjusted p
log2 − fold change
b
Adolescent-limited
Adolescent-persistent
Adulthood-onset
CCL25
FGF-21
HGF
4E-BP1
CCL11
CCL11
CDCP1
FGF-21
HGF
FGF-19
−log10 adjusted p
log2 − fold change


a
Adolescent-limited
Adolescent-persistent
Adulthood-onset
Creatinine
DHA/FA
DHA/FA
Omega-3/FA
MUFA/FA
$-\log_{10}$ adjusted p
$\log_2$ – fold change
b
Adolescent-limited
Adolescent-persistent
Adulthood-onset
Creatinine
DHA/FA
Omega-3/FA
MUFA/FA
PUFA/FA
ApoB/ApoA1
$-\log_{10}$ adjusted p
$\log_2$ – fold change


a
Adolescent-limited
Adolescent-persistent
Adulthood-onset
Marker
WBC
RBC
Hb
Hct
MCV
MCH
MCHC
Platelets
Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Insulin
Glucose
HOMA-IR
Cholesterol
Triglycerides
HDL
LDL
VLDL
CRP
GGT
ALT
AST
AST/ALT
P3NP
Hyaluronic acid
0.8
1.0
1.2
Estimate (95% CI)
b
Adolescent-limited
Adolescent-persistent
Adulthood-onset
Marker
WBC
RBC
Hb
Hct
MCV
MCH
MCHC
Platelets
Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Insulin
Glucose
HOMA-IR
Cholesterol
Triglycerides
HDL
LDL
VLDL
CRP
GGT
ALT
AST
AST/ALT
P3NP
Hyaluronic acid
0.8
1.0
1.2
Estimate (95% CI)