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RESEARCH PAPER

Circulatory levels of lysophosphatidylcholine species in obese adolescents: Findings from cross-sectional and prospective lipidomics analyses

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Asian Indians

Abstract *Background and aims:* Obesity has reached epidemic proportions, emphasizing the importance of reliable biomarkers for detecting early metabolic alterations and enabling early preventative interventions. However, our understanding of the molecular mechanisms and specific lipid species associated with childhood obesity remains limited. Therefore, the aim of this study was to investigate plasma lipidomic signatures as potential biomarkers for adolescent obesity.

Methods and results: A total of 103 individuals comprising overweight/obese (n = 46) and normal weight (n = 57) were randomly chosen from the baseline ORANGE (Obesity Reduction and Noncommunicable Disease Awareness through Group Education) cohort, having been followed up for a median of 7.1 years. Plasma lipidomic profiling was performed using the UHPLC-HRMS method. We used three different models adjusted for clinical covariates to analyze the data. Clustering methods were used to define metabotypes, which allowed for the stratification of subjects into subgroups with similar clinical and metabolic profiles. We observed that lysophosphatidylcholine (LPC) species like LPC.16.0, LPC.18.3, LPC.18.1, and LPC.20.3 were significantly ($p < 0.05$) associated with baseline and follow-up BMI in adolescent obesity. The

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association of LPC species with BMI remained consistently significant even after adjusting for potential confounders. Moreover, applying metabotyping using hierarchical clustering provided insights into the metabolic heterogeneity within the normal and obese groups, distinguishing metabolically healthy individuals from those with unhealthy metabolic profiles.
Conclusion: The specific LPC levels were found to be altered and increased in childhood obesity, particularly during the follow-up. These findings suggest that LPC species hold promise as potential biomarkers of obesity in adolescents, including healthy and unhealthy metabolic profiles.

1. Introduction

Obesity has been identified as a major risk factor for several chronic diseases and is associated with a higher risk of all-cause mortality [1]. The prevalence of overweight and obesity in children and adolescents is increasing worldwide [2], including in developing countries like India [3]. This is a significant challenge for Indian children and adolescents, as undernutrition with growth stunting frequently coexists with obesity, leading to a double burden of underweight and overweight [4]. Obesity is frequently associated with metabolic syndrome (MetS), which is a forerunner of type 2 diabetes (T2DM) and cardiovascular disease (CVD). Cardiovascular risk factors have been studied in younger populations like children, adolescents, and youth worldwide [5,6]. These studies have reported that atherosclerosis risk factors start early in childhood and track into adulthood [6]. A recent study from India showed that there are increased risk factors for atherosclerosis in adolescents and the young [7].

Higher prevalence of cardiovascular risk factors and association with insulin resistance have been shown even among Indian teenagers with normal weight [8]. According to Vikram et al., 2009 [6,8], while obesity can explain 45% of MetS in Asian Indian adolescents, other factors such as deep molecular phenotypes, including lipidomics/metabolomics markers are also important. Interestingly, a recent study by Misra et al. showed that abdominal obesity is highly prevalent among school-going adolescents [9]. Indeed, the recent Obesity Reduction and Awareness of Non-communicable diseases through Group Education (ORANGE) cohort study by our group showed that the prevalence of prediabetes in girls of 12–19 years was 5.4% [10] along with an incidence rate of 20.2 per 1000 person-years for developing dysglycemia (diabetes or prediabetes) [11]**.** These findings suggest that the risk factors for T2DM and cardiovascular disease have their manifestations in early adolescence. This necessitates the use of better and more robust biomarkers to detect metabolic changes in early life.

Obesity and related disorders are linked to conventional dyslipidemia; however, not all obese individuals exhibit dyslipidemia, and not all dyslipidemia individuals are obese. This prompts further studies on obesity, considering factors such as genetic background [12], the complex interplay of genetics and environment [13], and epigenetic mechanisms [14]. Further understanding of obesity and its comorbidities could be obtained from the plasma lipidome, which comprises several molecular lipid species [15]. It could also potentially highlight the early phase of pathophysiological changes associated with diseases. However, there are no studies that have investigated the role of lipidomic biomarkers among overweight/obese adolescents in India.

Previous studies have highlighted that Asian Indians with increased waist circumference and waist-hip ratio show a greater degree of central body obesity and insulin resistance, predisposing them to develop T2DM and coronary artery disease a decade earlier than Europeans [16]. This emphasizes a clear need to investigate lipidomic biomarkers that could aid in the understanding of the pathogenesis of obesity in Asian Indian adolescents. Therefore, we investigated baseline plasma lipidome using ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) to identify potential biomarkers for adolescent obesity in a follow-up ORANGE study cohort.

2. Methods

Study participants were recruited from the “ORANGE” study and also from the ORANGE follow-up cohort [11]. The methodology of the ORANGE study has been published elsewhere [17]. Briefly, the ORANGE program aims to assess the prevalence of obesity, hyperglycemia, metabolic syndrome, and dyslipidemias in a representative sample of children and adolescents aged 6–19 years from randomly selected residential apartments in Chennai, South India. A prospective follow-up of the ORANGE cohort was performed after a median of 7.1 years (n = 845; 5928 person-years of follow-up) [11].

For the current study, a total of 137 subjects, comprising overweight/obese and normal-weight subjects, were randomly chosen from the baseline ORANGE cohort, which had follow-up anthropometric and clinical data as well. The study participants include school-aged children, younger adolescents (teens), and older adolescents. Data quality control qualifies 103 individuals for the final data analysis. Overweight/obesity in adolescents was identified based on the WHO age and gender-specific cut points for children and adolescents [18].

Informed consent was obtained from one of the parents in the case of children below 18 years of age, along with

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the child's assent, while for those 18 years and older, the consent was obtained from the participants themselves. The study was conducted according to the ethical guidelines laid down by the Indian Council of Medical Research; approval of the study protocol and informed consent documents were obtained from the Ethics Committee of the Madras Diabetes Research Foundation before the study was undertaken. Anthropometric and clinical measurements were done using standardized methods [10,11,17].

3. Data measurement

The details of lipidomic measurements, including sample preparation, quality controls, data acquisition from the LC - Thermo Q-Exactive Orbitrap® HRMS, data cleaning, and post-processing, are provided in the supplemental file. The lipidomic data was acquired at baseline, and clinical data was acquired at baseline and follow-up from 137 study participants. Serum sample values of 207 metabolites (including four internal standards) were cleaned for further analysis. Firstly, metabolite relative standard deviation values greater than 20 were replaced with NA values in quality control data, resulting in the removal of batch 2 from the dataset. Secondly, metabolites with more than 60% missing values were removed from the data. The remaining samples with less than 60% metabolites missing were imputed with half of the minimum value (min/2) of each metabolite. Afterward, metabolite concentrations were log10-transformed and scaled (mean = 0, sd = 1) to ensure comparability between metabolites. For quality assurance, we have performed a dimension reduction method (principal component analysis) on concentrations for all metabolites, including internal standards in all samples. Internal standard metabolites were traceable among all the metabolites and used as a yardstick to identify other metabolites. We performed principal component analysis across samples to check for batch effect, and it was removed using the "removeBatchEffect" function from package *limma* version 3.54.2 in R. The final data set consisted of values on 41 lipids from 103 subjects.

4. Statistical methods

4.1. Multiple linear regression

Multiple linear regression models were run for the final dataset of 41 lipids from 103 subjects. Three linear regression models were run for each of the 41 lipids individually: (1) a basic model with body mass index (BMI) as dependent variable and metabolite as independent variable; (2) a second model adjusted for age and gender; and (3) a third model adjusted for age, gender, and triglycerides. For models using baseline BMI, covariates measured at baseline were used, and for models with follow-up BMI, covariates measured at follow-up were used. As a sensitivity analysis, we conducted multiple linear regressions separately for individuals who did not change their obesity class status at follow-up and for those whose obesity class changed at follow-up. In all three models for each metabolite, batch ID was added as a covariate to adjust for batch effect. To account for multiple testing, FDR-adjusted p values were used.

4.2. Assumptions of linear regression

The basic assumptions of the linear regression models, namely linearity of the relationship between dependent variable and independent variables, normality of residuals, uncorrelatedness, and homoscedasticity, were tested using the "gvlma" package of R. The four key assumptions are tested by one global statistic and 4 component statistics. One metabolite of each class was sufficiently representative of other class members. All assumptions were satisfied in the case of linear models with follow-up data; however, in linear models with baseline data, 3 out of 4 component statistics and the global statistic indicated a violation of model assumptions. An inverse transformation of baseline BMI rectified this problem. Beta coefficients obtained from linear regression with the transformed dependent variable, reciprocal of BMI in this case, are not very intuitive and are only presented as a complementary analysis. Since the results of linear regression analyses with untransformed BMI agreed with the results of analyses using transformed BMI, they are presented as the main analysis. Further details on the 'gvlma' package and its application to assess assumptions of linear regression can be found elsewhere [19]. For demonstration purposes, the results of 'gvlma' for the full model are shown for only one metabolite in the Supplementary Table S2.

4.3. Clustering

Clustering of metabolites within normal weight and overweight/obese individuals, both at baseline and at follow-up, was performed. The clustering technique used was agglomerative hierarchical clustering in R package "stats" version 4.2.1. Ward's method coupled with Euclidean distance as a dissimilarity metric was the clustering method employed. An optimal number of clusters was determined using 30 indices criteria of the R package "NbClust" version 3.0.1. Heatmaps were used to visualize metabolite distribution within the resulting clusters for each group. Nominal statistical significance was defined as $p < 0.05$. All statistical analyses were performed in RStudio using R version 4.2.1.

5. Results

5.1. Characteristics

Of the 103 participants in this study, 59% were male. Participants' median age was 14 years at the baseline and 22 years at the follow-up, for a median of 7.1 years. Initially, less than half of the participants (44.6%) were classified as overweight or obese, with a median BMI of 18.9 kg/m^2. However, in the follow-up assessment, this proportion increased to 56%, with a median BMI of 24.2 kg/m^2.

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Notably, the median triglyceride level among participants also changed over time, rising from 71 to 104 mg/dL from baseline to the follow-up. The summary of the baseline and follow-up characteristics of the study participants is provided in Table 1.

5.2. Correlation matrix

Lipidomics analysis of samples using UHPLC-HRMS identified 41 lipids composed of phosphatidylcholines, lysophosphatidylcholines (LPCs), triglycerides, sphingomyelins, and acylcarnitines. Fig. 1 illustrates correlations of significantly associated lipids with both baseline and follow-up clinical variables. Triglyceride (TG.54.4.NH4) and four LPCs, LPC (16:0), LPC (18:3), LPC (18:1), and LPC (20:3), were positively correlated with baseline BMI ($p < 0.05$) and follow-up BMI ($p < 0.05$).

5.3. Linear regression analyses

5.3.1. At baseline

In the initial model, where baseline BMI was the dependent variable, and metabolites were the independent variables, adjusting for batch ID, a total of 41 lipids were examined. Among them, only one triglyceride, TG (46:4), and four LPCs, namely LPC (16:0), LPC (18:3), LPC (18:1), and LPC (20:3), showed a significant positive association with baseline BMI.

In the second model, after additional adjustment for age and gender, LPC (16:0), LPC (18:3), and LPC (18:1) maintained their statistical significance. The third model included age, gender, triglycerides, and batch ID as covariates. Interestingly, the four LPCs (LPC (16:0), LPC (18:3), LPC (18:1), and LPC (20:3)) which were found to be statistically significant in the first model remained significant in the third model as well (Table 2). Supplementary Table S3 presents the complete results of the linear regression analysis using untransformed BMI.

5.3.2. At follow-up

The five lipids viz., TG (54:4), LPC (16:0), LPC (18:3), LPC (18:1), and LPC (20:3), which exhibited significant associations with baseline BMI in model 1 (covariates: Model 1- batch ID), were also found to be significantly and positively associated with follow-up BMI. LPC (18:1) and TG (54:4) continued to show significant positive associations with follow-up BMI in both model 2 (Model 2- batch ID, gender, and age) and model 3 (Model 3- batch ID, gender, age, and triglycerides) (Table 3). Notably, LPC (18:3) consistently demonstrated a significant association with BMI across all analyses. The results of the linear regression analysis, which examined the association between all 41 lipids and follow-up BMI, can be found in Supplementary Table S4.

5.4. Sensitivity analysis

In a separate sensitivity analysis, linear regression analyses were performed for individuals in the same obesity class at baseline and follow-up (Table S5), and for those in different obesity classes at these time points (Table S6).

Table 1 Clinical and biochemical characteristics of study participants. Data presented as mean ± SD. $^{*}p < 0.01$, $^{**}p < 0.001$ compared to baseline normal subjects; $^{\#}$ compared to follow-up normal subjects.

Variables	Baseline		Follow-Up	
	Normal subjects (n = 57)	Overweight/obese (n = 46)	Normal subjects (n = 45)	Overweight/obese (n = 58)
Demographic Profile				
Age (Years)	14.4 ± 2.0	14.9 ± 2.1	22.1 ± 2.2	22.0 ± 2.3
Male n (%)	33 (57.9%)	28 (60.9%)	25 (55.6%)	36 (62.1%)
Body Mass Index (BMI) (Kg/m^2)	17.6 ± 2.1	22.6 ± 5.1**	19.8 ± 2.3	28.1 ± 4.0$^{\#\#}$
Waist (cm)	64.0 ± 6.8	75.5 ± 11.4**	71.3 ± 7.5	91.8 ± 11.4$^{\#\#}$
Body fat (%)	14.1 ± 6.7	20.4 ± 9.0**	27.4 ± 8.8	24.1 ± 10.9
Systolic Blood Pressure (mm Hg)	110 ± 10	113 ± 13	113 ± 12	115 ± 12
Diastolic Blood Pressure (mm Hg)	69 ± 7	72 ± 10	73 ± 7	73 ± 9
Biochemical profile				
Fasting plasma glucose (mg/dL)	86.5 ± 5.5	89.9 ± 6.2*	88.2 ± 7.5	85.0 ± 5.6$^{\#}$
Serum cholesterol (mg/dL)	148.5 ± 18.9	144.6 ± 28.6	159.2 ± 30.9	157.3 ± 31
High density lipoprotein (mg/dL)	42.7 ± 14.1	39.8 ± 10.4	40.3 ± 7.3	40.5 ± 8.1
Low density lipoprotein (mg/dL)	85.3 ± 15.4	83.9 ± 21.6	97.8 ± 26.4	98.4 ± 24.9
Serum triglycerides (mg/dL)	71.2 ± 30.6	96.6 ± 61.9*	104.5 ± 66.5	92.0 ± 53.4
HOMA-IR	1.3 ± 0.6	3.6 ± 2.5**	1.80 ± 1.0	3.81 ± 1.87$^{\#\#}$
Hemogram				
Haemoglobin (HB) (g/dl)	13.6 ± 1.4	13.6 ± 1.7	13.9 ± 2.1	14.1 ± 1.6
Red blood cells (RBC) (millions/cumm)	4.2 ± 0.5	4.4 ± 0.6	4.0 ± 0.6	4.3 ± 0.5
White blood cells (WBC) (cells/cumm)	7280 ± 1178	7298 ± 1158	7186 ± 1685	7123 ± 1497
Platelets (lakh/cumm)	2.8 ± 0.5	2.7 ± 0.6	2.8 ± 0.7	2.7 ± 0.6
Lymphocytes (%)	37.4 ± 9.4	37.2 ± 6.3	34.4 ± 8.1	36.1 ± 6.4
Monocytes (%)	9.2 ± 1.8	9.0 ± 2.5	6.4 ± 1.4	6.5 ± 1.6
Eosinophils (%)	4.7 ± 2.6	4.9 ± 2.7	3.8 ± 2.7	4.0 ± 2.4
Neutrophil (%)	48.6 ± 10.1	48.7 ± 7.7	54.8 ± 9.2	53.04 ± 7.1

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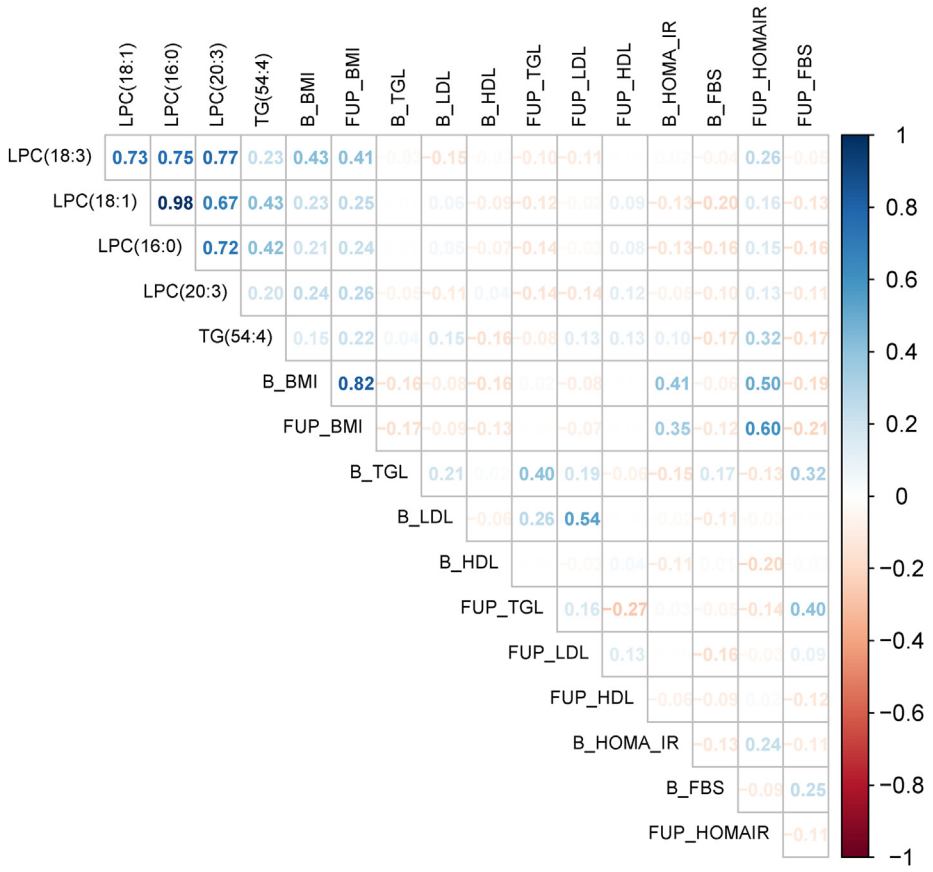

Figure 1 Correlation matrix of significantly associated lysophosphatidylcholine species and clinical variables. Positive correlation coefficient value is shown in blue whereas negative values are shown in red. B indicates variable at baseline and FUP indicates variable at follow-up. Abbreviations- BMI: Body Mass Index, TGL: Triglycerides, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, FBS: Fasting blood sugar. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2 Linear Regression Analyses with BMI as the dependent variable and metabolite (baseline) as the independent variable (n = 103).

At baseline (BMI)	Metabolite	Model 1			Model 2			Model 3		
		β	SE	P value	β	SE	P value	β	SE	P value
	LPC(18:3)	2.905	0.563	0.000[a]	2.831	0.616	0.000[a]	2.998	0.602	0.000[a]
	LPC(18:1)	1.468	0.570	0.012	1.221	0.592	0.042	1.409	0.586	0.018
	LPC(16:0)	1.409	0.598	0.021	1.184	0.611	0.055	1.427	0.606	0.021
	TG (46:4)	0.743	0.640	0.249	–	–	–	–	–	–
	LPC(20:3)	1.485	0.671	0.029	–	–	–	1.544	0.667	0.023

[a] p values significant after FDR adjustment. Covariates: Model 1- batch ID; Model 2- batch ID, gender, and age; Model 3- batch ID, gender, age, and triglycerides.

Table 3 Linear regression Analysis with BMI at follow-up as the dependent variable and metabolites (baseline) as the independent variable (n = 103).

At Follow-up (BMI)	Metabolite	Model 1			Model 2			Model 3		
		β	SE	P value	β	SE	P value	β	SE	P value
	LPC(16:0)	1.454	0.716	0.045	–	–	–	–	–	–
	LPC(18:3)	3.113	0.686	0.000[a]	3.200	0.749	0.000[a]	3.211	0.755	0.000[a]
	LPC(18:1)	1.532	0.683	0.027	–	–	–	–	–	–
	LPC(20:3)	1.680	0.799	0.038	–	–	–	–	–	–
	TG (54:4)	1.318	0.594	0.029	1.236	0.601	0.043	1.236	0.605	0.044

[a] p values significant after FDR adjustment. Covariates: Model 1- batch ID; Model 2- batch ID, gender, and age; Model 3- batch ID, gender, age, and triglycerides.

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The results of sensitivity analyses supported the original findings, although the overall set of lipids was found to be significant at the baseline and the follow-up changed partially. However, the direction and significance of the lipids' association with BMI were consistent with the main regression analyses. For instance, metabolites LPC.18.3 and TG.54.4.NH4 were significantly and positively associated with baseline and follow-up BMI in individuals whose obesity class did not change. In general, associations of lipids with follow-up BMI were more pronounced and significant than associations with baseline BMI.

In addition to the sensitivity analysis, the basic assumptions of the linear regression models, namely linearity of the relationship between dependent variables and independent variables, normality of residuals, uncorrelatedness, and homoscedasticity, were tested using the "gvlma" package of R (Table S2). One metabolite of each class was a representative of other class members. All assumptions were satisfied in the case of linear models with follow-up data, however, in linear models with baseline data, 3 out of 4 component statistics and the global statistics indicated a violation of model assumptions. An inverse transformation of baseline BMI rectified this problem. Beta coefficients obtained from linear regression with the transformed dependent variable, reciprocal of BMI in this case, are not very intuitive and are only presented as a complementary analysis. Since the results of linear regression analyses with untransformed BMI agreed with the results of analyses using transformed BMI (Table S7), they are presented as the main analysis.

5.5. Clustering subject stratification – metabotype

We further employed the metabotype approach to classify individuals into similar metabolic groups so as to identify subpopulations based on their metabolic phenotypes and deliver targeted dietary or medical intervention advice to individuals based on their specific metabolic characteristics [20,21]. To implement the metabotype approach, an unsupervised clustering method was utilized. Hierarchical clustering was performed on the data values of 41 lipids obtained from 103 subjects at both baseline and follow-up. The subjects were divided into four groups for clustering: 1) normal-weight subjects at baseline, 2) overweight/obese subjects at baseline, 3) normal-weight subjects at follow-up, and 4) overweight/obese subjects at follow-up.

The optimal cluster number, determined through the evaluation of 30 different indices criteria and the silhouette method, was found to be 2 for each of the four groups. The resulting clustering solutions are presented in heatmaps displayed in Figs. 2 and 3. From the heatmaps, it is evident that Cluster 1 within each clustering solution exhibited higher concentrations of metabolites compared to Cluster 2, except for three acylcarnitines (AC8 (10:0), AC (10:1), and AC (8:0)), which were found in higher concentrations in Cluster 2 among normal-weight individuals at baseline and follow-up. Furthermore, the metabolites that showed a significant association with BMI in linear regression analyses displayed varying concentration levels between the clusters. This observation suggests the presence of considerable

Figure 2 Agglomerative Hierarchical Clustering of metabolomics data values within 57 Normal weight (at baseline) and 46 Overweight/Obese (at baseline) subjects. The heatmap shows differences in the concentration of 41 metabolites between the identified clusters within each BMI class.

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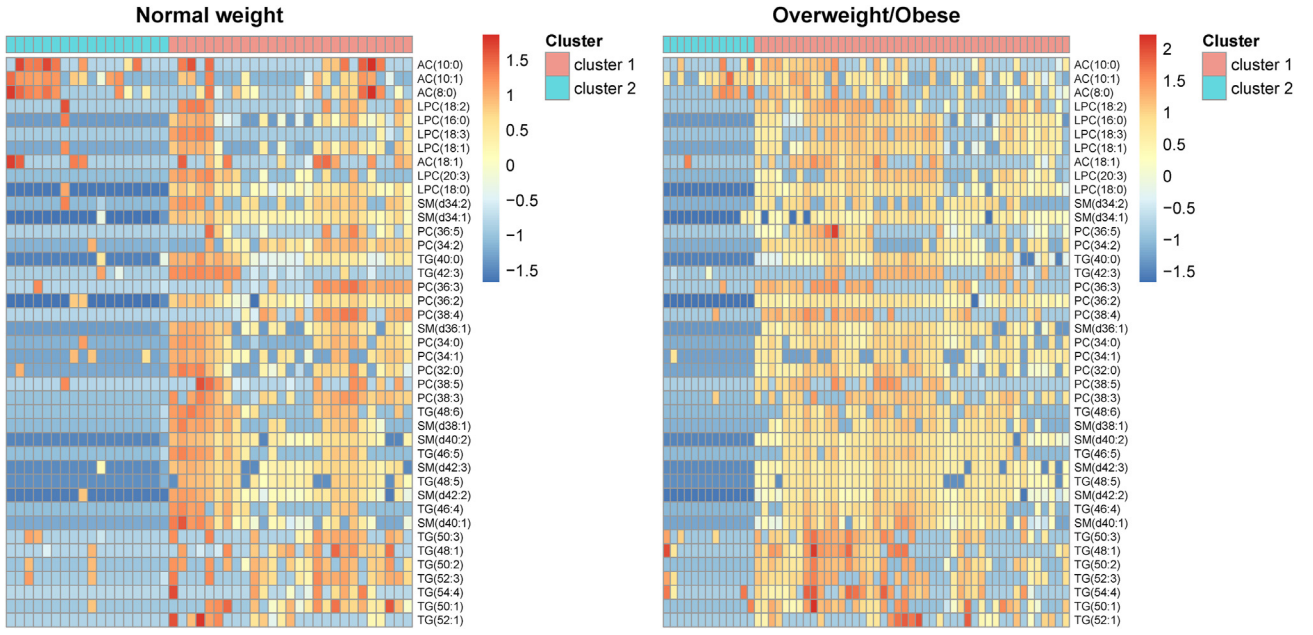

Figure 3 Agglomerative Hierarchical clustering of metabolomics data values within 45 Normal weight (at follow-up) and 58 Overweight/Obese (at follow-up) subjects. The heatmap shows differences in the concentration of 41 metabolites between the identified clusters within each BMI class.

metabolic heterogeneity even within the different classes of obesity.

6. Discussion and conclusion

Obesity causes a slew of metabolic issues and is a risk factor for a variety of chronic diseases [22]. Obesity, earlier only seen in adults, is now seen in adolescents [23]. There is, therefore, an urgent need for high-risk screening and effective intervention educational programs in children to prevent the rapid increase in obesity and other cardiovascular risk factors. Furthermore, there is an imperative need to investigate lipidomic indicators that could aid in the understanding of the pathophysiology of obesity as it is unclear how genetic backgrounds and lifestyle differences affect the performance of lipidomic biomarkers in distinct ethnic populations like Asian Indian adolescents. In this context, the lipidomics profiling conducted in this study reveals a positive association between LPC species and BMI, which becomes significant. The association of LPC species with BMI remained consistently significant even after adjusting for covariates in various models. Furthermore, the study found a significant association of LPC with BMI at the follow-up assessment, suggesting that it could serve as a potential long-term marker rather than just an early indicator of obesity. Finally, the concept of metabotyping was applied in this study, revealing the presence of subclusters within both the normal and overweight/obese groups.

Phospholipases are enzymes that play a crucial role in the metabolism of phospholipids, which are a major component of cell membranes [24]. These enzymes catalyze the hydrolysis of phospholipids into their constituent components, often yielding products such as fatty acids and lysophospholipids. LPC is a specific lysophospholipid that results from the hydrolysis of phosphatidylcholine (PC) by phospholipase A2. LPC can be derived from various sources, including cellular membranes, secreted enzymes, lipoproteins, and dietary sources [25]. There is increasing evidence to support the role of circulating LPC in obesity, and it has also been studied in the context of inflammation [26]. LPCs are signaling molecules with numerous biological roles, including the regulation of cellular proliferation, invasion, and inflammation [27]. Given that obesity is linked to persistent low-grade inflammation, one could anticipate a rise in LPCs because of obesity [28]. Indeed, higher plasma levels of LPCs have been reported in T2DM [29]. Additionally, studies on obese diabetic db/db mice have shown a higher level of liver and skeletal muscle LPCs, and this evidence supports the notion that LPC could be involved in mediating insulin resistance in obesity [28,30].

Although LPCs have been linked to obesity, the findings have not been consistent across investigations. A study by Wang et al. [31], demonstrated lower levels of LPC.18.1, LPC.18.2, LPC.20.0, LPC.20.1, and LPC.20.2 among obese children compared with non-obese children, out of which only LPC.18.1 is common. This is in contrast to our findings, where we reported that LPC species like LPC.16.0, LPC.18.3, LPC.18.1, and LPC.20.3 were significantly associated with both at the baseline and follow-up BMI in adolescent obesity. Another study by Bagheri et al. [32] showed a higher level of LPC 16:1 in the obese group, while other LPC species were present at higher levels in non-obese subjects. Interestingly, a study on monozygotic twins reported a positive correlation of LPC 16:0 and LPC 18:0 with BMI, regardless of genetic factors [28]. Kim et al. (2010) [33] also reported higher levels of LPC 14:0, LPC 16:0, and

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LPC 18:0 in overweight/obese men. Pro-inflammatory actions such as the expression of adhesion molecules, the release of chemotactic factors, and enhanced production of reactive oxygen species have been attributed to saturated and monounsaturated LPCs such as LPC16:0 and LPC18:1 [25]. The present study shows for the first time that four LPCs (16.0, 18.3, 18.1, and 20.3) had a significant and positive association with baseline BMI, even after adjusting for potential confounders. These findings support the interplay between BMI and LPCs, which may play an important role in adolescent obesity.

Our data are in support of recent studies demonstrating that plasma LPC levels are higher in individuals with obesity [28]. Thus, evidence is emerging to indicate that metabolic disorders are positively associated with elevated LPCs. Interestingly, we were unable to detect any differences in the LPC profile when comparing obese and healthy adolescents, which might be due to the issue of statistical power. Hence, further studies are needed to understand the mechanism underlying this association. Moreover, our analyses are based on single measurements of LPCs, which may not reflect LPC levels over time. Serial measurements of LPCs need to be done at different stages of insulin resistance/obesity to further clarify its role in these disorders.

It is well known that metabolic characteristics can provide insights into an individual's response to factors like health status, diet, and medication [34]. This understanding has led to the concept of personalization in healthcare. In the field of metabolomics, this concept has given rise to the idea of metabotyping, which involves classifying individuals into subgroups based on their metabolic characteristics or phenotypes. This approach utilizes the similarities and dissimilarities in metabolic profiles to identify distinct groups of individuals. By identifying these metabotypes or metabolic phenotypes, targeted dietary or medical interventions can be developed and tailored to the specific needs of each subgroup [20,21]. Our findings extend this observation to the adolescent population, indicating the presence of subclusters in metabolically healthy and unhealthy individuals within each group (normal/obese), highlighting the heterogeneity within these populations. This is consistent with our previous findings, which demonstrated that metabolic obesity could have different clinical repercussions [35] and is characterized by altered systemic inflammatory and adipokine profiles than phenotypic obesity [36]. While lifestyle factors are commonly recognized as influential in the development of obesity, the results of the present study suggest that other factors, such as genetics and environmental influences, may also contribute to the observed metabolic heterogeneity. The identification of subclusters within normal and obese groups provides insights into the metabolic heterogeneity of individuals, which could have implications for personalized healthcare strategies based on their specific metabolic phenotypes.

To our knowledge, this is the first study to report the circulating levels of LPCs in adolescent obesity in the Indian population. The potential value of these biomarkers lies in the early identification of high-risk individuals for obesity and other metabolic abnormalities in the adolescent population. One of the limitations of this study is that although lipidome screening showcases numerous valuable attributes in health science, challenges inherent to this approach continue to exist, especially in the accurate identification of metabolites, which is crucial for the biological interpretation and validation of lipidome data. Secondly, being a cross-sectional study with prospective analysis, the etiological mechanisms, or any causal inferences responsible for the association of LPCs with BMI could not be identified. Future studies will have the opportunity to measure LPCs at different time points of obesity and to simultaneously quantify both plasma LPCs with concomitant measurement of insulin sensitivity, inflammation, and other metabolic variables. Another limitation of the study is that the study participants were grouped according to their body mass index rather than randomly, and therefore, this may produce selection bias, and finally, the sample size is modest. Large-scale prospective studies in well-phenotyped cohorts are required to fairly establish the role of LPCs.

In summary, our study results show a positive association between LPC species and BMI at baseline and at follow-up. Thus, LPC could potentially serve as a long-term marker for obesity. The application of metabotyping revealed subclusters within the normal and obese groups, indicating the presence of metabolically healthy and unhealthy individuals within each group. There is a need for conducting additional studies with a focus on the potential pathophysiological role of LPCs in adolescent obesity, who are also at higher risk of developing metabolic disorders in future.

A statement on the originality

We wish to submit an article entitled **"Circulatory levels of lysophosphatidylcholine species in obese adolescents: findings from cross-sectional and prospective lipidomics analyses"** in Nutrition, Metabolism and Cardiovascular Diseases journal as an Original Article. This is to certify that this manuscript has not been submitted to any other journal and contains no material previously published.

Author's contributions

KG, AJ, VM, and VP conceptualized the work. RH, RMA, and VM helped in recruiting study participants. YVS, DP, SV, SK, DU, and VP were involved in lipidomics data curation and acquisition. Sapna Sharma, Sidra Sidra, HG, AJ, VP, and KG analyzed data and interpreted the results. Sapna Sharma, Sidra Sidra, HG, RMA, VM, AJ, JA, VP, MB, and KG drafted

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the manuscript, edited the manuscript, critically reviewed, and helped in drafting the final version.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [KG], upon reasonable request.

Financial interest

All authors have no relevant financial or non-financial interests to disclose.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2024.02.009.

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