nature medicine

Article

https://doi.org/10.1038/s41591-024-03279-x

Lipid profiling identifies modifiable signatures of cardiometabolic risk in children and adolescents with obesity

Received: 15 March 2024

Accepted: 30 August 2024

Published online: 20 September 2024

Check for updates

Yun Huang[1,10], Karolina Sulek[2,10], Sara E. Stinson[1], Louise Aas Holm[1,3], Min Kim[2], Kajetan Trost[1,2], Kourosh Hooshmand[2], Morten Asp Vonsild Lund[3,4], Cilius E. Fonvig[1,3,5], Helene Bæk Juel[1], Trine Nielsen[5,6], Lars Ängquist[1], Peter Rossing[2,5], Maja Thiele[7,8], Aleksander Krag[7,8], Jens-Christian Holm[1,3,5,11] ✉, Cristina Legido-Quigley[2,9,11] ✉ & Torben Hansen[1,11] ✉

Pediatric obesity is a progressive, chronic disease that can lead to serious cardiometabolic complications. Here we investigated the peripheral lipidome in 958 children and adolescents with overweight or obesity and 373 with normal weight, in a cross-sectional study. We also implemented a family-based, personalized program to assess the effects of obesity management on 186 children and adolescents in a clinical setting. Using mass spectrometry-based lipidomics, we report an increase in ceramides, alongside a decrease in lysophospholipids and omega-3 fatty acids with obesity metabolism. Ceramides, phosphatidylethanolamines and phosphatidylinositols were associated with insulin resistance and cardiometabolic risk, whereas sphingomyelins showed inverse associations. Additionally, a panel of three lipids predicted hepatic steatosis as effectively as liver enzymes. Lipids partially mediated the association between obesity and cardiometabolic traits. The nonpharmacological management reduced levels of ceramides, phospholipids and triglycerides, indicating that lowering the degree of obesity could partially restore a healthy lipid profile in children and adolescents.

Obesity, defined as an abnormal or excessive accumulation of fat mass that may impair health[1], can lead to serious cardiometabolic complications. The global prevalence of obesity in children and adolescents is on the rise, with projections indicating over 250 million will be affected by 2030 (ref. 2). While environmental changes are major contributors to this increase, genetic factors also play a significant role, with studies indicating a heritability rate of up to 67% (refs. 3,4). Obesity is not merely about excess fat accumulation; it leads to serious cardiometabolic complications. Increased body weight in children and adolescents can also result in prediabetes or diabetes, increased blood pressure of up to 25% among children with obesity and metabolic dysfunction-associated steatotic liver disease (MASLD), estimated to be 40% in children with body mass index (BMI) at or above 95th percentile[5]. Another critical aspect of obesity is the accumulation of adipose tissue and dyslipidemia, which is clinically manifested through altered lipid profiles such as increased cholesterol, low-density lipoprotein, triglycerides (TGs) and decreased high-density lipoprotein[6,7]. While these lipids are clinically validated, the number of lipid molecules in the human body is several orders of magnitude higher[8].

Lipid metabolism has been studied for decades with the aim to map their chemical diversity and functionality[9,10]. Recent advancements in mass spectrometry (MS) have enabled mapping of single

A full list of affiliations appears at the end of the paper. ✉e-mail: jhom@regionsjaelland.dk; cristina.legido_quigley@kcl.ac.uk; torben.hansen@sund.ku.dk

molecular structures[11]. In obesity, the lipidome profile is significantly altered and recent studies reported that sphingolipids and phosphatidylethanolamines (PE) are key drivers of cardiometabolic complications[12,13]. Despite these findings, there remains a gap in research specifically addressing pediatric health and the lipid profiles in children and adolescents with obesity.

Here, we performed MS-based plasma lipidomics and deep clinical phenotyping in children from the HOLBAEK study. This cross-sectional study includes 1,331 children and adolescents with normal weight, overweight or obesity[14,15]. Furthermore, the intervention study includes 186 children with overweight or obesity receiving the Holbaek obesity treatment, which is a family-based, individual-centered, comprehensive, nonpharmacological management with follow-up visits at the Children's Obesity Clinic, Holbaek Hospital, Denmark[14]. Our goal was to understand the role of lipid classes in pediatric obesity and to study single lipid associations of cardiometabolic risk profiles, including hepatic steatosis, dyslipidemia, insulin resistance, hyperglycemia and hypertension.

In our analysis, we identified plasma lipid signatures through 227 annotated lipids. These, when tested against cardiometabolic risk features such as hepatic steatosis, dyslipidemia, insulin resistance and cardiometabolic traits, related to liver function and glucose metabolism, indicating distinct class-wide lipid dynamics. Specifically, we observed that higher levels of ceramides (Cers), PE and phosphatidylinositols (PIs) were associated with worsened cardiometabolic risk profiles, whereas sphingomyelins (SMs) were protective and associated with reduced cardiometabolic risks. The mechanism behind the Cer production in the body is highly dependent on SM and fatty acids (FA)[16]. Moreover, SM depletion has been found to correlate with inflammation[12] and Cer in recent years has been linked with future development of cardiovascular disease (CVD)[17]. The rise in inflammatory cytokines and Cer in the circulation is proposed as a key mechanism in the development of atherosclerosis. Moreover, recent research has linked lipids from the PE and PI classes with conditions such as type 2 diabetes (T2D), CVD and steatotic liver disease, underscoring their significance in disease progression and potential treatment monitoring[18–22]. Our observations of the associations between these lipid species and cardiometabolic risk profiles in children and adolescents suggests that the impact of these lipids on metabolic disturbances emerges early in life.

The analysis of individual species demonstrated that children decreasing their degree of obesity exhibited decreased levels of TG, Cer, PE and PI species. Our findings suggest that specific lipids partly mediated cardiometabolic complications from obesity. Understanding which lipid molecules to target is key for potential interventions and treatments, thereby preventing the progression of pediatric obesity into severe complications.

Results

Study design and participant characteristics

The study population consisted of children and adolescents from the HOLBAEK study, previously known as the Danish Childhood Obesity Biobank[14,15], including an obesity clinic cohort, in which children and adolescents with a BMI ≥ 90th percentile (BMI standard deviation score (SDS) ≥ 1.28) according to a Danish ref. 23 participated in a multidisciplinary nonpharmacological obesity management program at the Children's Obesity Clinic, Holbæk Hospital; and a population-based cohort, recruited from schools across 11 municipalities in Zealand, Denmark.

Anthropometry, whole-body dual-energy X-ray absorptiometry (DXA) scan[24], proton magnetic resonance spectroscopy (^{1}H-MRS)[25] and blood parameters[26–32] were assessed as described previously. Untargeted lipidomic profiling was performed on 1,363 children and adolescents who had baseline examinations, as well as 186 participants who received obesity management at baseline and had a median follow-up duration of 1.1 years. We performed a cross-sectional analysis on 1,331 participants divided into normal weight (n = 373) and overweight/obesity groups (n = 958) and a longitudinal analysis on 186 children and adolescents with overweight or obesity. A schematic study design is shown in Fig. 1.

In the cross-sectional study, the overweight/obesity and normal weight groups differed significantly in anthropometrics and cardiometabolic risk profiles except for sex, lactate dehydrogenase (LDH) and hemoglobin A1c (HbA1c). Specifically, the overweight/obesity group had higher BMI SDS, waist, waist/hip ratio (WHR), body fat %, liver fat % and elevated levels of liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transferase (GGT). They also exhibited elevated levels of traditional lipids, including low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and TG, as well as higher glycemic parameters, including glucose, insulin, C-peptide and homeostasis model assessment of insulin resistance (HOMA-IR). Other notable differences included higher high-sensitivity C-reactive protein (hs-CRP), leptin, leptin/adiponectin ratio, glucagon, total glucagon-like peptide-1 (GLP-1), systolic blood pressure (SBP) SDS and diastolic blood pressure (DBP) SDS. They also had lower levels of high-density lipoprotein cholesterol (HDL-C) and adiponectin (Table 1). In comparison to the normal weight group, the overweight/obesity group exhibited a higher prevalence of hepatic steatosis defined as liver fat ≥ 5.0 % (15.6% versus 0%), high ALT (38.7% versus 12.6%), dyslipidemia (38.0% versus 10.8%), hyperglycemia (14.0% versus 8.6%), insulin resistance (35.0% versus 5.6%) and hypertension (15.9% versus 3.0%) (all $P < 0.05$).

Associations of lipid species with overweight or obesity

Differentially abundant lipids revealed a gradual change among the three weight statuses (normal weight, overweight and obesity), after adjusting for age and sex. Among 227 annotated lipid species, 142 exhibited significant differences across normal weight, overweight and obesity (P < 5% false discovery rate (FDR)). The pairwise comparisons revealed 121 lipids significantly differed between normal weight and obesity, 43 between overweight and normal weight and 60 between overweight and obesity (Fig. 2a).

Logistic regression analyses for normal weight versus overweight/obesity revealed 87 significant lipid species in 13 lipid classes, of which 52 were positively and 35 were negatively associated, independent of age and sex (P < 5% FDR). Notably, 20% measured Cers (3 of 15), 33% measured SMs (5 of 15), 47% TGs (25 of 53), all diacylglyceride (DG) (2 of 2) and 40% FAs (2 of 5) were positively associated, whereas the majority of glycerophospholipids, including 33% *N*, *N*-dimethyl-phosphatidylethanolamine (dMePE) (1 of 3), 30% lysophosphatidylcholine (LPC) (7 of 23) and 60% lysophosphatidyethanolamine (LPE) (3 of 5) displayed negative associations and PCs, PEs and PIs showed more divergent trends in associations: 14% and 20% PCs (9 and 13 of 64), 25% and 17% PEs (3 and 2 of 12) and 12% and 38% PIs (1 and 3 of 8) had significant positive and negative associations (Fig. 2b, Extended Data Fig. 1 and Supplementary Table 1).

The interaction of obesity with age related lipid species

A partial least squares-discriminant analysis (PLS-DA) score plot of lipids between three age groups in normal weight and overweight/obesity groups is shown in Fig. 3a,b. Three age groups (age group 1, girls aged <9 years and boys aged <10 years; age group 2, girls aged 9–15 years and boys aged 10–16 years; age group 3, girls aged >15 years and boys aged >16 years) were defined according to the approximate pubertal development[33]. Compared to the more pronounced separation between age groups 1 and 2 (larger than between age groups 2 and 3) in the normal weight group, age group 1 did not yield such a clear separation in the overweight/obesity group. We, therefore, assessed whether weight status modifies the association between continuous age and lipid species by including an interaction term

https://doi.org/10.1038/s41591-024-03279-x

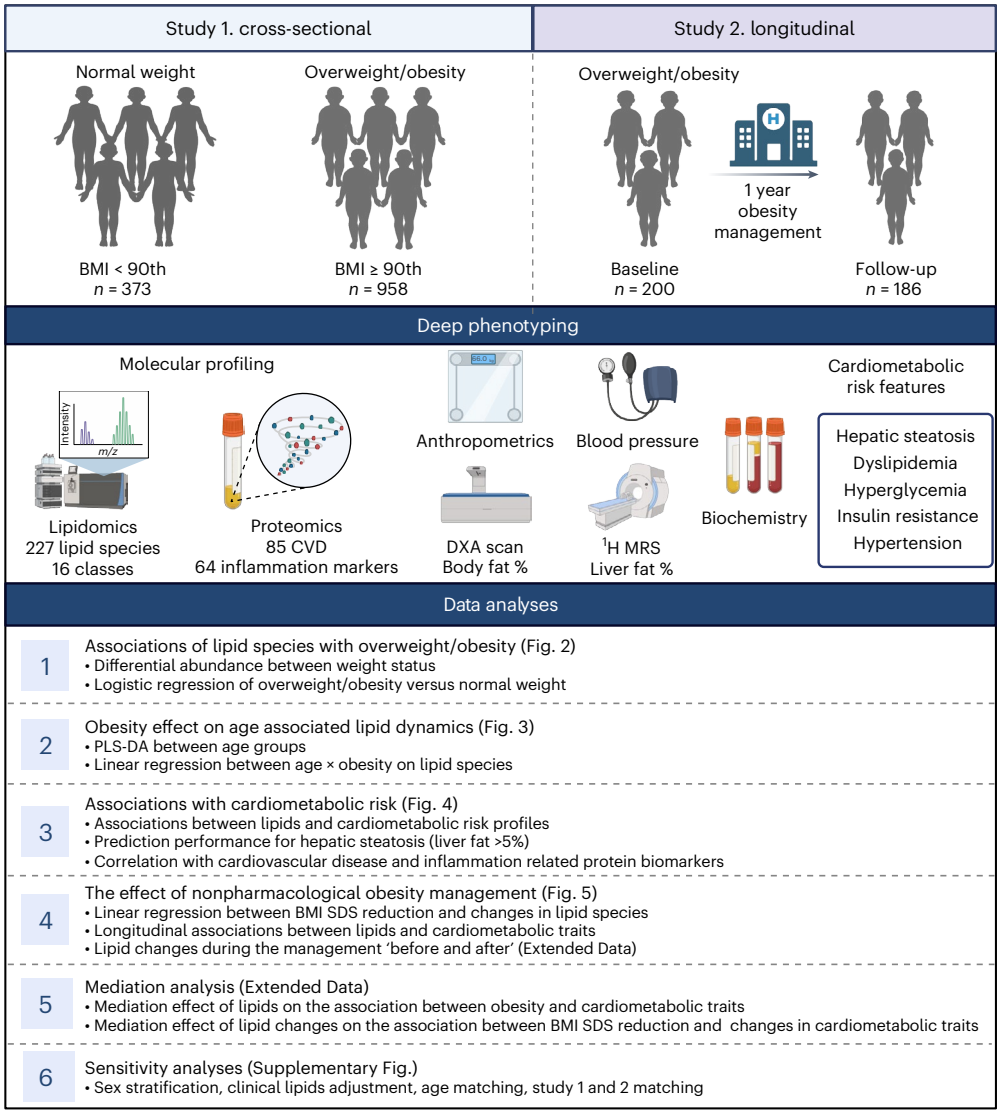


Fig. 1 | Schematic study design. Lipidomic profiles were measured in 373 children and adolescents with normal weight and 958 with overweight or obesity. We investigated lipid dysregulation in relation to overweight/obesity, cardiometabolic risk profiles, known CVD-related and inflammatory markers, and the predictive ability to detect hepatic steatosis. In addition, lipidomic profiles were measured in a subset of children and adolescents who received nonpharmacological obesity treatment. The lipidome response to BMI SDS reduction and longitudinal associations between lipids and cardiometabolic traits were examined. Mediation and sensitivity analyses were conducted. Created with BioRender.com.

((overweight/obesity versus normal weight) × age) for each lipid species, adjusting for sex. Significant interactions ($P < 0.05$) were detected in 26 lipid species, with lysophospholipids showing the most pronounced increase with age in the normal weight group compared to the overweight/obesity group, including lysodimethylphosphatidylethanolamine (LdMePE) (0:0 and 16:0) ($\beta = 0.40$ in normal weight versus $\beta = 0.23$ in overweight/obesity, $P = 0.005$), LdMePE (16:0 and 0:0) ($\beta = 0.33$ versus $\beta = 0.19$, $P = 0.016$), LPC (0:0 and 16:0) ($\beta = 0.39$ versus $\beta = 0.24$, $P = 0.007$), LPC (16:0 and 0:0) ($\beta = 0.38$ versus $\beta = 0.20$, $P = 0.001$) (Fig. 3c, Extended Data Fig. 2 and Supplementary Table 2). Two polyunsaturated FAs, FA(20:4) and FA(22:6), were decreased with age only in the overweight/obesity group. We detected statistically significant lower levels of these five lysophospholipids in age group 3 when comparing participants with normal weight to participants with overweight or obesity (Fig. 3d).

Considering the age gap between the overweight/obesity and normal weight groups, we matched individuals in the obesity group to the normal weight group by age and sex. The subanalysis confirmed a significant obesity interaction on lysophospholipids (Supplementary Fig. 1).

Associations of lipid species with cardiometabolic risk

A total of 135 lipids had at least one significant association ($P < 5\%$ FDR) with cardiometabolic risk feature after adjusting for age, sex and BMI SDS (Extended Data Fig. 3a and Supplementary Table 3). Seventeen sphingolipids, including 9 Cers, were associated with higher prevalence of hepatic steatosis, dyslipidemia and insulin resistance, whereas 8 SMs were associated with lower prevalence. Nine PEs and eight PIs were shown to have positive associations with hepatic steatosis, dyslipidemia and insulin resistance (Fig. 4a).

In total, 207 lipids had at least one significant association ($P < 5\%$ FDR) with cardiometabolic trait after adjusting for age, sex and BMI SDS (Extended Data Fig. 3b and Supplementary Table 4). We explored potential sex differences in the associations between lipid species and cardiometabolic traits, identifying 13 lipid species that demonstrated significant sex interactions ($P < 5\%$ FDR) with eight

https://doi.org/10.1038/s41591-024-03279-x

Table 1 | Characteristics of children and adolescents in the cross-sectional study

Group	*n*	Normal weight	*n*	Overweight/obesity	*P*
Sex, boys, *n* (%)	373	180 (48.3)	958	426 (44.5)	0.236
Age, years	373	8.35 (6.87–12.42)	958	11.90 (9.83–14.12)	<0.001
Age group, *n* (%)	373		958		<0.001
Age group 1		207 (55.5)		212 (22.1)	
Age group 2		126 (33.8)		612 (63.9)	
Age group 3		40 (10.7)		134 (14.0)	
BMI SDS	373	0.01 (−0.50–0.51)	958	2.87 (2.44–3.30)	<0.001
Body fat, %	93	24.34 (21.00–28.81)	745	43.90 (40.36–46.96)	<0.001
Waist, cm	263	62.90 (58.00–69.00)	884	91.50 (82.00–102.50)	<0.001
WHR	263	0.84 (0.80–0.89)	882	0.94 (0.90–0.99)	<0.001
Cardiometabolic traits					
Liver fat, %	32	0.50 (0.50–0.50)	454	1.00 (0.50–2.00)	<0.001
Plasma ALT, $U l^{-1}$	373	20.00 (17.00–24.00)	957	25.00 (20.00–32.00)	<0.001
Plasma AST, $U l^{-1}$	331	30.00 (25.00–35.00)	932	24.00 (20.00–30.00)	<0.001
Plasma GGT, $U l^{-1}$	373	15.00 (12.00–17.00)	956	17.00 (15.00–21.00)	<0.001
Plasma LDH, $U l^{-1}$	342	225.00 (193.00–252.00)	939	224.00 (193.00–254.50)	0.51
Plasma bilirubin, $\mu mol l^{-1}$	373	7.00 (5.00–9.00)	956	7.00 (6.00–10.00)	<0.001
Plasma HDL-C, $mmol l^{-1}$	372	1.50 (1.30–1.70)	957	1.20 (1.00–1.40)	<0.001
Plasma LDL-C, $mmol l^{-1}$	372	2.10 (1.80–2.50)	956	2.40 (2.00–2.80)	<0.001
Plasma TG, $mmol l^{-1}$	372	0.60 (0.40–0.70)	957	0.90 (0.70–1.30)	<0.001
Plasma TC, $mmol l^{-1}$	372	3.90 (3.50–4.40)	957	4.10 (3.60–4.60)	<0.001
Serum C-peptide, $nmol l^{-1}$	331	0.41 (0.31–0.54)	920	0.77 (0.57–1.01)	<0.001
HOMA-IR, $mIU l^{-1}$	212	1.06 (0.66–1.57)	934	3.13 (2.08–4.71)	<0.001
Serum insulin, $pmol l^{-1}$	366	39.15 (24.63–59.33)	946	83.72 (56.85–126.70)	<0.001
Plasma glucose, $mmol l^{-1}$	213	4.80 (4.50–5.00)	944	5.00 (4.80–5.30)	<0.001
Whole blood HbA1c, $mmol mol^{-1}$	372	34.00 (32.00–35.25)	953	34.00 (32.00–36.00)	0.35
Serum hs-CRP, $mg l^{-1}$	335	0.17 (0.06–0.49)	801	1.46 (0.50–3.91)	<0.001
Serum leptin, $ng l^{-1}$	334	4.96 (2.27–11.03)	812	29.54 (17.18–49.96)	<0.001
Serum adiponectin, $\mu g l^{-1}$	351	5.74 (3.50–9.01)	843	3.49 (2.36–5.21)	<0.001
Leptin:adiponectin ratio	334	0.90 (0.40, 2.50)	812	8.48 (4.37, 15.64)	<0.001
Plasma glucagon, $pmol l^{-1}$	366	5.98 (4.08–8.37)	939	8.47 (5.91–11.65)	<0.001
Plasma total GLP-1, $pmol l^{-1}$	367	2.81 (2.12–4.02)	946	3.07 (2.22–4.18)	0.028
SBP SDS	370	0.13 (−0.39–0.68)	891	0.73 (0.14–1.35)	<0.001
DBP SDS	370	−0.16 (−0.51–0.27)	891	0.27 (−0.15–0.71)	<0.001
Cardiometabolic risk features					
Liver fat ≥ 1.5%, *n* (%)	32	1 (3.1)	454	148 (32.6)	0.001
Liver fat ≥ 5.0%, *n* (%)	32	0 (0.0)	454	71 (15.6)	0.031
High ALT, *n* (%)	373	47 (12.6)	957	370 (38.7)	<0.001
Dyslipidemia, *n* (%)	372	40 (10.8)	957	364 (38.0)	<0.001
Hyperglycemia, *n* (%)	221	19 (8.6)	942	132 (14.0)	0.041
Insulin resistance, *n* (%)	180	10 (5.6)	919	322 (35.0)	<0.001
Hypertension, *n* (%)	370	11 (3.0)	891	142 (15.9)	<0.001

Data are expressed as median (IQR) or frequencies, *n* (%). Differences between the two groups were tested with the two-sided Wilcoxon rank-sum test or chi-squared test.

cardiometabolic traits (Supplementary Fig. 2). Sex-specific effect estimates are provided in Supplementary Table 5.

The associations between the 34 cardiometabolic risk feature-associated Cers, SMs, PEs and PIs with continuous cardiometabolic traits are shown (Fig. 4a). Specific Cers were positively associated with liver-related traits (liver fat %, ALT, AST and GGT), traditional lipids (LDL-C, TC and TG) and glycemic traits (C-peptide, HOMA-IR, insulin and glucose), glucagon and GLP-1, but not with hs-CRP or blood pressure. SMs showed negative associations with TG, liver and glycemic traits, leptin:adiponectin ratio and GLP-1. Furthermore, PIs and PEs were positively associated with liver and glycemic traits, leptin:adiponectin ratio and GLP-1. PIs were also linked to higher levels of hs-CRP,

https://doi.org/10.1038/s41591-024-03279-x

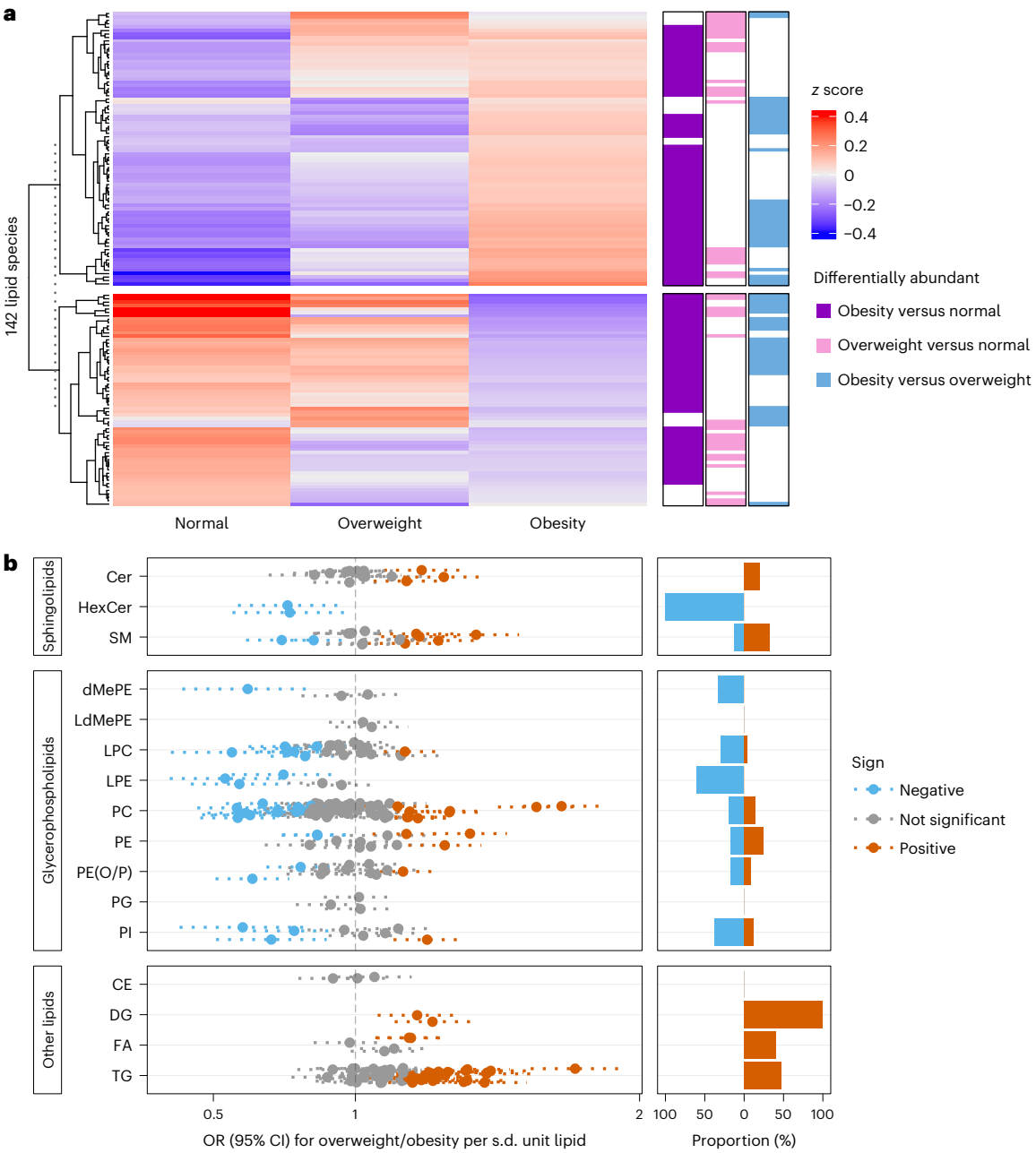


Fig. 2 | Overview of the plasma lipidome associated with overweight/obesity. **a**, Significant differences were identified in the mean normalized intensities of 142 lipid species across normal weight, overweight and obesity (n = 373, 192 and 766, respectively). Analysis was performed using an analysis of variance (ANOVA), with P values adjusted for multiple testing using the FDR method (P < 5% FDR). Three paired comparisons were subsequently conducted using Tukey's HSD test. **b**, Overall, 87 lipid species were associated with overweight/obesity compared to normal weight tested by logistic regression adjusted for age and sex (n = 958 and 373, P < 5% FDR). Bubble plot showing the odds ratio (OR) with error bars representing the 95% CI of lipid species in each lipid class. Gray circles denote nonsignificant associations. The proportion of significant associations (P < 5% FDR, orange denotes positive and blue denotes negative) in each lipid class are shown.

glucagon and DBP SDS. In a sensitivity analysis, further adjustment for TC and TG attenuated certain associations between lipids and liver and glycemic traits; however, the majority of distinct associations persisted (Supplementary Fig. 3)

We further tested the effect of weight status on the associations between the above-mentioned 34 Cers, SMs, PEs and PIs with cardiometabolic traits, adjusting for age and sex. Significant interactions were detected between 25 lipids with 14 traits (P < 5% FDR) (Extended Data Fig. 4 and Supplementary Table 6). In particular, significant associations were observed between Cers, SMs, PEs and PIs with ALT and GGT, SMs with insulin and PIs with hs-CRP, leptin, glucagon, GLP-1 and DBP SDS; a larger effect size in children and adolescents with overweight or obesity compared to participants with normal weight.

Predictive performance of lipids to detect hepatic steatosis

Given their clinical relevance, we explored the predictive potential of cardiometabolic-associated lipids for detecting hepatic steatosis, defined as liver fat above 5%. Employing feature selection techniques, we identified a three-lipid panel comprising PI(32:1), PE(36:1) and Cer(d42:0). This panel demonstrated a mean cross-validated receiver operating characteristic (ROC) area under the curve (AUC) of 0.79 (95% CI 0.77–0.81) through fivefold cross-validation repeated ten

https://doi.org/10.1038/s41591-024-03279-x

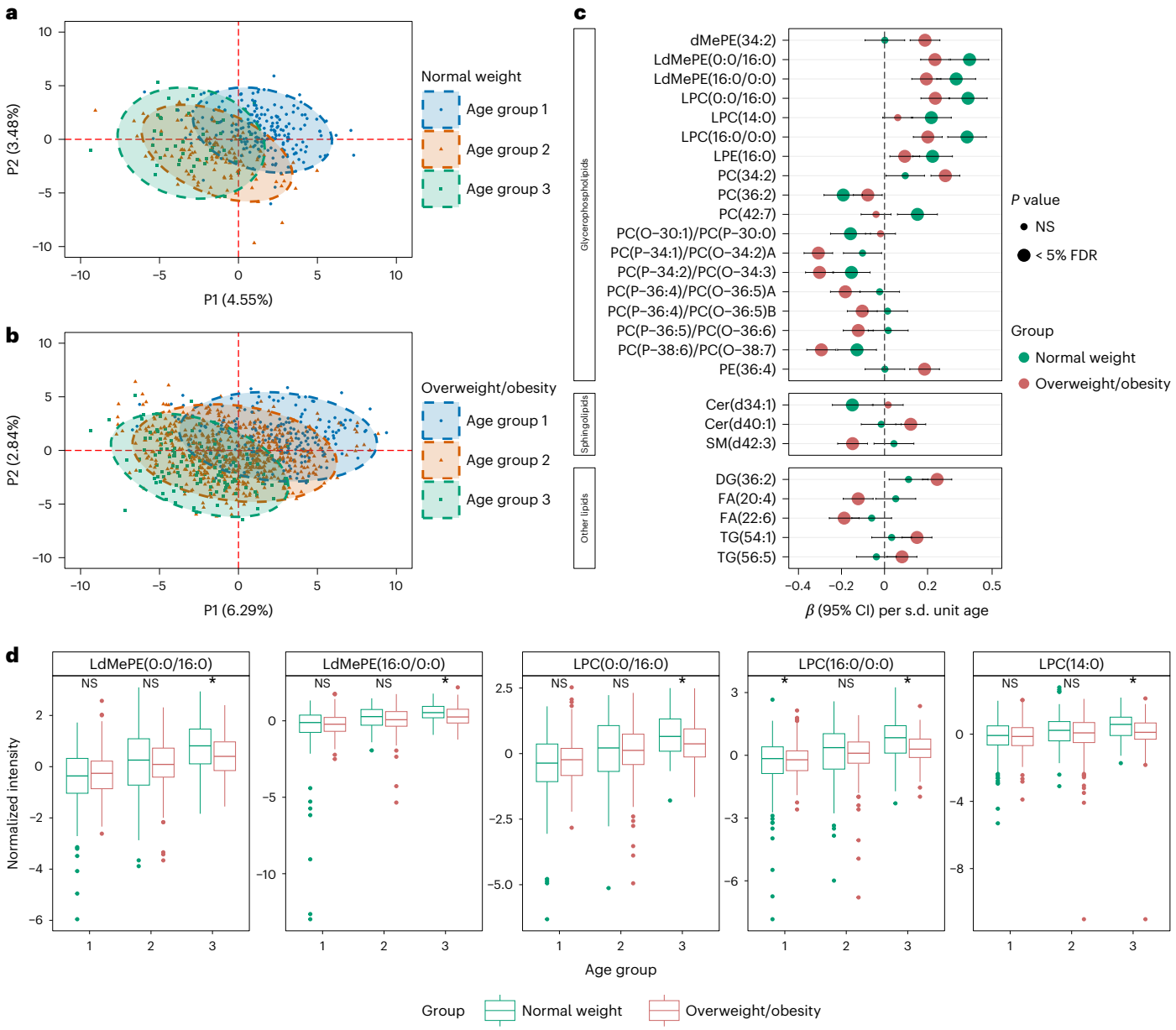


Fig. 3 | The interaction of obesity with age associated lipid species. a, PLS-DA score plot of lipid species in children with normal weight between three age groups. **b**, PLS-DA score plot of lipid species in children with overweight or obesity from three age groups. **c**, Associations between age and 26 lipid species that showed significant obesity (overweight/obesity versus normal weight) interaction ($P < 0.05$). Linear regression analysis was performed including an interaction term for obesity and adjusting for sex. The β-coefficients with error bars representing 95% CI were shown separately for the normal weight (green) and overweight/obesity group (red). $n = 958$ and 373 for overweight/obesity versus normal weight. **d**, Box plot showed the normalized intensities of five lysophospholipids that were most increased in normal weight children among three age groups. Data are presented as median values, box edges are IQR (25th to 75th percentiles) and whiskers represent 1.5 × IQR. An asterisk indicates a significant difference between two groups ($P < 0.05$). NS, not significant. $n = 207$, 126 and 40 and 212, 612 and 134 for age group 1, 2 and 3 in the normal weight and overweight/obesity group, respectively.

times (Fig. 4b). Furthermore, integration of this lipid panel with three liver enzymes (ALT, AST and GGT) significantly increased the AUC from 0.78 (95% CI 0.76–0.8) to 0.82 (95% CI 0.81–0.84) (as determined by DeLong's test, $P < 0.05$).

Correlations of lipids with CVD and inflammatory markers

Correlation between the 34 cardiometabolic-associated Cers, SMs, PEs and PIs with markers from CVD and INF panels revealed significant correlations between nine sphingolipids and ten CVD markers and Cer(d42:0) with inflammatory marker CDCP1. Additionally, 10 PEs and PIs correlated with 15 CVD markers and 6 PEs and PIs correlated with 6 inflammatory markers (Spearman correlation $r > 0.2$ and $P < 5\%$ FDR) (Fig. 4c and Supplementary Table 7). Positive correlations were detected between Cers, PEs and PIs with CVD markers, including FGF21, PRSS8, SPON2, HAOX1, LEP and ADM, whereas SMs were negatively correlated. PEs and PIs also correlated with inflammatory markers, including VEGFA, IL-18R1 and HGF, among others (Fig. 4d).

Mediation effect of lipids on cardiometabolic traits

We conducted mediation analysis to explore the role of 87 obesity-associated lipids on cardiometabolic traits, adjusting for age and sex. Overall, 83 lipids significantly mediated the effect of obesity on

19 cardiometabolic traits, with a median mediation proportion of 5% (Extended Data Fig. 5 and Supplementary Table 8). Notably, certain PCs and TGs exhibited particularly substantial mediation proportions, surpassing 20% in traditional lipid profiles. Specifically, TG(52:1) demonstrated partial mediation effects across cardiometabolic traits. Additionally, SMs, LPEs and LPCs were found to exert a negative mediation effect on the association between obesity and glycemic traits, with mediation proportions ranging from 5% to 23% for glucose.

The effect of personalized obesity management

A subset of 186 children and adolescents with overweight or obesity, comprising 84 boys and with a median age of 11.6 years (interquartile range (IQR) 9.9–13.7), underwent obesity management for a median duration of 1.1 years (IQR 1.0–1.2). Among them, 154 participants experienced a decrease in BMI SDS, whereas 32 maintained or increased their BMI SDS. Across all participants, there was a median reduction in BMI SDS of −0.39 (IQR −0.76 to −0.07) from baseline to follow-up, accompanied by a median decrease in body fat content of −2.85 (IQR −6.53 to −0.38) ($P < 0.001$). Furthermore, significant improvements were observed in WHR, LDH, TC, HDL-C, LDL-C, HbA1c and DBP SDS (all $P < 0.001$) (Extended Data Table 1). Liver enzymes (ALT, AST, GGT and bilirubin) and glycemic traits (C-peptide, insulin and glucose) did not show significant improvements in all participants or the subgroup with decreased BMI SDS, but worsened in those who increased BMI SDS.

Before investigating the lipidome response to obesity management, we examined the baseline associations of BMI SDS with lipids and how these associations evolved with BMI SDS reduction. Utilizing the baseline data from the intervention study as a replication set, we found that 25 of 58 lipids associated with BMI SDS in children with overweight/obesity from the cross-sectional study ($P < 5\%$ FDR, $n = 958$) also exhibited significant and consistent associations at baseline in the intervention study ($P < 5\%$ FDR, $n = 186$). Additionally, 24 lipids showed directionally consistent trends but did not reach statistical significance (Supplementary Fig. 4).

A comparison of lipid profiles before and after obesity management revealed significant changes. Among the 145 lipids examined, significant increases were observed in 44 lipids, including PCs, LPCs and LPEs ($P < 5\%$ FDR). In contrast, 23 lipids, including Cers, SM and TGs were significantly decreased (Extended Data Fig. 6 and Supplementary Table 9).

Furthermore, we investigated the lipidome changes in response to continuous BMI SDS reduction and their associations with cardiometabolic traits. A total of 62 lipid species demonstrated significant associations with BMI SDS reduction, out of which 45 were also significantly associated with BMI SDS at baseline ($P < 5\%$ FDR). Of note, TGs exhibited the greatest reduction following BMI SDS reduction. Additionally, nine sphingolipids including Cers and SMs and four glycerophospholipids, including PEs and PIs were significantly decreased (Fig. 5a, Extended Data Fig. 7 and Supplementary Table 10).

Longitudinal analyses revealed associations between changes in Cers, SMs, PEs and PIs families with changes in cardiometabolic traits that were independent of age, sex, treatment duration, baseline BMI SDS and change in BMI SDS at nominal significance ($P < 0.05$) (Fig. 5b, Extended Data Fig. 8 and Supplementary Table 11). Changes in these cardiometabolic-associated lipid profiles were significantly associated with changes in traditional lipids. Changes in Cer(d42:0) and Cer(d42:1) were positively associated with changes in ALT at a nominal significance level. Changes in SM(d36:1) and SM(d36:2) were associated with changes in bilirubin and negatively associated with changes in HOMA-IR and glucose. Longitudinal positive associations were observed between certain PEs and PIs with ALT, HOMA-IR and glucose.

Mediation effect of lipid changes on cardiometabolic traits

We investigated whether changes in lipid species potentially mediate the relationship between reductions in BMI SDS and changes in cardiometabolic traits. A reduction in BMI SDS was associated with changes in 11 cardiometabolic traits and changes in 70 lipids. Out of 253 tested paths, 216 paths exhibited a significant indirect effect, with a median proportion of 23% (Extended Data Fig. 9 and Supplementary Table 12). Changes in 65 lipids significantly mediated the association of BMI SDS reduction and changes in traditional lipids, with mediation proportions ranging from 7% to 60%. Furthermore, changes in Cer(d42:0) mediated an 18% reduction in ALT, while some PCs and TGs mediated improvements in HOMA-IR and insulin levels, with proportions ranging from 9% to 26%.

To consolidate the intervention results a subset of lipids ($n = 25$) from seven lipid classes replicated with baseline BMI SDS using data from overweight/obesity group in the cross-sectional and baseline data from children with obesity in the intervention study were selected. Twenty-two of these lipids in six lipid classes were significantly ($P < 5\%$ FDR) decreased with BMI SDS reduction. Of these, 21 lipids mediated changes in cardiometabolic traits (Fig. 5c and Supplementary Table 13).

Discussion

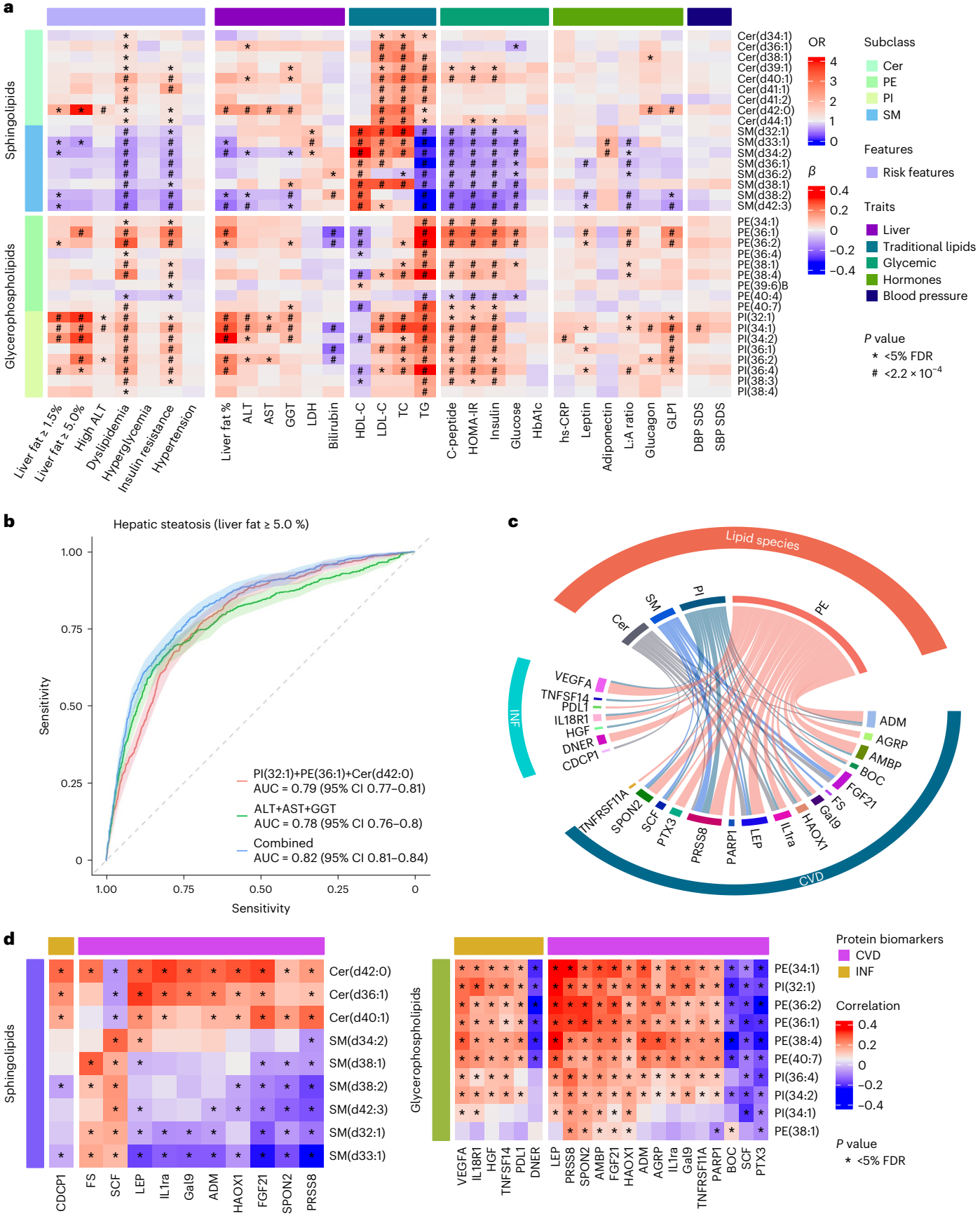
Obesity impacts multiple organs[34]. In our study, children and adolescents with overweight or obesity were heavily burdened by cardiometabolic risk. Nearly 38% had dyslipidemia, over 30% displayed hepatic steatosis and over 15% exhibited hypertension.

We found elevated plasma concentrations of Cer and TG in children and adolescents with overweight or obesity. Cer lipid molecules, emerging as early biomarkers for CVD[35], play crucial roles in cellular stress, inflammation signaling and apoptosis[36,37]. Large TG, particularly those containing 54 and 56 carbons and little FA unsaturation, were elevated; however, more evidence is needed to understand specific TG biological function in obesity[38].

Our investigation also explored lipidome variations across age in children and adolescents with obesity compared to normal-weight individuals. The main driver in age-stratification was phospholipid metabolism. We observed reductions in the levels of LdMePE(16:0), LPC(16:0) and LPC(14:0). While little is known about specific FA chains in lysophospholipids, these are active lipids involved in FA transport in the brain[39]. Furthermore, children with obesity had depleted levels of omega-3 FAs, such as docosahexaenoic acid, indicating a diet poor in essential FAs[40] and potentially impacting availability of docosahexaenoic acid in the brain. The implications of these alterations for

Fig. 4 | Associations of lipid species with cardiometabolic risk. a, The 34 lipids including Cer, SM, PE and PI species having at least one significant association with one cardiometabolic risk feature ($P < 5\%$ FDR). Logistic regression analysis was performed adjusting for age, sex and BMI SDS. Their associations with cardiometabolic traits tested by linear regression are shown in parallel. **b**, The discriminant accuracy of three lipids and liver enzymes for diagnosing hepatic steatosis, defined as liver fat ≥5.0%. The analysis includes data from 479 participants, among whom 71 cases of hepatic steatosis were identified. Each curve is accompanied by its corresponding 95% CI, depicted as a shaded area. The mean AUC values with their respective 95% CI are also provided for each ROC curve. **c**, Correlations of these cardiometabolic-associated lipid species with CVD and inflammation (INF)-related protein biomarkers were calculated using two-sided Spearman correlation. The size of the link represents the number of significant correlations (Spearman $r > 0.2$ and $P < 5\%$ FDR). Nine sphingolipids correlated with ten CVD markers, one sphingolipid correlated with one INF marker. Ten PEs and PIs correlated with 15 CVD markers and six PEs and PIs correlated with six INF markers. **d**, Two-sided Spearman correlations are shown. $^{*}P < 5\%$ FDR; $^{\#}P < 2.2 \times 10^{-4}$. The sample size ($n$) for each feature/trait is listed in Table 1; the maximum observed is 1,330.

https://doi.org/10.1038/s41591-024-03279-x



https://doi.org/10.1038/s41591-024-03279-x

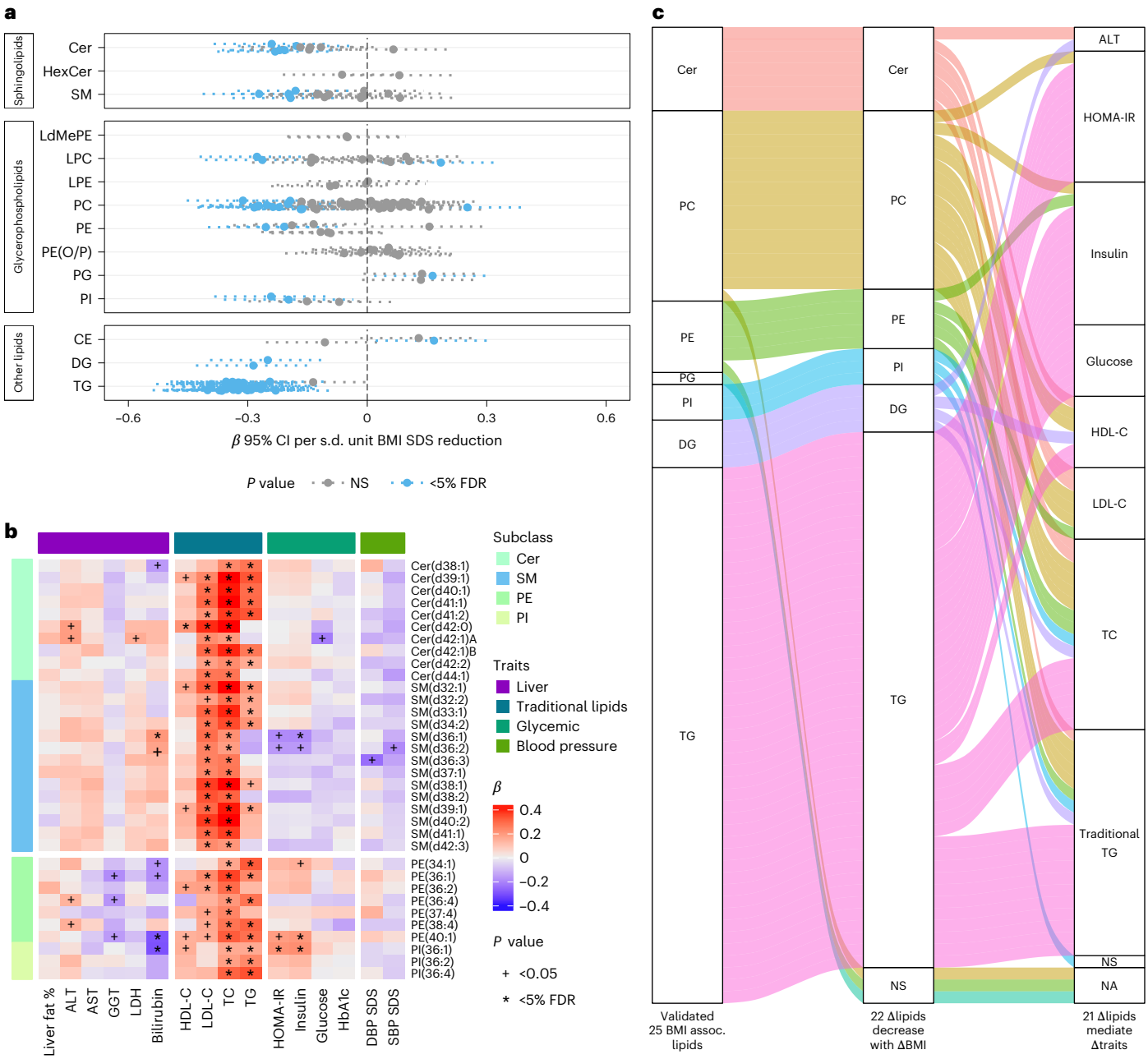


Fig. 5 | The effect of nonpharmacological obesity management. a, BMI SDS reduction was associated with changes in 62 lipids tested by linear regression, adjusting for age, sex and treatment duration ($n = 186$, $P < 5\%$ FDR). The β-coefficients with error bars representing 95% CI are shown. **b**, Changes in Cers, SMs, PEs and PIs were associated with changes in cardiometabolic traits tested by linear regression, adjusting for age, sex, treatment duration, baseline BMI SDS and change in BMI SDS ($P < 5\%$ FDR). BMI SDS reduction was calculated as the difference between BMI SDS at baseline and BMI SDS at follow-up. Changes in lipid profiles and cardiometabolic traits were calculated as the difference between the values at follow-up and those at baseline. $^{+}P < 0.05$; $^{*}P < 5\%$ FDR. **c**, Alluvial plot to illustrate the overall lipid class inter cohort validation, response to weight loss and proportion of mediation links: 25 validated lipids in seven lipid classes associated with baseline BMI SDS using data from overweight/obesity group in the cross-sectional ($n = 958$) and baseline data from children with obesity ($n = 186$) in the intervention study (left). Twenty-two of these lipids in six classes were significantly ($P < 5\%$ FDR) decreased with BMI SDS reduction (middle). The significant mediator role of changes in these lipids in the association between changes in BMI SDS and changes in cardiometabolic traits (right, $n = 21$). The colors of curved lines represent different lipid classes. NA indicates that three lipids, which did not significantly change with BMI SDS reduction, were not applicable for mediation effect. NS, nonsignificant indirect effect from mediation analysis. The sample size (n) for each trait is listed in Supplementary Table 9; the maximum observed is 185.

developmental outcomes like puberty onset and cognitive development warrant further dedicated research[41,42].

Integration of lipidomics with cardiometabolic risk profiles revealed that increased Cer and decreased SM were associated with dyslipidemia and insulin resistance. C-peptide, HOMA-IR, insulin and glucose levels, correlated with Cer level and negatively with SM. SM and Cer are closely linked metabolically and elevated Cer in blood is linked to a risk of developing T2D[43], hepatic steatosis[44] and CVD[45]. Although the mechanisms are still elusive, general inflammatory signals such as cytokines are thought to upregulate Cer synthesis[46]. Cer also correlated

with FGF21, a reported CVD biomarker[47] and the inflammatory cytokine CDCP1, which has been linked with myocardial infarction and nonalcoholic steatohepatitis[48,49].

Increased levels of specific PIs and PEs were associated with hepatic steatosis in children and adolescents. Notably, PI(32:1) showed a strong association with liver traits and inflammatory cytokines. A previous study involving 42 children with obesity reported links between hepatic steatosis and elevated levels of PEs[50], as have adult MASLD studies[21,22]. We therefore compared the diagnostic accuracy of a three-lipid panel with that of liver enzymes (ALT, AST and GGT) in assessing hepatic steatosis in children. The lipid panel performance was in line with liver enzymes, and integrating enzymes and lipids yielded 82% overall diagnostic accuracy, highlighting the potential for routine clinical evaluations.

We examined the mediating effects of lipids on the association between obesity and cardiometabolic outcomes and found that most lipids demonstrated partial effects on cardiometabolic traits. Notably, certain TGs mediated effects across glycemic and liver traits, whereas SMs exhibited a negative mediation effect on glycemic traits, by lowering glucose levels. These findings suggest that SMs, LPEs and LPCs may play a protective role in regulating glucose homeostasis in children.

Our intervention study aimed to reduce the degree of obesity and cardiometabolic risk, with 83% of participants reducing their BMI SDS. The clinical profile improved, including decreases in total body and liver fat, circulating lipoproteins and blood pressure; however, circulating total TG did not change, though specific TGs investigated with lipidomics did. Approximately 17% participants did not reduce their BMI SDS and this group exhibited increased circulating C-peptide levels and potential prediabetes risk[51].

Dietary and exercise interventions have long been recognized as tools to improve dyslipidemia in obesity[52]. In our study, the overall lipidome changes were clear: BMI SDS reduction was associated with reductions in all lipid classes. TGs were drastically reduced, while cardiometabolic-associated Cer, PE and PI also decreased with BMI SDS reduction. Particularly Cer(42:0) and Cer(40:1), linked to all-cause mortality in adults with diabetes[45], decreased in response to BMI SDS reduction. Changes in Cer were associated with changes in ALT levels after adjusting for baseline BMI SDS and change in BMI SDS, indicating improved liver function. Changes in PE(40:1) and PI(36:1) were associated with changes in HOMA-IR and insulin levels, suggesting a potential role in modulating insulin resistance.

Mediation analysis revealed that changes in 66 lipids partially affected cardiometabolic traits, with an average mediation proportion of 23%. Changes in Cer(d42:0) partially mediated reduced ALT levels, whereas changes in phosphatidylcholine (PC) and TG mediated improvements in HOMA-IR and insulin levels; however, given the smaller cohort size of the intervention group, we acknowledge the limitations of relying on nominal significance.

Overall, our findings emphasize that lipid dysregulation and potential lipid-mediated damage can be reversed through personalized, clinically based obesity management in children and adolescents. However, given the heterogeneous nature of obesity, pharmacological interventions might be necessary for specific lipids if lifestyle management is ineffective, as seen with liraglutide lowering Cer(42:1) independently of weight loss in adults[53].

Limitations of this study include the lack of ethnic diversity, as it was conducted in a majority white pediatric population. Genetic factors, diet and exercise likely impacted the baseline lipidome. The steatosis liver predictor was calculated in 71 cases and a larger diagnostic group is needed for validation. Notably, without a control group it was not possible to definitively attribute changes in lipid species solely to obesity management. A strength of this study was the inclusion of two large cohorts of deeply phenotyped children and adolescents from a population-based study.

In conclusion, lipidomics profiling has highlighted lipids potentially involved in the disease pathology of childhood obesity and associated with cardiometabolic complications. Personalized obesity management can beneficially modify the overall lipidome in children and adolescents.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03279-x.

References

1. World Health Organization. *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (2024).
2. Jebeile, H., Kelly, A. S., O'Malley, G. & Baur, L. A. Obesity in children and adolescents: epidemiology, causes, assessment, and management. *Lancet Diabetes Endocrinol.* **10**, 351–365 (2022).
3. Loos, R. J. F. & Yeo, G. S. H. The genetics of obesity: from discovery to biology. *Nat. Rev. Genet.* **23**, 120–133 (2022).
4. Maes, H. H., Neale, M. C. & Eaves, L. J. Genetic and environmental factors in relative body weight and human adiposity. *Behav. Genet.* **27**, 325–351 (1997).
5. Vajravelu, M. E., Tas, E. & Arslanian, S. Pediatric obesity: complications and current day management. *Life* https://doi.org/10.3390/life13071591 (2023).
6. Stewart, J., McCallin, T., Martinez, J., Chacko, S. & Yusuf, S. Hyperlipidemia. *Pediatrics Rev.* **41**, 393–402 (2020).
7. Mooradian, A. D. Dyslipidemia in type 2 diabetes mellitus. *Nat. Clin. Pract. Endocrinol. Metab.* **5**, 150–159 (2009).
8. Wenk, M. R. The emerging field of lipidomics. *Nat. Rev. Drug Discov.* **4**, 594–610 (2005).
9. Han, X. Lipidomics for studying metabolism. *Nat. Rev. Endocrinol.* **12**, 668–679 (2016).
10. Han, X. The emerging role of lipidomics in prediction of diseases. *Nat. Rev. Endocrinol.* **18**, 335–336 (2022).
11. Leaptrot, K. L., May, J. C., Dodds, J. N. & McLean, J. A. Ion mobility conformational lipid atlas for high confidence lipidomics. *Nat. Commun.* **10**, 985 (2019).
12. Thiele, M. et al. Sphingolipids are depleted in alcohol-related liver fibrosis. *Gastroenterology* https://doi.org/10.1053/j.gastro.2023.02.023 (2023).
13. Cadby, G. et al. Comprehensive genetic analysis of the human lipidome identifies loci associated with lipid homeostasis with links to coronary artery disease. *Nat. Commun.* **13**, 3124 (2022).
14. Holm, J.-C. et al. Chronic care treatment of obese children and adolescents. *Int. J. Pediatr. Obes.* **6**, 188–196 (2011).
15. Lausten-Thomsen, U. et al. Reference values for serum total adiponectin in healthy non-obese children and adolescents. *Clin. Chim. Acta* **450**, 11–14 (2015).
16. Galadari, S., Rahman, A., Pallichankandy, S., Galadari, A. & Thayyullathil, F. Role of ceramide in diabetes mellitus: evidence and mechanisms. *Lipids Health Dis.* **12**, 98 (2013).
17. Peterson, L. R. et al. Ceramide remodeling and risk of cardiovascular events and mortality. *J. Am. Heart Assoc.* https://doi.org/10.1161/JAHA.117.007931 (2018).
18. Eichelmann, F. et al. Deep lipidomics in human plasma: cardiometabolic disease risk and effect of dietary fat modulation. *Circulation* **146**, 21–35 (2022).
19. Miao, G. et al. Longitudinal plasma lipidome and risk of type 2 diabetes in a large sample of American Indians with normal fasting glucose: the Strong Heart Family study. *Diabetes Care* **44**, 2664–2672 (2021).

20. Huynh, K. et al. High-throughput plasma lipidomics: detailed mapping of the associations with cardiometabolic risk factors. *Cell Chem. Biol.* **26**, 71–84.e4 (2019).
21. Ooi, G. J. et al. Hepatic lipidomic remodeling in severe obesity manifests with steatosis and does not evolve with non-alcoholic steatohepatitis. *J. Hepatol.* **75**, 524–535 (2021).
22. Ma, D. W. L. et al. Plasma phospholipids and fatty acid composition differ between liver biopsy-proven nonalcoholic fatty liver disease and healthy subjects. *Nutr. Diabetes* **6**, e220 (2016).
23. Nysom, K., Mølgaard, C., Hutchings, B. & Michaelsen, K. F. Body mass index of 0 to 45-y-old Danes: reference values and comparison with published European reference values. *Int. J. Obes. Relat. Metab. Disord.* **25**, 177–184 (2001).
24. Nielsen, T. R. H. et al. Childhood obesity treatment: effects on BMI SDS, body composition, and fasting plasma lipid concentrations. *PLoS ONE* **13**, e0190576 (2018).
25. Chabanova, E., Fonvig, C. E., Bøjsøe, C., Holm, J.-C. & Thomsen, H. S. 1H MRS assessment of hepatic fat content: comparison between normal- and excess-weight children and adolescents. *Acad. Radiol.* **24**, 982–987 (2017).
26. Johansen, M. J. et al. The effect of overweight and obesity on liver biochemical markers in children and adolescents. *J. Clin. Endocrinol. Metab.* https://doi.org/10.1210/clinem/dgz010 (2020).
27. Frithioff-Bøjsøe, C. et al. Glucose metabolism in children and adolescents: population-based reference values and comparisons to children and adolescents enrolled in obesity treatment. *Pediatr. Diabetes* **20**, 538–548 (2019).
28. Stinson, S. E. et al. Hyperglucagonemia in pediatric adiposity associates with cardiometabolic risk factors but not hyperglycemia. *J. Clin. Endocrinol. Metab.* https://doi.org/10.1210/clinem/dgac108 (2022).
29. Nielsen, T. R. H. et al. Dyslipidemia and reference values for fasting plasma lipid concentrations in Danish/North-European white children and adolescents. *BMC Pediatr.* **17**, 116 (2017).
30. Frithioff-Bøjsøe, C. et al. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. *Pediatr. Diabetes* **21**, 194–202 (2020).
31. Stinson, S. E. et al. Fasting Plasma GLP-1 is associated with overweight/obesity and cardiometabolic risk factors in children and adolescents. *J. Clin. Endocrinol. Metab.* **106**, 1718–1727 (2021).
32. Lund, M. A. V. et al. Low-grade inflammation independently associates with cardiometabolic risk in children with overweight/obesity. *Nutr. Metab. Cardiovasc. Dis.* **30**, 1544–1553 (2020).
33. Juul, A. et al. Pubertal development in Danish children: comparison of recent European and US data. *Int. J. Androl.* **29**, 247–255 (2006).
34. Caprio, S., Santoro, N. & Weiss, R. Childhood obesity and the associated rise in cardiometabolic complications. *Nat. Metab.* **2**, 223–232 (2020).
35. Jensen, P. N. et al. Circulating ceramides and sphingomyelins and the risk of incident cardiovascular disease among people with diabetes: the strong heart study. *Cardiovas. Diabetol.* **21**, 167 (2022).
36. Bikman, B. T. & Summers, S. A. Ceramides as modulators of cellular and whole-body metabolism. *J. Clin. Investig.* **121**, 4222–4230 (2011).
37. Levade, T. et al. Sphingolipid mediators in cardiovascular cell biology and pathology. *Circ. Res.* **89**, 957–968 (2001).
38. Astrup, A. et al. Saturated fats and health: a reassessment and proposal for food-based recommendations. JACC state-of-the-art review. *J. Am. Coll. Cardiol.* **76**, 844–857 (2020).
39. Chua, G.-L. et al. Mfsd2a utilizes a flippase mechanism to mediate omega-3 fatty acid lysolipid transport. *PNAS* **120**, e2215290120 (2023).
40. Madore, C. et al. Essential omega-3 fatty acids tune microglial phagocytosis of synaptic elements in the mouse developing brain. *Nat. Commun.* **11**, 6133 (2020).
41. Leonibus et al. Timing of puberty and physical growth in obese children: a longitudinal study in boys and girls. *Pediatr. Obes.* **9**, 292–299 (2014).
42. Reinehr, T. & Roth, C. L. Is there a causal relationship between obesity and puberty? *Lancet Child Adolesc. Health* **3**, 44–54 (2019).
43. Turpin, S. M. et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* **20**, 678–686 (2014).
44. Xie, C. et al. Activation of intestinal hypoxia-inducible factor 2α during obesity contributes to hepatic steatosis. *Nat. Med.* **23**, 1298–1308 (2017).
45. Wretlind, A. et al. Ceramides as risk markers for future cardiovascular events and all-cause mortality in long-standing type 1 diabetes. *Diabetes* **72**, 1493–1501 (2023).
46. Teichgräber, V. et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat. Med.* **14**, 382–391 (2008).
47. Geng, L., Lam, K. S. L. & Xu, A. The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic. *Nat. Rev. Endocrinol.* **16**, 654–667 (2020).
48. Valdes-Marquez, E., Clarke, R., Hill, M., Watkins, H. & Hopewell, J. C. Proteomic profiling identifies novel independent relationships between inflammatory proteins and myocardial infarction. *Eur. J. Prevent. Cardiol.* **30**, 583–591 (2023).
49. Jia, X. et al. Identification and multicentric validation of soluble CDCP1 as a robust serological biomarker for risk stratification of NASH in obese Chinese. *Cell Rep. Med.* https://doi.org/10.1016/j.xcrm.2023.101257 (2023).
50. Draijer, L. G. et al. Lipidomics in nonalcoholic fatty liver disease: exploring serum lipids as biomarkers for pediatric nonalcoholic fatty liver disease. *J. Pediatr. Gastroenterol. Nutr.* **71**, 433–439 (2020).
51. Hassan, A. A. et al. Urinary C-peptide and urinary C-peptide creatinine ratio as markers for insulin resistance in obese children and adolescents. *Pediatr. Res.* **92**, 805–809 (2022).
52. Bray, G. A., Frühbeck, G., Ryan, D. H. & Wilding, J. P. H. Management of obesity. *Lancet* **387**, 1947–1956 (2016).
53. Wretlind, A. et al. Ceramides are decreased after liraglutide treatment in people with type 2 diabetes: a post hoc analysis of two randomized clinical trials. *Lipids Health Dis.* **22**, 160 (2023).

[1]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark. [2]Steno Diabetes Center Copenhagen, Copenhagen, Denmark. [3]The Children's Obesity Clinic, accredited European Centre for Obesity Management, Department of Paediatrics, Copenhagen University Hospital Holbæk, Holbæk, Denmark. [4]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [5]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [6]Medical Department, Zealand University Hospital, Roskilde, Denmark. [7]Center for Liver Research, Department of Gastroenterology and Hepatology, Odense University Hospital, Odense, Denmark. [8]Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark. [9]Institute of Pharmaceutical Science, King's College London, London, United Kingdom. [10]These authors contributed equally: Yun Huang, Karolina Sulek. [11]These authors jointly supervised this work: Jens-Christian Holm, Cristina Legido-Quigley, Torben Hansen. ✉e-mail: jhom@regionsjaelland.dk; cristina.legido_quigley@kcl.ac.uk; torben.hansen@sund.ku.dk

Methods

Ethics

According to the Declaration of Helsinki, written informed consent was obtained from all participants. An informed oral assent was given by the participant if the participant was younger than 18 and then the parents gave informed written consent. The study was approved by the Ethics Committee of Region Zealand, Denmark (SJ-104) and by the Danish Data Protection Agency (REG-043-2013). The HOLBAEK study is registered at ClinicalTrials.gov (NCT00928473).

Study population

Both cohorts from the HOLBAEK study were enrolled between January 2009 and April 2019. Among 1,363 children and adolescents with available lipidome profiles in the cross-sectional study, participants were excluded based on diagnosed type 1 diabetes mellitus or T2D ($n = 3$); intake of medications including statins, insulin, metformin and liraglutide ($n = 3$); meeting T2D criteria based on blood sample (fasting plasma glucose ≥ 7.0 mmol l^{-1} and/or HbA1c ≥ 48 mmol mol^{-1}) ($n = 2$); the interval between visit and blood sample collection >90 days ($n = 12$); underweight (BMI < 5th percentile (BMI SDS < −1.64)) ($n = 12$). As a result, 1,331 participants were included in the cross-sectional analysis and divided into normal weight (BMI ≥ 5th and BMI < 90th percentile (BMI SDS ≥ −1.64 and <1.28), $n = 373$) and overweight/obesity (BMI ≥ 90th percentile (BMI SDS ≥ 1.28), $n = 958$) groups. Participants with obesity (BMI ≥ 99th percentile (BMI SDS ≥ 2.33), $n = 766$) were further stratified as indicated.

Children and adolescents with overweight or obesity (BMI SDS ≥ 1.28) enrolled in the multidisciplinary, family-based and individual-centered obesity clinic cohort received comprehensive management using an evidence-based treatment protocol which comprises a range of recommendations on nutrition, including meal exercises, picky eating, exercise, inactivity, border setting promoting growth, development and improved physical, mental and social thriving, as previously described[14]. The intervention study included 186 children and adolescents with overweight or obesity, who were followed for a median of 1.1 years (IQR 1.0–1.2). Their lipidomic profiles were available at both baseline and follow-up.

Anthropometric measurements

In the obesity clinic cohort, anthropometrics were obtained at clinical examinations, whereas the population-based group was assessed in a mobile laboratory by medical professionals[31]. Weight, height, waist and WHR were measured. BMI SDS was calculated based on a Danish ref. 23. For SBP and DBP, mean values for the last two measurements of blood pressure were calculated and converted to blood pressure SDS based on age-, sex- and height-specific reference values from the American Academy of Pediatrics[54].

Lipidomics

EDTA plasma or serum sample preparation for lipidomic analysis has been described previously[55]. In brief, 10 µl plasma was mixed with 10 µl 0.9% *w*/*v* NaCl(aq) and internal standards containing 120 µl chloroform:methanol (2:1) mixture. The lipid containing chloroform was analyzed using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight MS (Agilent LC-Q-TOF 6200 with MassHunter Data Acquisition v.B.09.00). Samples were analyzed in a randomized order with quality control pooled plasma samples at regular intervals throughout the run. The lipidomics data were pre-processed with MZmine2 (ref. 56) and lipid features were normalized to internal standards and log transformed. The data were cross matched with an in-house library where 227 lipid features from 16 different lipid classes were identified at level 1 and 2 (ref. 57). We excluded lipids with >20% missing data across all samples and relative standard deviation (RSD) values > 20% across quality control samples. Serum lipidomics raw files from the intervention study were pre-processed with Skyline v.22.2.0.351 (ref. 58), where a list of targets were generated from the in-house library applied to the first study. Lipids were normalized to internal standards and log transformed. Features with RSD > 20% were excluded and only 145 lipids found in the cross-sectional study were considered in the intervention analysis.

The identified lipids from both studies were standardized to have a mean of 0 and s.d. of 1. Lipids were classified into classes: cholesteryl ester (CE), Cer, DG, dMePE, FA, hexosylceramides (HexCer), LdMePE, LPC, LPE, PC, PE, alkyl or alkenyl ether PEs (PE-O/P), phosphatidylglycerol (PG), PI, SM and TG. In particular, lipid species were classified into three major classes: sphingolipids (Cer, HexCer and SM), glycerophospholipids (dMePE, LdMePE, LPC, LPE, PC, PE, PE-O/P, PG and PI) and other lipids (CE, DG, TG and FA).

DXA examination

Whole-body DXA scans were performed and total body fat percentage was quantified in the overweight/obesity ($n = 745$), normal weight ($n = 93$) groups and 125 children and adolescents with overweight or obesity who received the obesity management, using a GE Lunar Prodigy (DF+10031, GE Healthcare) until October 2009 and thereafter using a GE Lunar iDXA (ME+200179, GE Healthcare)[24].

^{1}H-MRS examination

Liver fat content was quantified in the overweight/obesity ($n = 454$) and normal weight ($n = 32$) groups and 100 children and adolescents with overweight or obesity received obesity management, using a 3T Achieva MR imaging system (Philips Medical Systems), as previously described[25]. Data postprocessing was performed by an experienced senior magnetic resonance physicist.

Biochemical analyses

Venous blood samples were collected after overnight fasting. Fasting biochemical measurements including in plasma: ALT, AST, GGT, LDH and bilirubin[26], HDL-C, LDL-C, TC, TG[29], glucose[27], glucagon[28] and GLP-1 (ref. 31), in serum: insulin, C-peptide[27], hs-CRP[32], leptin, adiponectin, leptin:adiponectin ratio[30] and in whole blood HbA1c[27], as previously described.

Defining cardiometabolic risk features

Hepatic steatosis was defined using two cutoffs of liver fat: ≥5.0%, cutoff used in adults histological[59]; and ≥1.5%, a cutoff used by our group that has shown to represent more accurately the upper normal limit of liver fat content in children and adolescents[25]. We also defined high ALT (above 24.5 U l^{-1} in girls and above 31.5 U l^{-1} in boys), which was found to be the optimal cutoff for diagnosing hepatic steatosis (liver fat > 1.5%) by our group[26]. Hyperglycemia was defined as fasting plasma glucose ≥5.6–6.9 mmol l^{-1} and/or HbA1c ≥ 39–47 mmol mol^{-1}, according to the American Diabetes Association guidelines for prediabetes[60]. Insulin resistance was defined based on HOMA-IR value above the 90th percentile of previously published age- and sex-specific population-based reference values from our group[27]. HOMA-IR was calculated as (insulin mU l^{-1} × glucose mM)/22.5. Dyslipidemia was defined as values above the 95th percentile according to pediatric guidelines, corresponding to TC ≥ 200 mg dl^{-1} (5.2 mM), LDL-C ≥ 130 mg dl^{-1} (3.4 mM), TG ≥ 100 mg dl^{-1} (1.1 mM) for 0–9 years or ≥130 mg dl^{-1} (1.5 mM) for 10–19 years or HDL-C < 40 mg dl^{-1} (1.0 mM)[61]. Hypertension was defined as a SBP and/or DBP above the 95th percentile for age, height and sex[62].

CVD-related and inflammatory markers

A proximity extension assay was performed using the Target 96 Cardiovascular II (CVDII) and Target 96 Inflammation (INF) panels from Olink Proteomics on EDTA plasma, as previously described[63]. Proximity extension assay technology uses nucleic acid labeling of antibodies in combination with qPCR, producing normalized protein expression values as an arbitrary unit on a $\log_2$ scale. Overall, 85 markers from

CVDII and 64 markers from INF were included as >80% of individuals were above the detection limit.

Statistical analyses

Statistical analyses were performed using R software v.4.2.2 (ref. 64). Data are expressed as median (IQR) for continuous variables or frequencies and percentages for categorical variables. The Wilcoxon rank-sum test (for continuous variables) and the chi-squared test (for categorical variables) were used to test differences in characteristics between two groups.

Differential expression analysis was applied to weight status (normal weight, overweight and obesity) using ANOVA adjusting for age and sex, followed by a Tukey's honestly significant difference (Tukey's HSD) post hoc test for pairwise comparisons. Logistic regression was used to examine the association of each lipid individually with overweight/obesity versus normal weight adjusted for age and sex. PLS-DA was performed to examine the lipidomes differences between the three age groups, separately for obesity/overweight and normal weight groups using ropls v.1.30.0 package[65]. Tenfold cross-validation and 300 permutations were used. The first two component scores were plotted in a score plot, where each point represents an individual.

The effect of obesity on the association between continuous age and individual lipid species was tested by a corresponding interaction model including an interaction term (age × overweight/obesity versus normal weight) adjusting for sex. Cardiometabolic traits were log transformed except for BMI SDS, SBP SDS and DBP SDS. The associations of lipids with cardiometabolic risk features and cardiometabolic traits were examined using multiple logistic and linear regressions adjusted for age, sex and BMI SDS when pooling the normal weight and overweight/obesity groups. The interaction between sex and lipid species (lipid × sex) was examined in linear regression models for cardiometabolic traits, adjusted for age and BMI SDS. The obesity interaction (lipid × overweight/obesity versus normal weight) was also tested in the linear regression models for cardiometabolic traits. The reported estimates (β or OR) are based on a 1-s.d. unit increase in independent variables. Spearman correlations between lipids with CVD and inflammatory markers were tested and the correlation coefficients and the P values were obtained using the rcorr function in the Hmisc v.4.7.2 package[66]. Multiple testing correction was performed based on FDR at 5% and a stringent Bonferroni adjusted $P < 2.2 \times 10^{-4}$ (0.05 of 227 lipids tested), separately for each outcome. $P < 5\%$ FDR was considered statistically significant. In all figures, only those lipids with at least one outcome association reaching FDR significance were included. Changes in lipid profiles before and after obesity management were assessed while adjusting for age and sex. Linear mixed models were employed using the gls function from the nlme package v.3.1.160 in R[67]. The effects of BMI SDS reduction on lipid profiles were analyzed using linear regressions controlling for age, sex and treatment duration. The associations between changes in lipid profiles and changes in continuous cardiometabolic traits were examined using linear regressions controlling for age, sex, treatment duration, baseline BMI SDS and change in BMI SDS. BMI SDS reduction was calculated as the difference between BMI SDS at baseline and BMI SDS at follow-up. Changes in lipid profiles and cardiometabolic traits were calculated as the difference between the values at follow-up and those at baseline. The chord diagram and heatmaps were created using the circlize v.0.4.15 (ref. 68) and ComplexHeatmap v.2.14.0 (ref. 69) R packages.

Prediction model. We performed feature selection for hepatic steatosis, defined as liver fat above 5%, using the maximum relevance and minimum redundancy method[70], implemented in the njab Python package (https://njab.readthedocs.io/en/stable/). Through fivefold cross-validation repeated ten times, this analysis identified a three-lipid panel that achieved the highest mean ROC AUC. Furthermore, we evaluated the discriminative performance of three clinical used liver enzymes (ALT, AST and GGT), both individually and in combination with the lipid panel using the same cross-validation method. To mitigate imbalanced class distribution, a downsampling approach was applied to the majority class within each cross-validation fold. The statistical comparison of AUCs was conducted using DeLong's test. These analyses were performed using the caret v.6.0.94 (ref. 71) and pROC v.1.18.0 R package[72].

Mediation analysis. In the cross-sectional study, mediation analysis was performed to explore the mediating role of obesity-associated lipids on cardiometabolic traits. Bootstrapping with 1,000 iterations was employed to estimate direct, indirect and total effects across obesity–lipid–trait triangles adjusted for age and sex. We examined 518 potential paths identifying significant associations between obesity → lipid → traits at a significance level of $P < 5\%$ FDR. The proportion of the effect mediated from obesity through the lipid was determined by dividing its indirect effect by the total effect. In the intervention study, mediation analysis was performed to examine the mediation effect of lipid changes on the association between BMI SDS reduction and changes in cardiometabolic traits adjusting for age, sex and treatment duration. We tested 253 possible paths (BMI SDS reduction → lipid change → trait change associations at nominal significance $P < 0.05$). Bootstrap confidence intervals were used to assess the statistical significance of the mediation effects. Mediation analyses were performed using the mediation v.4.5.0 R package[73].

Sensitivity analyses. We matched individuals in the obesity group to those in the normal weight group by age and sex using the MatchIt R package. The matched obesity group has a median age of 9.10 (IQR 7.53–10.18) (n = 373, 175 boys), while the normal weight group has a median age of 8.35 (6.87–12.42) (n = 373, 180 boys). We performed a subanalysis to investigate the interaction between obesity and the association of age with 26 previously identified lipids using age- and sex-matched overweight/obesity and normal weight groups (n = 373 versus n = 373). To explore the impact of TC and TG on lipid–trait association, we tested the association between 34 cardiometabolic-associated lipids and traits adjusted for age, sex, BMI SDS, TC and TG. To identify common lipids associated with BMI SDS in both the cross-sectional and intervention studies, we performed linear regression analyses between BMI SDS and lipid species adjusting for age and sex. These analyses utilized data from the overweight/obesity group in the cross-sectional study and baseline data from the intervention study.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All results from statistical and bioinformatics analysis are provided in Supplementary Tables 1–13. Mean levels of lipids have been deposited in the GitHub repository at https://github.com/yunhuanghy/Lipidomic/tree/main/average_data. In line with the current regulation of General Data Protection Regulation (https://gdpr-info.eu/) to maintain patient confidentiality, individual-level clinical and lipidomics data generated in this study cannot be made publicly available. Lipidomics datasets are available from the authors upon request by contacting T.H. at torben.hansen@sund.ku.dk. The obesity management protocol is available upon request to J.-C.H. at jhom@regionsjaelland.dk. Access to the data can be granted through the Danish Data Protection Agency and the ethics committee for the Region Zealand of Denmark by obtaining proper approvals and in accordance with patient information and processing agreements. The time frame for response to requests from the authors is within 1 month. When applying and processing data, restrictions apply: (1) a data-processing agreement must be signed between the data controller and processor; (2) data must not be processed for

purposes other than statistical and scientific studies; (3) personal data must be deleted, anonymized and destroyed at the end of investigation; and (4) data must not be passed on to a third party or individuals who are not authorized to access the data.

Code availability

The code used for data analysis is available on GitHub: https://github.com/yunhuanghy/Lipidomic.

References

54. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* **114**, 555–576 (2004).
55. Israelsen, M. et al. Comprehensive lipidomics reveals phenotypic differences in hepatic lipid turnover in ALD and NAFLD during alcohol intoxication. *JHEP Rep.* **3**, 100325 (2021).
56. Pluskal, T., Castillo, S., Villar-Briones, A. & Oresic, M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinform.* **11**, 395 (2010).
57. Schrimpe-Rutledge, A. C., Codreanu, S. G., Sherrod, S. D. & McLean, J. A. Untargeted metabolomics strategies-challenges and emerging directions. *J. Am. Soc. Mass. Spectrom.* **27**, 1897–1905 (2016).
58. Kirkwood, K. I. et al. Utilizing Skyline to analyze lipidomics data containing liquid chromatography, ion mobility spectrometry and mass spectrometry dimensions. *Nat. Protoc.* **17**, 2415–2430 (2022).
59. Kleiner, D. E. et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **41**, 1313–1321 (2005).
60. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. *Diabetes Care* **44**, S15–S33 (2021).
61. National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* **128**, S213–S256 (2011).
62. Flynn, J. T. et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics* https://doi.org/10.1542/peds.2017-1904 (2017).
63. Stinson, S. E. et al. High plasma levels of soluble lectin-like oxidized low-density lipoprotein receptor-1 are associated with inflammation and cardiometabolic risk profiles in pediatric overweight and obesity. *J. Am. Heart Assoc.* https://doi.org/10.1161/JAHA.122.027042 (2023).
64. R Core Team. R: a language and environment for statistical computing. *R Foundation for Statistical Computing*. https://www.R-project.org/ (2021).
65. Thévenot, E. A., Roux, A., Xu, Y., Ezan, E. & Junot, C. Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *J. Proteome Res.* **14**, 3322–3335 (2015).
66. Harrell, F. E. Jr. Harrell Miscellaneous. R package Hmisc version 5.1-3. (Comprehensive R Archive Network (CRAN), 2024).
67. Pinheiro, J., Bates, D. & R Core Team. nlme: linear and nonlinear mixed effects models. R package nlme version 3.1-166. (Comprehensive R Archive Network (CRAN), 2024).
68. Gu, Z., Gu, L., Eils, R., Schlesner, M. & Brors, B. circlize implements and enhances circular visualization in R. *Bioinformatics* **30**, 2811–2812 (2014).
69. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).
70. Ding, C. & Peng, H. Minimum redundancy feature selection from microarray gene expression data. In *Computational Systems Bioinformatics. Proc. 2003 IEEE Bioinformatics Conference. CSB2003*, 523–528 (IEEE Comput. Soc., 2003).
71. Kuhn, M. Building predictive models in R using the caret package. *J. Stat. Soft.* https://doi.org/10.18637/jss.v028.i05 (2008).
72. Robin, X. et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform.* **12**, 77 (2011).
73. Tingley, D., Yamamoto, T., Hirose, K., Keele, L. & Imai, K. mediation: R package for causal mediation analysis. *J. Stat. Soft.* https://doi.org/10.18637/jss.v059.i05 (2014).

Acknowledgements

We thank all volunteers and their parents who participated in *The HOLBAEK Study*. We also thank colleagues from Translational Medicine at Steno Diabetes Center Copenhagen, Hansen Group at Novo Nordisk Foundation Center for Basic Metabolic Research and The Children's Obesity Clinic for fruitful discussions. In particular, we thank A. Frost Bjerre, B. Holløse, O. Troest, T. Larsen, A. Forman and T. Hvidtfeldt Lorentzen for technical assistance with sample preparation. We thank A. Wretlind from Translational Medicine for supporting the lipidomics data merge. We thank L. Skovborg Just and L. Ryborg for managing the GALAXY and MicrobLiver consortia. We thank H. Webel, R. Thielemann, P. Proitsi and Y. Fan for their inputs and assistance with the revision. We acknowledge the funding agencies that supported this study: the Innovation Fund Denmark (grant no. 0603-00484B), the Novo Nordisk Foundation (grant no. NNF15OC0016544 to T.H.), the Novo Nordisk Foundation Challenge Program (grant no. NNF15OC0016692 to the MicrobLiver consortium (T.H. and A.K. in this study)). Furthermore, K.S. was supported by the Novo Nordisk Foundation Excellence Emerging Investigator Grant: Endocrinology and Metabolism 2022 (grant no. NNF 0074491). S.E.S. was funded by the NNF Copenhagen Bioscience PhD Program (grant no. NNF18CC0033668). L.A.H. was supported by the Danish Cardiovascular Academy, which is funded by the Novo Nordisk Foundation (grant no. NNF20SA0067242) and the Danish Heart Foundation (grant no. PhD2023009-HF). C.E.F. was supported by the BRIDGE-Translational Excellence Program (grant no. NNF18SA0034956), Steno Diabetes Center Sjælland and the Region Zealand Health Scientific Research Foundation (grant no. R32-A1191). The European Union's Horizon 2020 research and innovation program (grant no. 668031 to the GALAXY consortium (A.K., T.H., C.L.-Q. and M.T. in this study)). The LundbeckFonden Ascending Investigator Program was awarded to C.L.-Q. (LFR344-2020-989). We also thank the Novo Nordisk Foundation for supporting the Novo Nordisk Foundation Center for Basic Metabolic Research (grant nos. NNF18CC0034900 and NNF23SA0084103).

Author contributions

The paper was drafted by K.S., Y.H. and C.L.-Q. Y.H. performed the bioinformatics analysis and generated figures for the paper. S.E.S. contributed to bioinformatics analysis and results interpretation. K.S., K.T., K.H. and M.K. performed and interpreted the lipidomics experiments. J.-C.H. and T.H. designed and coordinated the clinical cohort. M.A.V.L., C.E.F. and L.A.H. recruited participants in clinical cohorts, collected samples and clinical data. L.Ä. supported statistical analysis. H.B.J., T.N., P.R., M.J. and A.K. contributed to data coordination and management. J.-C.H, C.L.-Q. and T.H. developed the present project concept and protocol and supervised the project. All authors reviewed the paper before submission.

Competing interests

The data and paper were prepared while K.S. was employed at the Steno Diabetes Center Copenhagen. In the process of revision and publication, K.S. has started a position at Novo Nordisk. C.L.-Q. has received consultancy fees from Pfizer. C.L.-Q. has received honoraria, travel or speakers' fees from Biogen and research funds from Pfizer and Novo Nordisk. C.L.-Q. is the director of the company BrainLogia. All of these activities are unrelated to this study. The other authors declare no competing interests.

Additional information

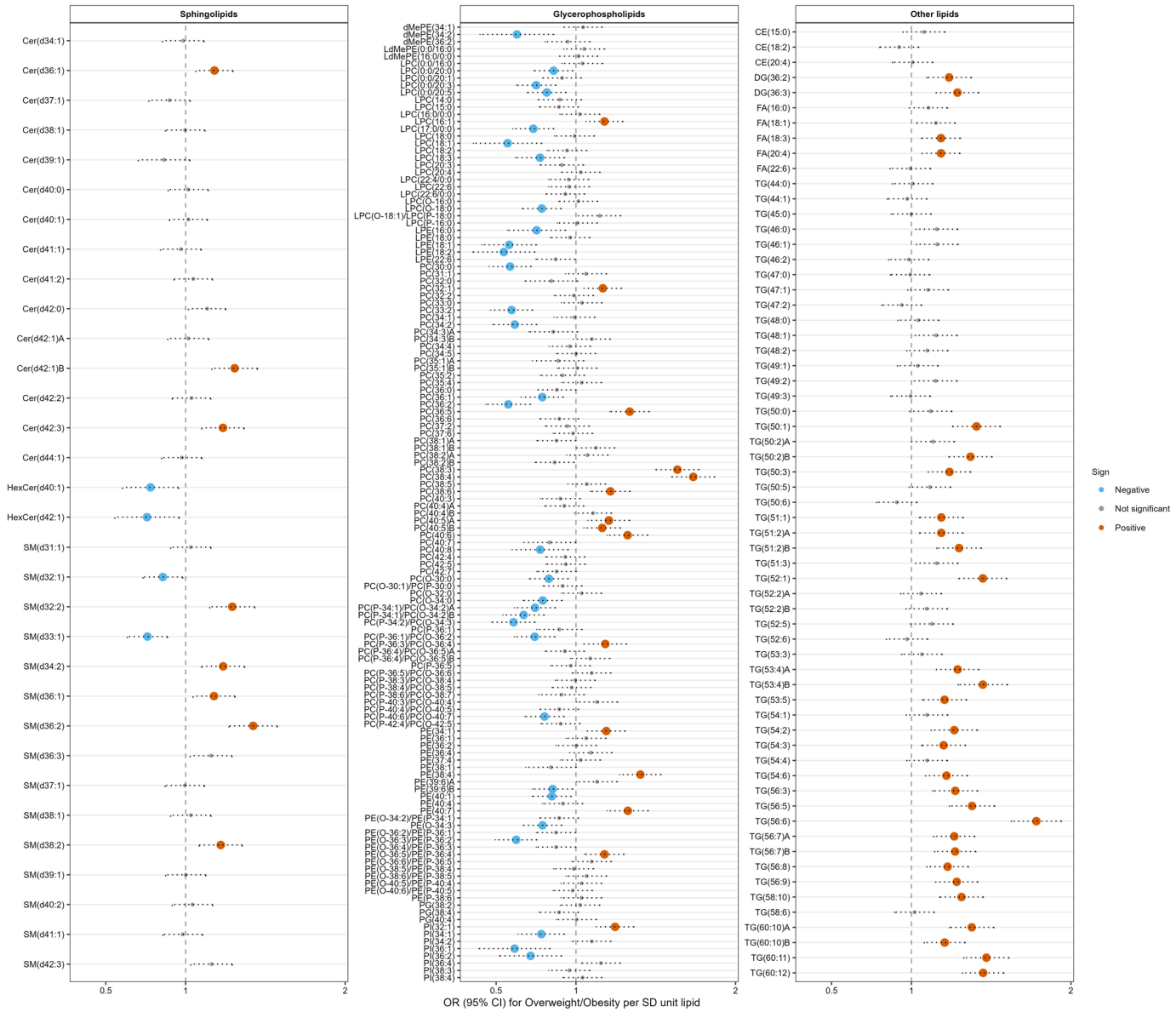
Extended data is available for this paper at https://doi.org/10.1038/s41591-024-03279-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-024-03279-x.

Correspondence and requests for materials should be addressed to Jens-Christian Holm, Cristina Legido-Quigley or Torben Hansen.

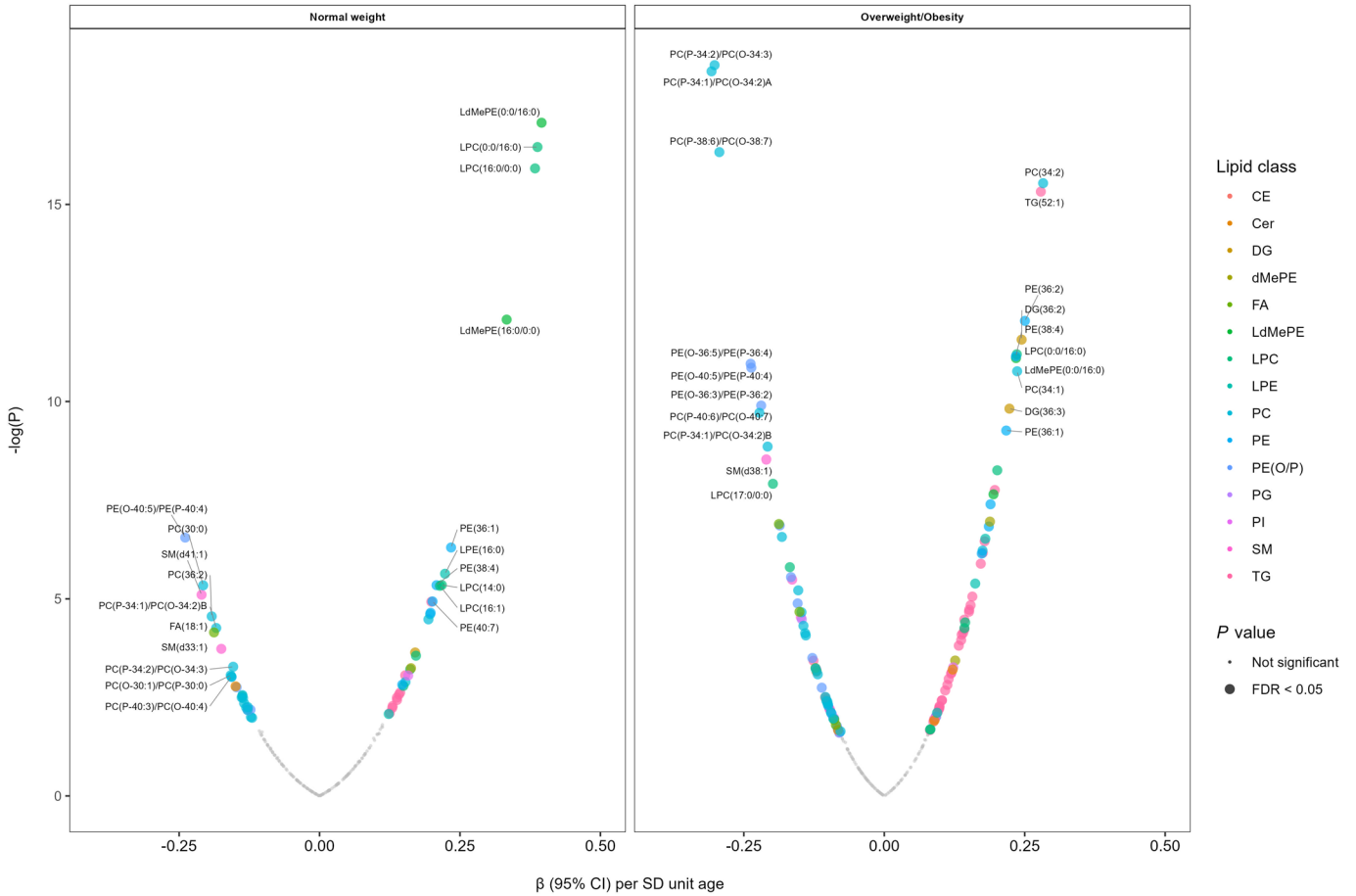
Peer review information *Nature Medicine* thanks Andreas Birkenfeld, Ram Weiss and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Sonia Muliyil, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-024-03279-x



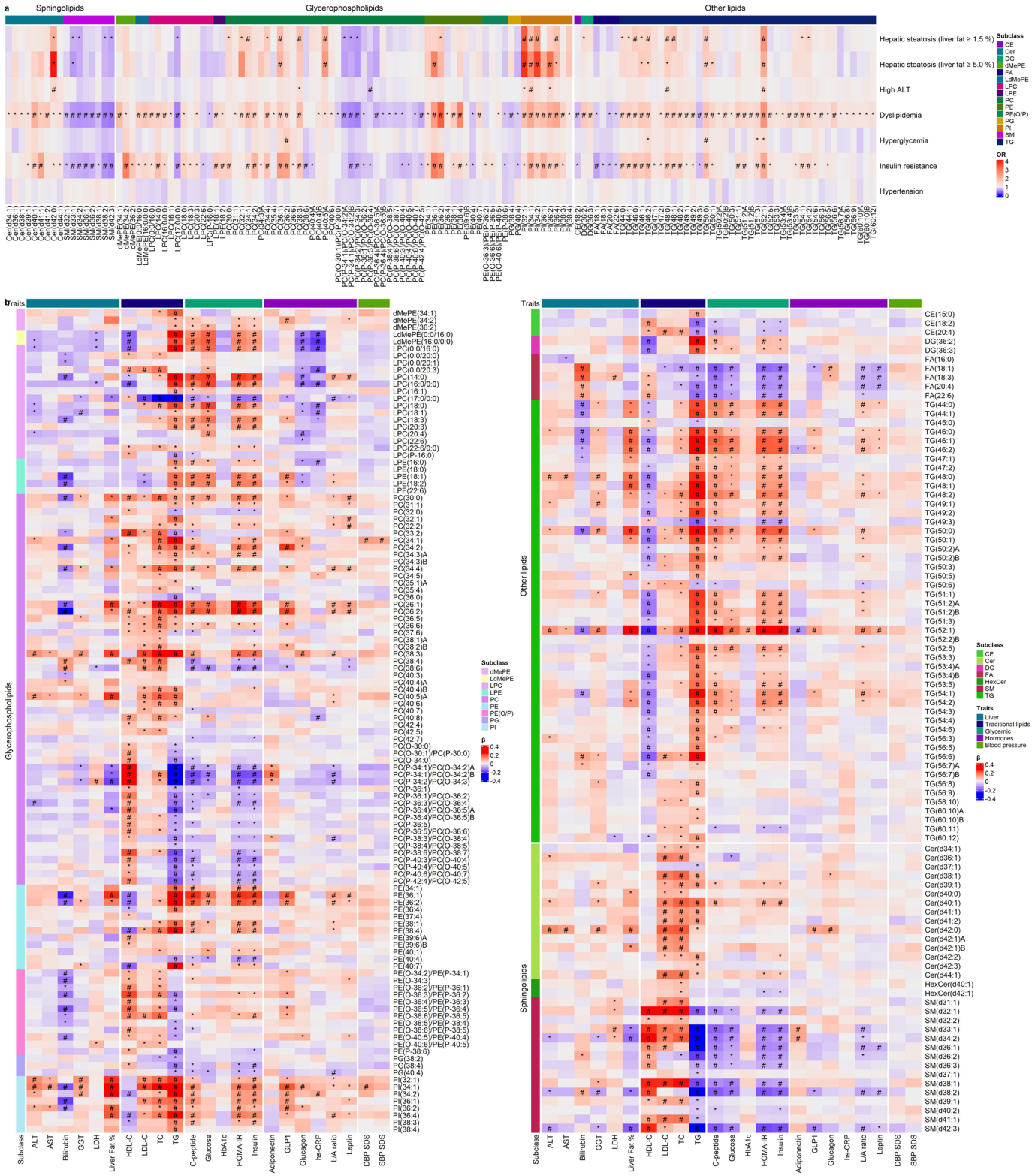
Extended Data Fig. 1 | Associations between 227 lipid species and overweight/obesity. Logistic regression was performed adjusting for age and sex. The odds ratio (OR) with error bars representing 95% CI of each lipid species are shown. Circles: Gray denotes no significant associations; orange and blue denote positive and negative significant associations adjusted for multiple testing ($P < 5\%$ FDR), respectively. n = 958/373 for overweight/obesity and normal weight groups.

https://doi.org/10.1038/s41591-024-03279-x



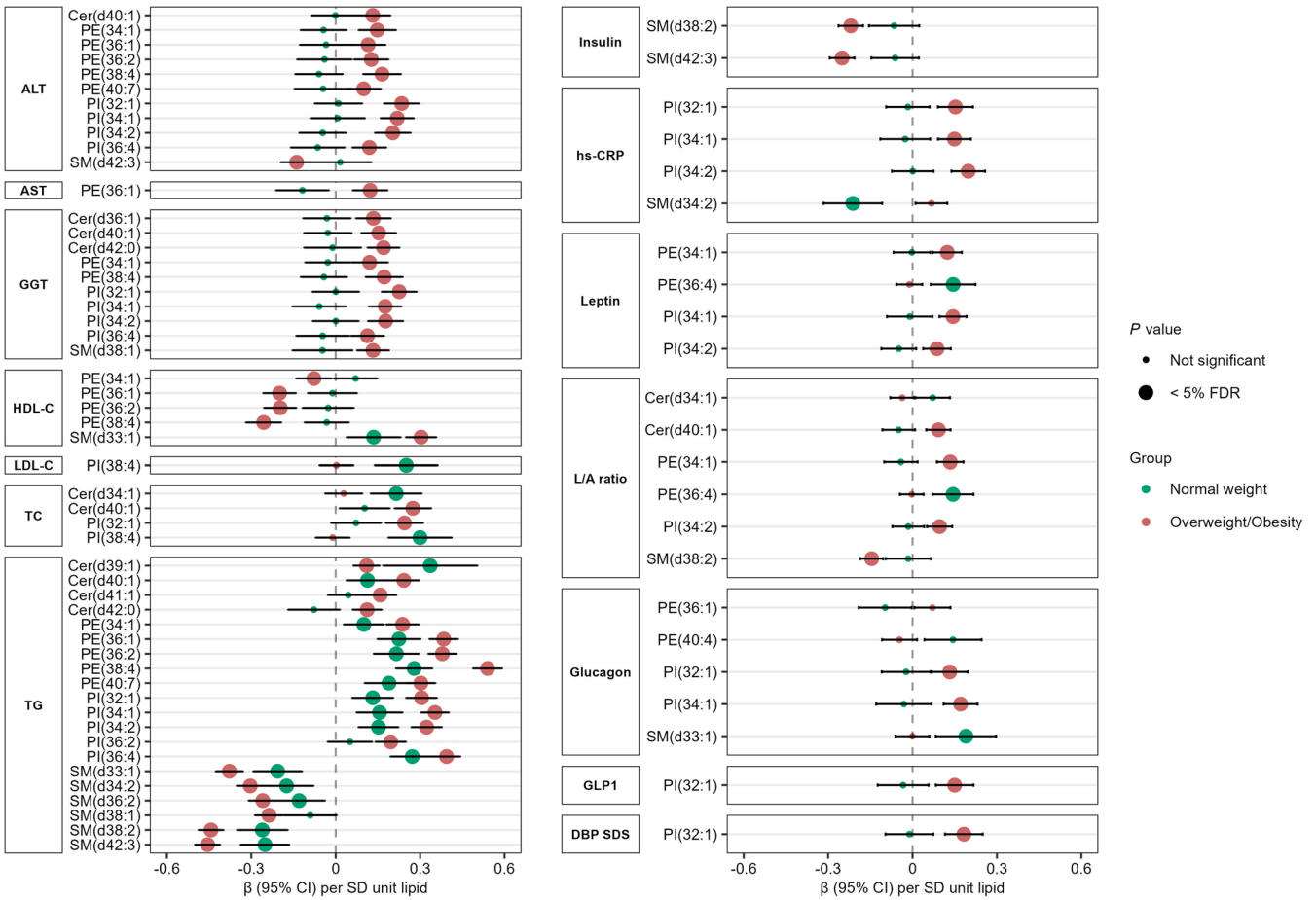
Extended Data Fig. 2 | Obesity interaction on the association between age and lipid species. Linear regression analysis was performed including an interaction term for obesity and adjusting for sex. The top 10 lipid species within each directionality of association (10 positive and 10 negative) in normal weight (n = 373) and overweight/obesity (n = 958) groups are labeled.

https://doi.org/10.1038/s41591-024-03279-x



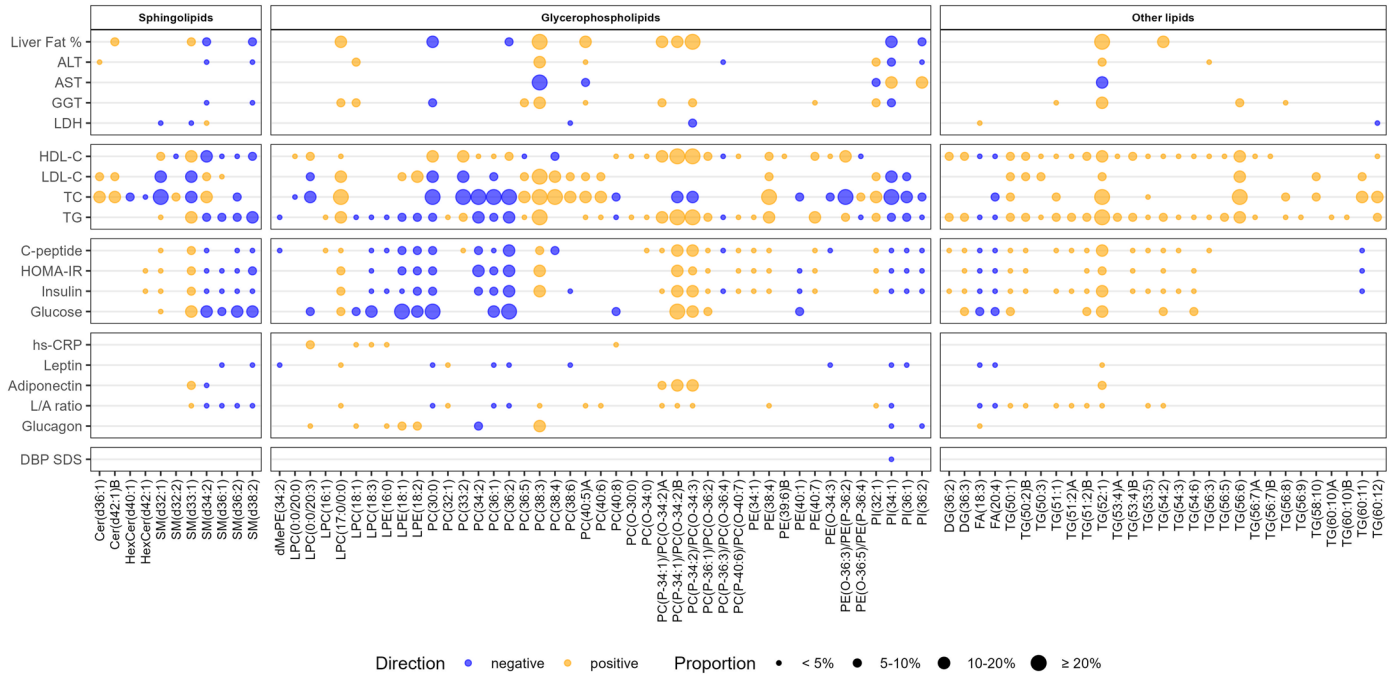
Extended Data Fig. 3 | Associations between lipid species and cardio-metabolic risk. a, 135 lipids have at least one significant association (P < 5% FDR) with one risk feature tested by logistic regression, adjusting for age, sex and BMI SDS. **b**, 207 lipids have at least one significant association (P < 5% FDR) with one trait tested by linear regression, adjusting for age, sex and BMI SDS. An asterisk indicates P < 5% FDR; a hash indicates $P < 2.2 \times 10^{-4}$. The sample size (n) for each feature is listed in Table 1, the maximum observed is 1,330.

https://doi.org/10.1038/s41591-024-03279-x



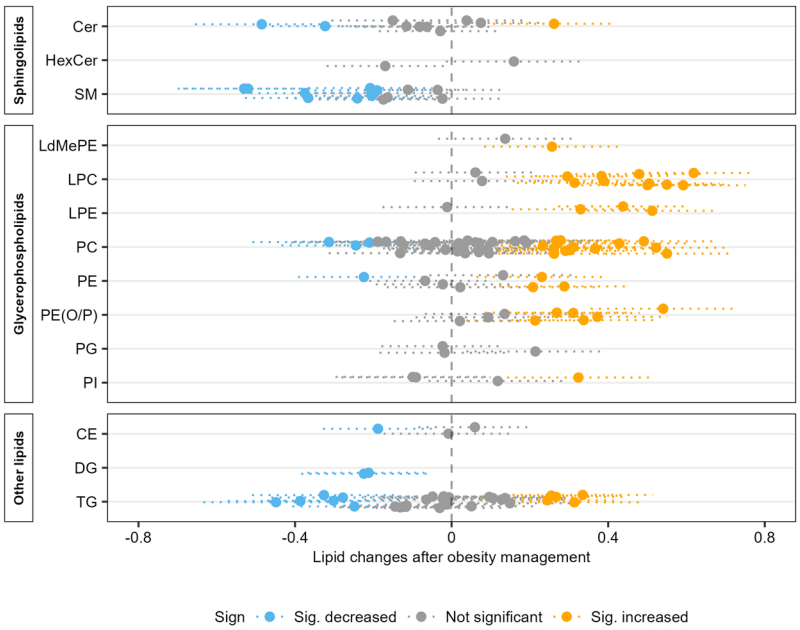
Extended Data Fig. 4 | Associations between 25 cardiometabolic-associated lipids with 14 traits that showed significant obesity interaction (P < 5% FDR). Linear regression analysis was performed including an interaction term for obesity (overweight/obesity vs. normal weight) and adjusting for sex. The β-coefficients with error bars representing 95% CI are shown separately for the normal weight (green) and overweight/obesity group (red). The sample size (n) for each trait is listed in Table 1.

https://doi.org/10.1038/s41591-024-03279-x



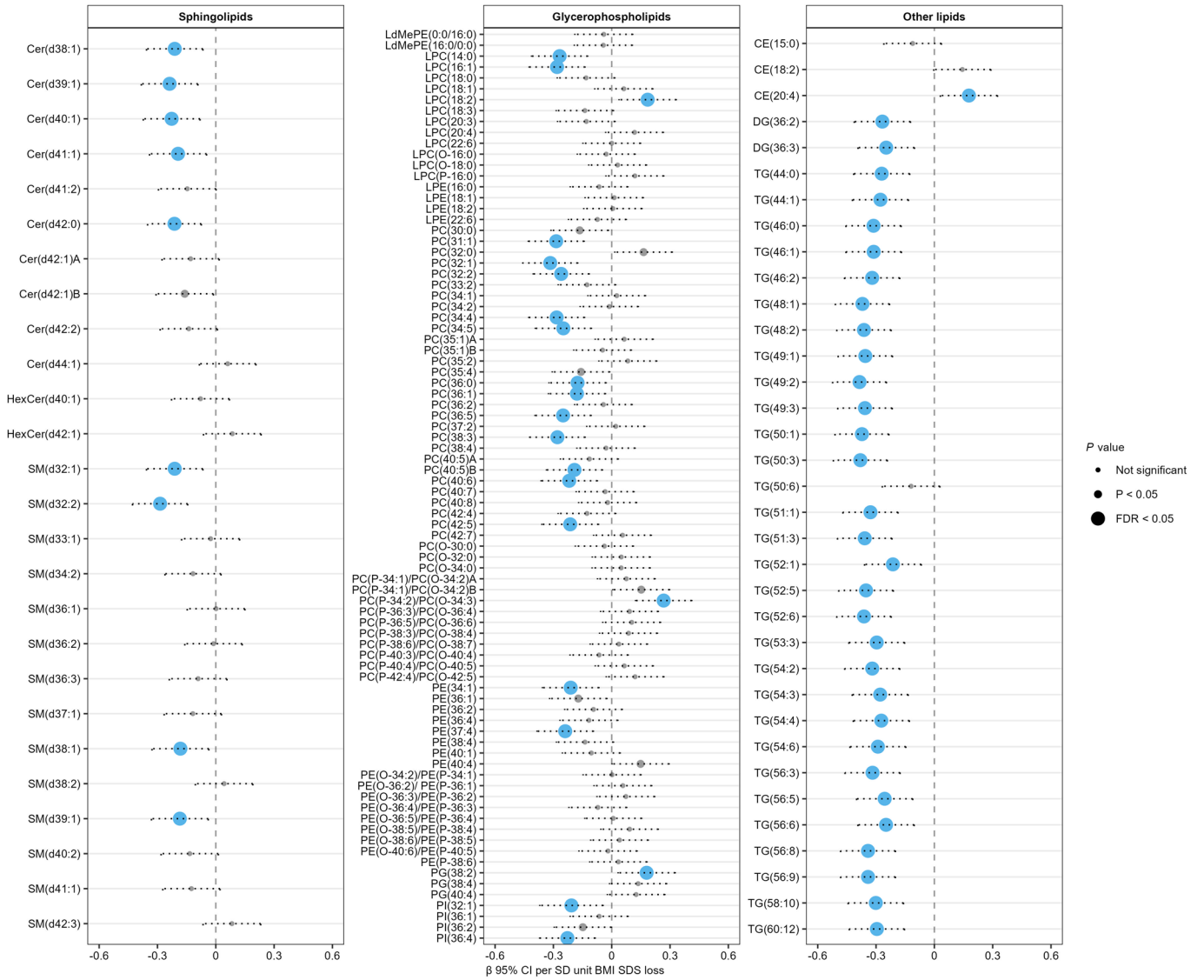
Extended Data Fig. 5 | The mediation proportion of 83 lipid profiles on the association between obesity and 19 cardiometabolic traits. Mediation analysis was performed adjusting for age and sex. Each dot represents a significant indirect effect (P < 5% FDR), with dot size indicating the mediation proportion categorized into <5%, 5–10%, 10–20%, and ≥ 20%. Colors denote the direction of effects, with orange indicating positive and blue negative. The sample size (n) for each trait is listed in Table 1.

https://doi.org/10.1038/s41591-024-03279-x



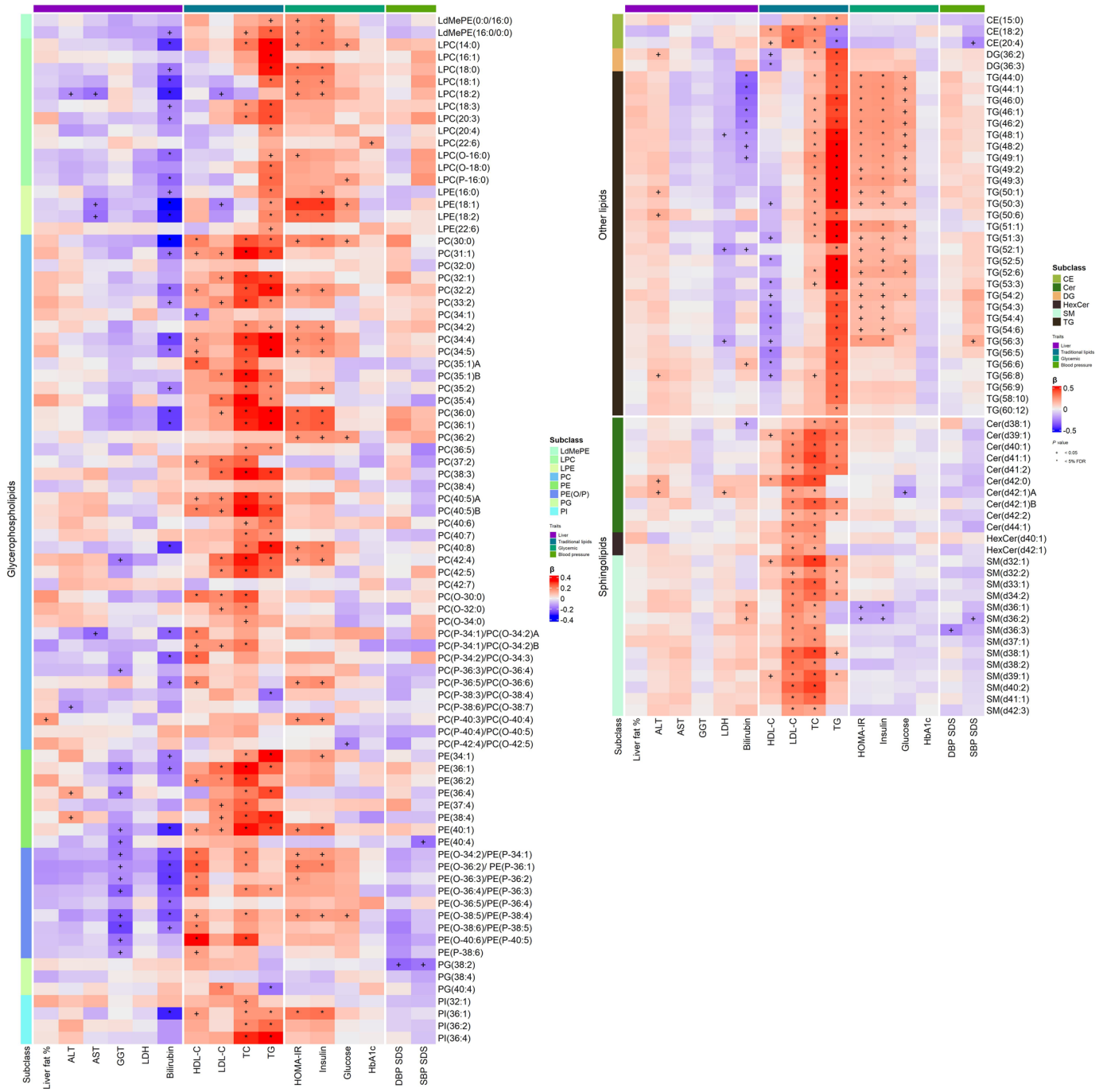
Extended Data Fig. 6 | Lipid changes associated with obesity management. Sixty-seven lipids showed significant changes (n = 186, P < 5% FDR), 23 decreased (blue) and 44 increased (orange). These changes were tested by a linear mixed model, adjusting for age and sex. The β-coefficients with error bars representing 95% CI were shown.

https://doi.org/10.1038/s41591-024-03279-x



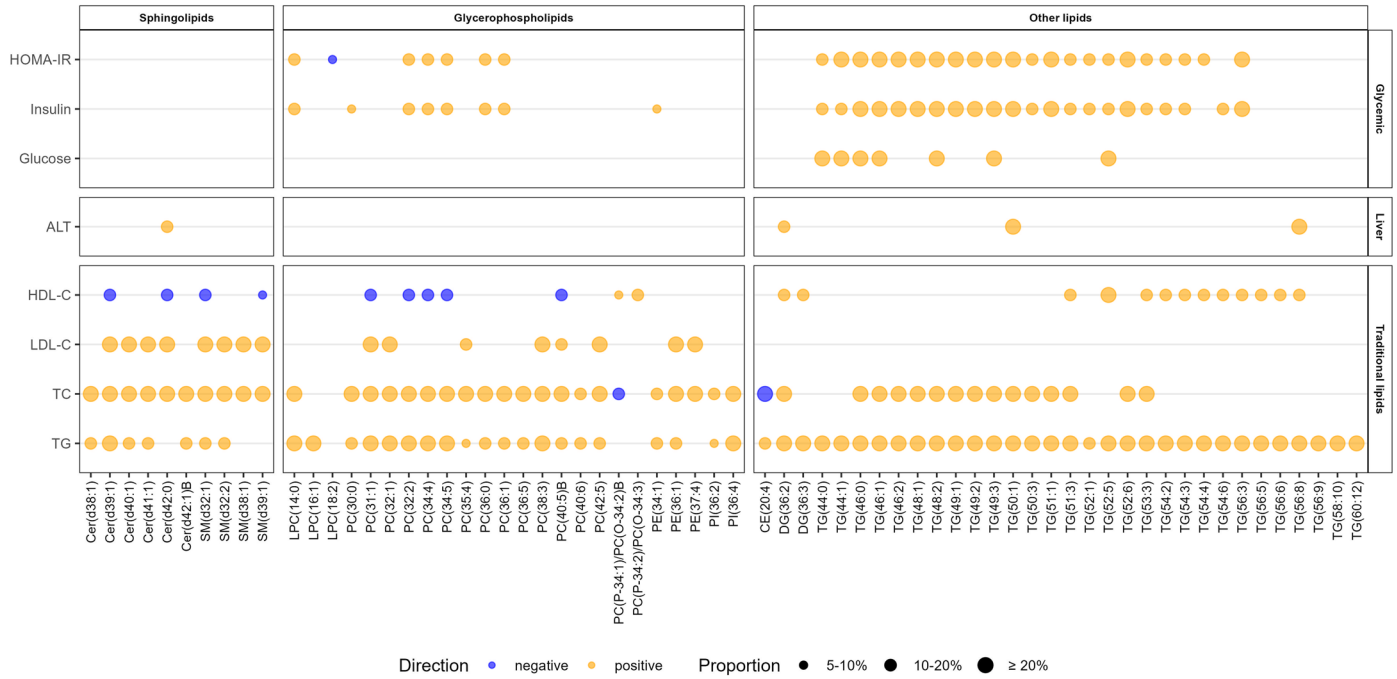
Extended Data Fig. 7 | Associations between BMI SDS reduction and changes in 145 lipid species. Linear regression analysis was performed adjusting for age, sex, and treatment duration year (n = 186, P < 5% FDR). The β-coefficients with error bars representing 95% CI are shown. BMI SDS loss was calculated by BMI SDS at baseline - BMI SDS at follow-up. Changes in lipid profiles were calculated by value at follow-up - value at baseline.

https://doi.org/10.1038/s41591-024-03279-x



Extended Data Fig. 8 | Associations between changes in 145 lipid species and changes in cardiometabolic traits. Linear regression analysis was performed adjusting for age, sex, treatment duration, baseline BMI SDS, and change in BMI SDS. A plus sign indicates P < 0.05; an asterisk indicates P < 5% FDR. The sample size (n) for each trait is listed in Extended Data Table 1, the maximum observed is 185.

https://doi.org/10.1038/s41591-024-03279-x



Extended Data Fig. 9 | The proportion mediated by changes of 66 lipid profiles on the association between BMI SDS reduction and changes in eight cardiometabolic traits. Mediation analysis was performed adjusting for age, sex, and treatment duration. Each dot represents a significant indirect effect, with dot size indicating the mediation proportion categorized into 5–10%, 10–20%, and ≥ 20%. Colors denote the direction of effects, with orange indicating positive and blue negative. BMI SDS reduction was calculated as the difference between BMI SDS at baseline and BMI SDS at follow-up. Changes in lipid profiles and cardiometabolic traits were calculated as the difference between the values at follow-up and those at baseline. The sample size (n) for each trait is listed in Extended Data Table 1.

https://doi.org/10.1038/s41591-024-03279-x

Extended Data Table 1 | Changes in participants characteristics after nonpharmacological obesity management

	All			Decreased BMI SDS			Increased BMI SDS		
Outcome	n	Change	P	n	Change	P	n	Change	P
BMI SDS	186	-0.39 [-0.76, -0.07]	<0.001	154	-0.47 [-0.81, -0.23]	<0.001	32	0.13 [0.08, 0.27]	<0.001
Body fat, %	125	-2.85 [-6.53, -0.38]	<0.001	106	-3.23 [-6.79, -1.55]	<0.001	19	0.56 [-0.75, 2.22]	0.169
Waist, cm	124	-1.00 [-7.00, 4.02]	0.193	105	-3.00 [-7.00, 3.00]	0.003	19	6.50 [2.60, 11.85]	0.001
WHR	124	-0.02 [-0.07, 0.02]	0.003	105	-0.02 [-0.07, 0.02]	0.002	19	0.01 [-0.05, 0.03]	0.891
Liver fat, %	100	0.00 [-1.42, 0.00]	0.021	81	0.00 [-1.60, 0.00]	<0.001	19	0.30 [0.00, 1.35]	0.078
Plasma ALT, U/L	185	-1.00 [-6.00, 5.00]	0.115	153	-1.00 [-7.00, 4.00]	0.018	32	2.00 [-4.50, 9.00]	0.181
Plasma AST, U/L	180	-2.00 [-7.00, 4.00]	0.071	149	-2.00 [-7.00, 4.00]	0.099	31	-2.00 [-6.50, 3.50]	0.44
Plasma GGT, U/L	185	0.00 [-3.00, 2.00]	0.841	153	-1.00 [-3.00, 2.00]	0.071	32	2.50 [0.00, 4.25]	0.005
Plasma LDH, U/L	181	-12.00 [-33.00, 5.00]	<0.001	151	-12.00 [-33.00, 2.00]	<0.001	30	-14.00 [-27.00, 11.50]	0.082
Plasma bilirubin, umol/L	185	-0.30 [-2.00, 1.00]	0.013	153	-0.30 [-2.00, 1.00]	0.028	32	0.00 [-2.25, 1.00]	0.261
Plasma TG, mmol/L	185	0.00 [-0.30, 0.20]	0.37	153	0.00 [-0.30, 0.20]	0.132	32	0.10 [-0.13, 0.30]	0.264
Plasma TC, mmol/L	185	-0.10 [-0.50, 0.20]	<0.001	153	-0.20 [-0.50, 0.20]	<0.001	32	-0.05 [-0.42, 0.40]	0.688
Plasma HDL-C, mmol/L	185	0.10 [0.00, 0.30]	<0.001	153	0.10 [0.00, 0.30]	<0.001	32	0.05 [-0.10, 0.20]	0.418
Plasma LDL-C, mmol/L	185	-0.20 [-0.60, 0.10]	<0.001	153	-0.20 [-0.60, 0.10]	<0.001	32	-0.15 [-0.40, 0.20]	0.239
Serum C-peptide, nmol/L	144	0.03 [-0.13, 0.18]	0.112	119	0.02 [-0.15, 0.15]	0.883	25	0.16 [0.019, 0.32]	0.001
HOMA-IR, mIU/L	182	0.34 [-5.79, 6.72]	0.518	150	-0.44 [-6.52, 5.98]	0.626	32	4.32 [-1.85, 13.75]	0.017
Serum insulin, pmol/L	184	1.10 [-24.93, 29.91]	0.573	152	-3.05 [-26.00, 22.41]	0.554	32	17.92 [-10.56, 57.52]	0.015
Plasma glucose, mmol/L	184	0.10 [-0.20, 0.30]	0.011	152	0.10 [-0.20, 0.30]	0.016	32	0.10 [-0.13, 0.30]	0.356
Whole blood HbA1c, mmol/mol	184	-1.00 [-2.00, 1.00]	<0.001	152	-1.00 [-2.00, 1.00]	<0.001	32	-0.50 [-2.00, 1.00]	0.431
SBP SDS	122	-0.28 [-0.92, 0.48]	0.011	104	-0.38 [-0.95, 0.44]	0.001	18	-0.05 [-0.49, 1.01]	0.369
DBP SDS	122	-0.30 [-0.73, 0.02]	<0.001	104	-0.29 [-0.69, -0.00]	<0.001	18	-0.35 [-0.73, 0.26]	0.13

Changes from baseline to follow-up are expressed as median (interquartile range), with significance determined using the two-sided Wilcoxon signed rank test. Abbreviations: BMI, body mass index; SDS, standard deviation score; WHR, waist-to-hip ratio; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, hemoglobin A1c; DBP, diastolic blood pressure; SBP, systolic blood pressure.

nature portfolio

Corresponding author(s): Torben Hansen, Cristina Legido-Quigley, Jens-Christian Holm

Last updated by author(s): Aug 19, 2024

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	We used MassHunter (Agilent), MassHunter Workstation Software LC/MS Data Aquasition for 6200 series TOF/6500 series Q-TOF, Version B.09.00. MZMine 2.0 for lipidomics data.
Data analysis	We used R software version 4.2.2 for data analysis (main packages: ropls v1.30.0, circlize v0.4.15, Hmisc v4.7.2, ComplexHeatmap v2.14.0, caret v6.0.94, pROC v1.18.0, nlme v3.1.160, mediation v4.5.0). The code is available at https://github.com/yunhuanghy/Lipidomic

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Average levels of lipids have been deposited in the GitHub repository: https://github.com/yunhuanghy/Lipidomic/tree/main/average_data. In line with the current regulation of General Data Protection Regulation (https://gdpr-info.eu/) to maintain patient confidentiality, individual-level clinical and lipidomics data generated in

this study cannot be made publicly available. Lipidomics datasets are available from the authors upon request by contacting Prof. Torben Hansen at torben.hansen@sund.ku.dk. The obesity management protocol is available upon request to Jens-Christian Holm at jhom@regionsjaelland.dk. Access to the data can be granted through the Danish Data Protection Agency and the ethics committee for the Region Zealand of Denmark by obtaining proper approvals and in accordance with patient information, and processing agreements. The time frame for response to requests from the authors is within a 1-month period. When applying and processing data, restrictions apply: (1) a data processing agreement must be signed between the data controller and processor; (2) data must not be processed for purposes other than statistical and scientific studies; and (3) personal data must be deleted, anonymized and destroyed at the end of investigation and (4) must not be passed on to a third party or individuals who are not authorized to access the data.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We always use sex for the biological separation of boys and girls. We include sex as a covariate in most of our analyses. We explored potential sex differences in the associations between lipid species and cardiometabolic traits. Sex-specific effect estimates were provided.
Reporting on race, ethnicity, or other socially relevant groupings	The study was conducted in a majority Caucasian pediatric population, with ethnicity determined through self-reporting by participants.
Population characteristics	In the cross-sectional study, the normal weight group consisted of 373 participants with a median age of 8.3 years (interquartile range [IQR]: 6.9-12.4), of whom 48% were boys. The overweight/obesity group included 958 participants, with a median age of 11.9 years (IQR: 9.8-14.1) and 44% boys. The intervention study included 186 participants with a median age of 11.6 years (IQR: 9.9-13.7) and 45% boys.
Recruitment	The study population consisted of a random sample of children and adolescents from The HOLBAEK Study, previously known as The Danish Childhood Obesity Biobank. Participants in the obesity clinic cohort were recruited from the Children's Obesity Clinic, which provides the multidisciplinary childhood obesity management program at the Children's Obesity Clinic, Department of Pediatrics, Holbæk Hospital. Eligibility criteria was a BMI SDS above the 90th percentile according to Danish reference values. Participants in a population-based cohort were recruited from schools across 11 municipalities in Zealand, Denmark. Both cohorts were enrolled between January 2009 and April 2019. There is potential self-selection bias in the obesity clinic cohort and the population-based cohort. Families attending the obesity clinic may have a higher awareness of obesity, and those who consented to participate may differ in socioeconomic profiles, health awareness, and other factors from those who declined. Moreover, the recruitment is based on the Zealand region in Denmark. These factors might potentially affect the generalizability of the findings.
Ethics oversight	According to the Declaration of Helsinki, written informed consent was obtained from all participants. An informed oral assent was given by the participant if the participant was younger than 18, and then the parents gave informed written consent. The study was approved by the Ethics Committee of Region Zealand, Denmark (SJ-104) and by the Danish Data Protection Agency (REG-043-2013). The HOLBAEK Study are registered at ClinicalTrials.gov (NCT00928473).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	958 children and adolescents with overweight or obesity and 373 with normal-weight were included in the cross-sectional study, and 186 within the intervention study, where blood samples underwent lipidomics analysis. No sample size calculation was performed. Cross-sectional dataset has been previously used in other publications, mainly focused on clinical outputs. There we could see that the sample size was sufficient to provide statistical significance. The intervention study was restricted to the feasibility criteria, driven by clinical time and budget available, as omics study always requieres high number of participants.
Data exclusions	Participants were excluded based on: diagnosed type 1 diabetes mellitus or T2D (n=3); intake of medications including statins, insulin, metformin, and liraglutide (n=3); meeting T2D criteria based on blood sample (fasting plasma glucose ≥ 7.0 mmol/L and/or HbAlc ≥ 48 mmol/mol) (n=2); the interval between baseline visit and blood sample collection> 90 days (n=12); underweight (BMI < 5th percentile [BMI SDS < -1.64]) (n=12).
Replication	We did not perform technical or biological replication of the samples within lipidomics analysis. However, in the intervention study we could observe a replicated patterns of obesity-associated lipids.
Randomization	Samples within each cohort were randomised for the lipidomics experiments. Other clinical tests did not require a randomization, as data

Randomization	collection happened at the same time as the clinical visit. We do not present here a randomized clinical trial.
Blinding	Scientists performing the lipidomics analysis were blinded with no access to the clinical data of the individuals. For experiments other than lipdidomics and proteomics, blinding was not relevant, since first cohort was a cross-sectional study and second was intervention, where all participants were under the same type of weight management.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov registration number: NCT00928473
Study protocol	The study protocol was approved by the ethics committee for the Region Zealand (protocol no. SJ-104) and available upon request by contacting Prof. Torben Hansen at torben.hansen@sund.ku.dk. This information was also stated in the manuscript
Data collection	Participants in The HOLBAEK Study were enrolled between January 2009 and April 2019. Clinical data were collected at the participants' baseline visit at The Children's Obesity Clinic, Department of Paediatrics, Holbaek Hospital in Denmark.
Outcomes	In the cross-sectional study, the primary outcome was the identification of lipid species associated with obesity, achieved by comparing the lipid profiles of children and adolescents with overweight or obesity, defined as a BMI SDS above 1.28, to those of normal weight. Secondly, we examined the association between lipid species and cardiometabolic risk profiles. In the intervention study, the primary outcome focused on the changes in lipid species associated with reductions in BMI SDS. The secondary outcome involved assessing the changes in lipid species in relation to cardiometabolic risk profiles.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*