Glycerophosphocholine Metabolites and Cardiovascular Disease Risk Factors in Adolescents

A Cohort Study

Editorial, see p 1651

BACKGROUND: Glycerophosphocholine (GPC) metabolites modulate atherosclerosis and thus risk for cardiovascular disease (CVD). Preclinical CVD may start during adolescence. Here, we used targeted serum lipidomics to identify a new panel of GPCs, and tested whether any of these GPCs are associated, in adolescence, with classical risk factors of CVD, namely excess visceral fat (VF), elevated blood pressure, insulin resistance, and atherogenic dyslipidemia.

METHODS: We studied a population-based sample of 990 adolescents (12–18 years, 48% male), as part of the Saguenay Youth Study. Using liquid chromatography-electrospray ionization-mass spectrometry, we identified 69 serum GPCs within the 450 to 680 *m/z* range. We measured VF with MRI.

RESULTS: We identified several novel GPCs that were associated with multiple CVD risk factors. Most significantly, PC16:0/2:0 was negatively associated with VF ($P=1.4\times10^{-19}$), blood pressure ($P=7.7\times10^{-5}$), and fasting triacylglycerols ($P=9.0\times10^{-5}$), and PC14:1/0:0 was positively associated with VF ($P=3.0\times10^{-7}$), fasting insulin ($P=5.4\times10^{-32}$), and triacylglycerols ($P=1.4\times10^{-29}$). The Sobel test of mediation revealed that both GPCs mediated their respective relations between VF (as a potential primary exposure) and CVD risk factors (as outcomes, P values$<1.3\times10^{-3}$). Furthermore, a GPC shown recently to predict incident coronary heart disease in older adults, PC18:2/0:0, was associated with several CVD risk factors in adolescents; these associations were less strong than those with the newly identified GPCs.

CONCLUSIONS: We identified novel GPCs strongly associated with multiple CVD risk factors in adolescents. These GPCs may be sensitive indicators of obesity-related risk for CVD outcomes in adults, and may improve biological understanding of CVD risk.

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Clinical Perspective

What Is New?

- This is the first adolescent study of serum glycerophosphocholine (GPC) metabolites, some of which are involved in systemic oxidative stress and inflammation.
- We used targeted serum lipidomics to identify a new panel of GPCs, including previously nonstudied GPCs.
- Levels of PC18:2/0:0, recently shown to predict cardiovascular disease (CVD) outcomes in older adults, were associated with CVD risk factors already in adolescents.
- Newly identified GPCs, PC16:0/2:0 and PC14:1/0:0, demonstrated even stronger associations with multiple CVD risk factors.
- Mediation analyses reveal that the former may mediate the relation between excess visceral fat and blood pressure, whereas the latter may mediate the relations between excess visceral fat and both fasting insulin and triacylglycerols.

What Are the Clinical Implications?

- Recent research in older individuals suggests that some GPC metabolites may improve the prediction of CVD outcomes in older adults.
- The newly identified GPCs in this study, associated with CVD risk factors already in adolescents, warrant further investigation in prospective adult studies.
- The development of a lipidomics signature that could facilitate early intervention or treatment of those at risk for CVD, and monitor response to interventions, could help triage limited healthcare resources.
- Further research on GPCs associated with CVD risk might improve biological understanding of disease and identify potential drug targets to impede CVD.

Cardiovascular disease (CVD) is a leading cause of death, disability, and loss of productivity worldwide.[1] Obesity and its cardiometabolic sequelae, namely elevated blood pressure (BP), insulin resistance, and atherogenic dyslipidemia, are major risk factors for CVD.[2,3] It is well established that CVD has a long preclinical phase, which may start as early as during childhood and adolescence.[4]

Obesity, defined by excess body fat, promotes the development of CVD through endocrine/paracrine effects of expanding adipose tissue.[5] These effects may vary by the predilection site of body fat deposition; individuals who accumulate excess fat viscerally rather than subcutaneously are at a higher risk for CVD.[6–12]

One of the key cardiometabolic sequelae of obesity is atherogenic dyslipidemia; it is traditionally assessed with high-abundance lipids, such as cholesterols and triacylglycerols, which circulate at millimolar blood levels. Current advancements in mass spectrometry (MS) now allow the discovery and study of new low-abundance lipids, which circulate at micro- or nanomolar blood levels.[13–16] Recent research in older individuals suggests that some of these low-abundance lipids, eg, glycerophosphocholine (GPC) metabolites, may improve the prediction of CVD outcomes.[17–20] These lipids have not been studied in youth.

GPC metabolites include lysophosphocholines (LPCs) and platelet-activating factors (PAFs), which are groups of lipids that modulate systemic oxidative stress and inflammation.[21–23] Both LPCs and PAFs were thought to consist of a handful of lipid species, but recent MS advancements revealed that they may each comprise many more different lipid species.[15] Structurally, LPCs and PAFs consist of a glycerol backbone to which (1) 1 fatty acid moiety is attached at the *sn*-1 position, (2) another fatty acid moiety is attached at the *sn*-2 position (only in non-lyso species), and (3) a phosphocholine head is attached at the *sn*-3 position.[24] Individual LPCs and PAFs differ by the length and the degree of saturation of the fatty acid moieties, and by the type of linkage these moieties are attached with to the glycerol backbone. Experimental studies demonstrate that these relatively subtle structural differences lead to profound functional differences.[21,22] For the most part, however, structure-function relations of individual LPCs and PAFs remain to be investigated.

Given the above, the present study was aimed at examining a new panel of LPCs and PAFs, in adolescence, for their association with classical CVD risk factors, namely excess visceral fat (VF), elevated BP, insulin resistance, and atherogenic dyslipidemia.[2] We performed this study in a population-based sample of 990 adolescents who have undergone high-fidelity phenotyping, including the identification of the novel panel of LPCs and PAFs with state-of-the-art MS (ie, liquid chromatography-electrospray ionization-mass spectrometry [LC-ESI-MS]) and VF quantification with MRI.[25]

METHODS

Cohort

Adolescent participants (n=1028; age 12–18 years) were recruited from the Saguenay Lac St. Jean region of Quebec, Canada, as part of the SYS (Saguenay Youth Study).[25] The SYS is a cross-sectional study of adolescents and their parents aimed at investigating the etiology and early stages of common cardiometabolic and brain diseases.[25] The SYS includes 486 nuclear families. Written consent (parents) and assent (adolescents) were obtained in accordance with the research ethics committees of the Chicoutimi Hospital (Chicoutimi, QC, Canada) and the Hospital for Sick Children (Toronto, ON, Canada). Targeted serum lipidomics was completed in 990 (479 males and 511 females) of the 1028 participants, from 476 of the 486 families (an average of 2 adolescents per family), and these were the adolescents investigated in the present study.

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Measurements

Targeted Serum Lipidomics

Sera from fasting blood samples were used to identify and quantify GPC species within the 450 to 680 *m/z* range using LC-ESI-MS.[14] We chose the 450 to 680 *m/z* range, because it was expected to contain LPCs and PAFs.[14] LC-ESI-MS is the method of choice for identification of new lipid species with low abundance.[13] A total of 69 distinct serum GPC species were identified and quantified (online-only Data Supplement Table I). As mentioned above, GPCs consist of a glycerol backbone to which a phosphocholine head and 1 or 2 fatty acid moieties are attached. The nomenclature of GPCs, as adopted by the LIPID MAPS consortium,[24] is based on their structure, with the head group being specified first and the individual fatty acid moieties specified second. The head group of GPCs is always a phosphocholine, abbreviated as PC. Individual fatty acid moieties are defined by their length (the first number) and degree of saturation (the second number), with the absence of a prefix implying an acyl linkage, whereas the O and P prefixes indicate alkyl and vinyl linkages, respectively. For example, PC 18:2/0:0 has a fatty acid moiety with 18 carbons and 2 unsaturated bonds attached with an acyl linkage at the *sn*-1 position, and no fatty acid moiety attached at the *sn*-2 position. PC O-16:0/2:0, in contrast, has a fatty acid moiety with 16 carbons and 0 unsaturated bonds attached with an alkyl linkage at the *sn*-1 position, and a 2-carbon acetate attached by an acyl linkage at the *sn*-2 position.

Visceral Adiposity

Visceral adiposity was assessed as a volume of VF measured from T1-weighted MRIs acquired on a 1.0 T scanner (Gyroscan NT; Philips Healthcare) using a semiautomatic method described previously[26]; for this purpose, a 10-mm axial slice (with in-plane resolution 1.56×1.56 mm^2) at the level of the umbilicus was used.

Blood Pressure

Beat-by-beat brachial systolic BP and diastolic BP were measured using Finometer (FMS Finapres). The Finometer is a reliable device for tracking BP in adults and children >6 years of age.[27,28] BP was averaged over a 5-minute period after participants had been seated at rest for 5 minutes. Because (1) systolic rather than diastolic hypertension is predominant among obese children[29] and young adults,[30] and (2) population variance in systolic BP vastly exceeds that in diastolic BP,[31] we chose to study systolic BP and not diastolic BP, as 1 of the 5 main outcomes. Nevertheless, data with diastolic BP are shown in online-only Data Supplement Figure.

Insulin Resistance and Dyslipidemia

Blood samples drawn between 8:00 AM and 10:00 AM following an overnight fast were used to measure serum concentrations of insulin (as an index of insulin resistance) and both triacylglycerols (TGs) and high-density lipoprotein cholesterol (HDL-cholesterol) (as indices of atherosclerotic dyslipidemia[2]). These measurements were made in the Biochemistry Department of Chicoutimi Hospital (Chicoutimi, QC, Canada).

Dietary Intake

Dietary intake was assessed with a 24-hour recall, which is a well-established means of assessing diet; it has been used, for example, in the US National Health and Nutrition Examination Surveys, which are the only nationally representative dietary surveys in the United States.[32,33] All participants underwent the 24-hour recall on a Saturday; it was conducted as an in-person interview by a trained nutritionist.[34] This instrument has been validated for youth in Quebec.[35] The data on total fat (g), saturated fat (% of total) and polyunsaturated fat (% of total), total fiber (g), and total carbohydrate (g) intake were analyzed in the present study.

Physical Activity

Physical activity was assessed with a questionnaire as the number of days per week that the participant engaged in an exercise session lasting at least 20 minutes. This instrument has been validated for youth in Quebec.[35]

Statistical Methods

The main aim of the present study was to examine whether any of the 69 identified GPCs are associated with 5 primary outcome variables: (1) VF (as an index of abdominal adiposity); (2) BP (as a measure of elevated BP); (3) fasting insulin (as an index of insulin resistance); (4) TG; and (5) HDL-cholesterol, as indices of atherogenic dyslipidemia.[2] Before these analyses, statistical outliers (values >3 or <3 standard deviations from the mean) were excluded for all outcome measures, resulting in <1% of available data being removed (average of 0.5%). Skewed data were log transformed. Linear mixed regression modeling assessed the associations between GPCs and the 5 primary outcome variables. All models were adjusted for sex, age, height, and family relatedness. The latter was performed by adding family-specific random intercepts and representing the within-family correlations using the compound symmetry covariance structure of residuals.[36] To examine potentially confounding effects of diet and physical activity, further models adjusted additionally for dietary intake of total fat (g), saturated fat (% of total fat) and polyunsaturated fat (% of total fat), total fiber (g), and total carbohydrates (g), and for physical activity (number of sessions [per week] lasting at least 20 minutes), as well. Statistical analyses were performed by using the lme4 package[37] in R.[38] $P<1.4\times10^{-4}$ was considered significant after Bonferroni correction for multiple comparisons (345 comparisons=5 primary outcomes×69 GPCs). The Sobel test of mediation[39] was used to determine whether selected GPCs mediated the directed relations between VF (as a potential primary exposure) and other CVD risk factors (as outcomes). This test assumes normalcy of product distribution, which is a requirement usually met with a sample size >500. Although our sample size was >500 (n=990), we further assessed the significance of mediation effect with Monte Carlo–based confidence intervals.[40,41]

RESULTS

Basic Characteristics of the Studied Sample of Adolescents

The sample included 990 adolescents (12–18 years, 48% male, Table). The prevalences of overweight (15%) and obesity (12%) were similar to those in the Canadian adolescent population at large.[42] In this sample, VF was associated positively with BP ($P=7.8\times10^{-4}$), insu-

Table. Characteristics of Studied Adolescents

	Median	Interquartile Range
Age, y	14.8	13.4–16.3
Height, cm	162.8	156.3–170.0
Weight, kg	56.5	48.5–65.8
Waist circumference, cm	71.3	66–78
Body mass index, kg/m^2	20.9	18.8–23.6
Visceral fat, cm^3	15.8	10.8–26.3
Total body fat, kg	10.7	6.4–16.4
Systolic blood pressure, mm Hg	120.7	113.5–130.4
Diastolic blood pressure, mm Hg	77.9	71.8–83.3
Fasting insulin, pmol/L	61.0	42.0–84.0
Triacylglycerols, mmol/L	0.91	0.67–1.24
High-density lipoprotein cholesterol, mmol/L	1.37	1.18–1.58

lin (P=3.3×10^{-47}), and TG (P=2.7×10^{-23}), and was associated negatively with HDL-cholesterol (P=3.8×10^{-12}, online-only Data Supplement Table II).

Serum Lipidomics of GPCs

LC-ESI-MS identified a total of 69 serum GPC species within the 450 to 680 *m/z* range[14]; these included 30 LPCs, 19 PAFs, 7 lyso-PAFs, 6 acyl-PAFs, 5 diacyl-GPCs, 1 plasmenyl-PAF, and 1 lyso-plasmalogen (online-only Data Supplement Table I). The LPCs and PAFs contributed the most to the total GPC concentration (74.7% and 23.6%, respectively). GPCs with the highest contributions were LPC 16:0, LPC 18:0, LPC 18:1, and LPC 18:2 (11.9%–17.0%), and PAF 14:0 (6.9%, online-only Data Supplement Table I).

GPCs and CVD Risk Factors

All associations between GPCs and CVD risk factors described below survived Bonferroni correction for multiple comparisons (P<1.4×10^{-4}; 345 comparisons=69 GPCs×5 CVD risk factors).

Visceral Adiposity

Two GPCs were associated positively and 4 GPCs were associated negatively with VF (Figure). The GPC with the strongest positive association was PC 14:1/0:0 (P=3.0×10^{-7}), and the GPC with the strongest negative association was PC 16:0/2:0 (P=1.4×10^{-19}, Figure).

Blood Pressure

Only 1 GPC was associated with systolic BP, and this association was negative (Figure). The associated GPC was PC 16:0/2:0, which was the GPC also most strongly negatively associated with VF (P=7.7×10^{-5}, Figure). Associations with diastolic BP were similar to those with systolic BP (online-only Data Supplement Figure).

Insulin Resistance

A total of 5 GPCs were associated with fasting insulin, and all these associations were positive (Figure). The most strongly associated GPC was PC 14:1/0:0 (P=5.4×10^{-32}), which was the GPC also most strongly positively associated with VF (Figure).

Atherogenic Dyslipidemia

Six GPCs were associated positively and 8 GPCs were associated negatively with fasting TG (Figure). The strongest association was observed with PC 14:1/0:0 (P=1.4×10^{-29}, Figure), which was the GPC also most strongly positively associated with VF and fasting insulin; as with VF and fasting insulin, the association with TG was positive. Regarding HDL-cholesterol, 8 GPCs were associated positively and no GPC was associated negatively. The strongest association was seen with PC 15:1/0:0 (P=1.1×10^{-7}); it was not associated with other CVD risk factors (Figure).

Taken together, in the present sample of adolescents, we observed a total of 34 significant associations between the tested GPCs and CVD risk factors; 21 GPCs were associated with at least 1 CVD risk factor, and 9 of these GPCs were associated with 2 or 3 CVD risk factors (Figure). The GPCs demonstrating the strongest associations with multiple CVD risk factors were PC 16:0/2:0, which showed the strongest negative associations with VF and BP, and PC 14:1/0:0, which showed the strongest positive associations with VF, insulin, and TG (Figure). The associations of these 2 GPCs were robust; they remained virtually unchanged after additional adjusting for dietary intake of total fat, polyunsaturated fat, saturated fat, total fiber or total carbohydrates, or for physical activity (online-only Data Supplement Tables III and IV).

Last, PC 18:2/0:0, which is a GPC that was recently shown to be associated with CVD risk factors in older adults,[17] was also associated with CVD risk factors in the current sample of adolescents. Similarly to the older adults, this GPC was associated negatively with fasting TG (P=2.5×10^{-5}) and VF (P=2.0×10^{-3}), and it was associated positively with HDL-cholesterol (P=7.4×10^{-3}). As such, these results in adolescents replicate previous results in older adults.[17]

GPCs May Mediate the Relations Between VF and Other CVD Risk Factors

Excess visceral adiposity promotes the development of other CVD risk factors, including elevated BP, insulin resistance, and atherogenic dyslipidemia.[8–12] Here, we tested the possibility that the GPCs identified as being strongly associated with VF and BP (PC 16:0/2:0) and with VF, insulin, and TG (PC 14:1/0:0) could mediate the relations between VF and the other CVD risk factors. The Sobel test of mediation, which we used for this purpose, showed that PC 16:0/2:0 did mediate the directed relation between VF

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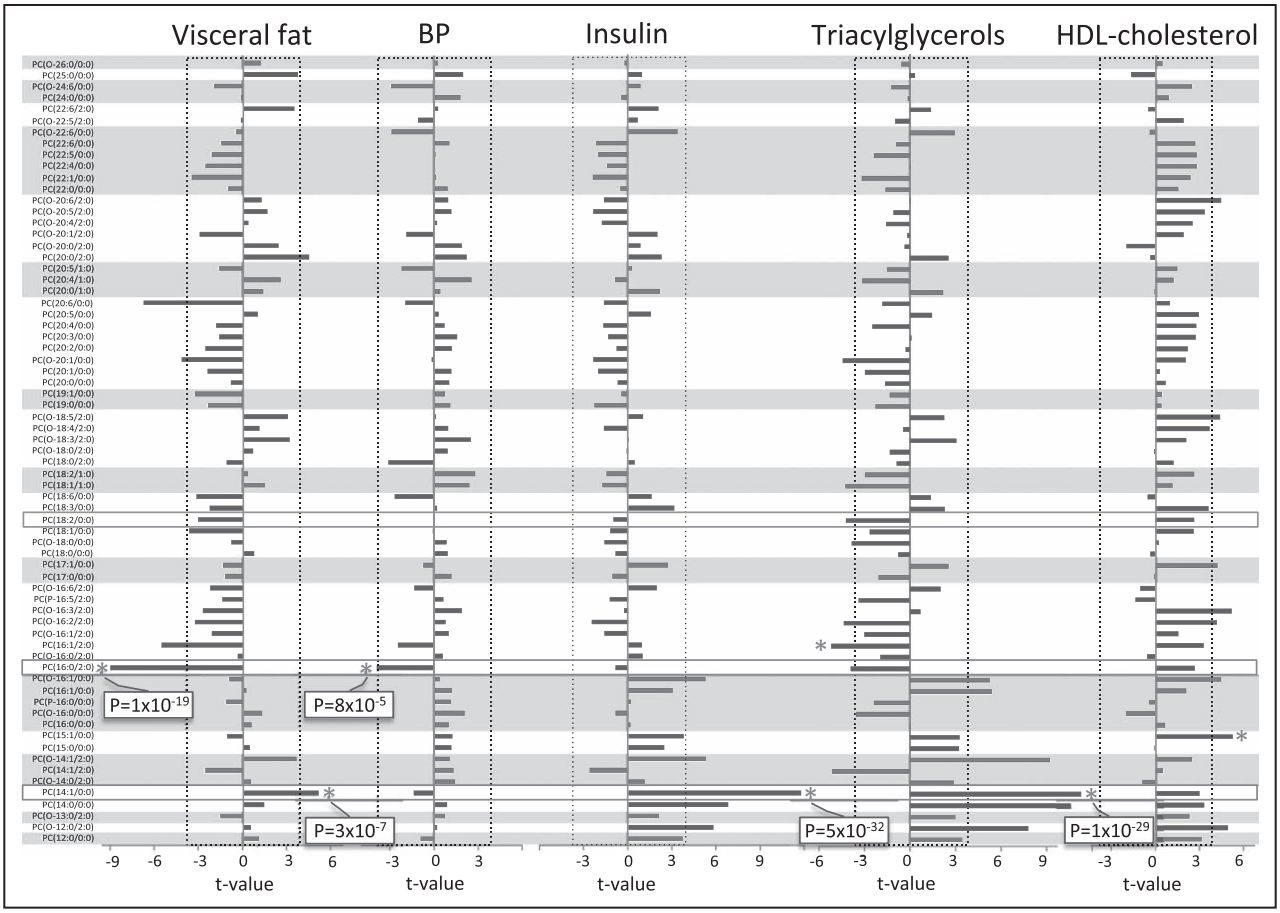

Figure. Associations of 69 identified GPCs with VF, BP, insulin, TGs, and HDL-cholesterol.
Statistical strengths of associations (*t* values) of individual GPCs with VF, BP, insulin, TG, and HDL-cholesterol were assessed with linear mixed regression modeling that adjusted for sex, age, height, and family relatedness. The dotted lines indicate the boundaries of statistical significance after Bonferroni correction for multiple comparisons ($P<1.4\times10^{-4}$, 5 primary outcomes×69 GPC species=345 comparisons); stars indicate the most significant positive and negative associations for each main outcome. Bottom 2 boxes highlight the 2 GPCs that were associated with multiple CVD risk factors in the present study (PC 14:1/0:0 and PC 16:0/2:0) and the top box highlights a GPC (PC 18:2/0:0) associated with CVD risk factors in adults in a previous study.[17] GPCs are ordered according to the length and degree of saturation of fatty acid moieties at *sn*-1 and *sn*-2; with gray and white bands indicating different groups of GPCs on the basis of these features. Associations of GPCs with BP in this figure are shown for systolic BP; those with diastolic BP are presented in online-only Data Supplement Figure. BP indicates blood pressure; CVD, cardiovascular disease; GPC, glycerophosphocholine; HDL, high-density lipoprotein; TG, triacylglycerol; and VF, visceral fat.

(as a potential primary exposure) and BP (as an outcome, $P=1.3\times10^{-4}$, online-only Data Supplement Table V), and that PC 14:1/0:0 did mediate the directed relations between VF (as a potential primary exposure) and each fasting insulin and TG (as outcomes $P=3.9\times10^{-6}$ and 4.6×10^{-6}, respectively, online-only Data Supplement Table V).

DISCUSSION

The present study of a population-based sample of ≈1000 adolescents identified 2 novel lipid species that were associated with multiple CVD risk factors, namely, excess VF, elevated BP, insulin resistance, and atherogenic dyslipidemia. Mediation analyses suggested that 1 of these species may mediate the relation between excess VF and elevated BP, whereas the other species may mediate the relations between excess VF and both insulin resistance and raised serum TG.

The 2 identified lipid species, ie, PC 16:0/2:0 (an acyl-PAF) and PC 14:1/0:0 (an LPC), have not been studied previously. Current advancements in MS now enable the identification and quantification of new, low abundance, lipid species, such as the studied LPCs and PAFs.[21] Targeted LC-ESI-MS used in the present study is an appropriate technique for this purpose, because it is of both high sensitivity and high specificity.[13,14] This technique enables not only the identification of individual fatty acid moieties, but also the elucidation of the type of linkage with which these moieties are attached to the glycerol backbone.[14] This is in contrast with other MS techniques,

such as direct MS, which provide only the total number of carbons and double bonds across attached fatty acid moieties.[15] Thus, using targeted LC-ESI-MS, we were able to distinguish individual lipid species (1) with the same total number of carbons and double bonds but different fatty acid moieties (eg, PC 16:0/2:0 [palmitic acid] and PC 18:0/0:0 [stearic acid]), or (2) with the same fatty acid moieties but different linkages these moieties are attached with to the glycerol backbone (eg, PC 16:0/2:0 [ester linkage] and PC O-16:0/2:0 [ether linkage]). Importantly, such structural variations lead to functional differences.[21,22] In the present study, PC 16:0/2:0 was strongly associated with VF and BP, whereas the above structural homologs, PC 18:0/0:0 and PC O-16:0/2:0, were not associated with any CVD risk factors (Figure).

LPCs and PAFs have been profiled previously but not at the same levels of detail and extent as in the present study.[18,19,43,44] Here we identified and quantified a total of 30 LPCs, 19 PAFs, 7 lyso-PAFs, 6 acyl-PAFs, 5 diacyl-GPCs, 1 plasmenyl-PAF, and 1 lyso-plasmalogen in ≈1000 individuals. One of the profiled species was LPC 18:2 (PC 18:2/0:0). In a previous prospective study of older adults, circulating levels of this species predicted CVD outcomes and were associated with preclinical CVD risk factors.[17] Specifically, lower circulating levels of LPC 18:2 were associated with higher risk of developing myocardial infarction or unstable angina over a 4- to 10-year period (inverse association); they were also associated with higher adiposity and fasting serum TG.[17] Similarly, in our cohort of adolescents, lower circulating levels of LPC 18:2 were associated with higher VF ($P=2.0\times10^{-3}$) and fasting serum TG ($P=2.5\times10^{-5}$); these associations, however, were less strong than those we observed with the newly identified GPCs (Figure).

Sobel tests of mediation suggested the identified lipid species might mediate the observed relations between VF and BP (acyl-PAF 16:0), and between VF and fasting insulin (LPC 14:1). This is consistent with a large body of research indicating that excess body fat (and visceral fat, in particular) promotes BP elevation, insulin resistance, and atherogenic dyslipidemia through the action of fat tissue–produced adipocytokines that can enhance insulin resistance and induce systemic inflammation and oxidative stress.[5–12,45] The specific biological functions of the 2 identified lipid species are not known at present, but experimental research suggests that LPCs and PAFs are modulators of systemic inflammation and oxidative stress.[23,46,47]

The current study has some limitations. It is an observational study, and, as such, it cannot infer causality or directionality of observed relations. Nevertheless, it is a large-scale study investigating ≈1000 individuals with advanced lipidomics profiling (LC-ESI-MS). Further strengths of the current study are the facts that (1) all lipidomics analyses were performed with fasting blood samples and (2) visceral adiposity was assessed with MRI.

In the present study, adjusting for lifestyle factors, such as diet and physical activity, did not alter the observed associations between GPCs and CVD risk factors. These results indicate that diet and physical activity do not confound these associations, but they do not indicate that altering CVD risk factors (eg, visceral adiposity with exercise) would not change circulating levels of relevant GPCs. In the current study, for example, lower exercise is associated with higher VF ($t=-3.96$ $P=7.5\times10^{-5}$) and lower PC 16:0/2:0 ($t=3.0$, $P=2.3\times10^{-3}$), but adjusting for exercise does not affect the association between VF and PC 16:0/2:0 (online-only Data Supplement Table III).

In summary, we identified novel lipid species strongly associated with multiple CVD risk factors in adolescents. Whether these GPCs may serve as novel biomarkers of preclinical CVD requires further investigation in prospective studies. The development of a lipidomics signature that could facilitate early intervention or treatment of those at risk for CVD, and monitor response to interventions, could help triage limited healthcare resources. Our results are relevant to preclinical CVD, because CVD pathology can be seen already during childhood and adolescence and can be reversed if addressed appropriately.[4,48]

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DISCLOSURES

Dr Abrahamowicz is a James McGill Professor at McGill University. Dr Paus is the Tanenbaum Chair in Population Neuroscience at Baycrest and University of Toronto.

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FOOTNOTES

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REFERENCES

1. Mendis S, Puska P, Norrving B, eds. Global Atlas on Cardiovascular Disease Prevention and Control: Policies, Strategies and Interventions. Geneva, Switzerland: World Health Organization; 2011. http://www.who.int/entity/cardiovascular_diseases/publications/atlas_cvd/en/index.html Accessed July 6, 2016.
2. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640–1645. doi: 10.1161/CIRCULATIONAHA.109.192644.
3. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation*. 2005;112:3066–3072. doi: 10.1161/CIRCULATIONAHA.105.539528.
4. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med*. 1998;338:1650–1656. doi: 10.1056/NEJM199806043382302.
5. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. *Cell*. 2014;156:20–44.
6. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB Sr, O'Donnell CJ. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;116:39–48. doi: 10.1161/CIRCULATIONAHA.106.675355.
7. Demerath EW, Reed D, Rogers N, Sun SS, Lee M, Choh AC, Couch W, Czerwinski SA, Chumlea WC, Siervogel RM, Towne B. Visceral adiposity and its anatomical distribution as predictors of the metabolic syndrome and cardiometabolic risk factor levels. *Am J Clin Nutr*. 2008;88:1263–1271.
8. Hayashi T, Boyko EJ, Leonetti DL, McNeely MJ, Newell-Morris L, Kahn SE, Fujimoto WY. Visceral adiposity is an independent predictor of incident hypertension in Japanese Americans. *Ann Intern Med*. 2004;140:992–1000.
9. Abraham TM, Pedley A, Massaro JM, Hoffmann U, Fox CS. Association between visceral and subcutaneous adipose depots and incident cardiovascular disease risk factors. *Circulation*. 2015;132:1639–1647. doi: 10.1161/CIRCULATIONAHA.114.015000.
10. Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, Grundy SM, Khera A, McGuire DK, de Lemos JA. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *JAMA*. 2012;308:1150–1159. doi: 10.1001/2012.jama.11132.
11. Hwang YC, Fujimoto WY, Hayashi T, Kahn SE, Leonetti DL, Boyko EJ. Increased visceral adipose tissue is an independent predictor for future development of atherogenic dyslipidemia. *J Clin Endocrinol Metab*. 2016;101:678–685. doi: 10.1210/jc.2015-3246.
12. Nicklas BJ, Penninx BW, Cesari M, Kritchevsky SB, Newman AB, Kanaya AM, Pahor M, Jingzhong D, Harris TB; Health, Aging and Body Composition Study. Association of visceral adipose tissue with incident myocardial infarction in older men and women: the Health, Aging and Body Composition Study. *Am J Epidemiol*. 2004;160:741–749. doi: 10.1093/aje/kwh281.
13. Hinterwirth H, Stegemann C, Mayr M. Lipidomics: quest for molecular lipid biomarkers in cardiovascular disease. *Circ Cardiovasc Genet*. 2014;7:941–954. doi: 10.1161/CIRCGENETICS.114.000550.
14. Xu H, Valenzuela N, Fai S, Figeys D, Bennett SA. Targeted lipidomics: advances in profiling lysophosphocholine and platelet-activating factor second messengers. *FEBS J*. 2013;280:5652–5667. doi: 10.1111/febs.12423.
15. Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. *Nat Rev Mol Cell Biol*. 2010;11:593–598. doi: 10.1038/nrm2934.
16. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res*. 2010;51:3299–3305. doi: 10.1194/jlr.M009449.
17. Ganna A, Salihovic S, Sundström J, Broeckling CD, Hedman AK, Magnusson PK, Pedersen NL, Larsson A, Siegbahn A, Zilmer M, Prenni J, Arnlöv J, Lind L, Fall T, Ingelsson E. Large-scale metabolomic profiling identifies novel biomarkers for incident coronary heart disease. *PLoS Genet*. 2014;10:e1004801. doi: 10.1371/journal.pgen.1004801.
18. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation*. 2014;129:1821–1831. doi: 10.1161/CIRCULATIONAHA.113.002500.
19. Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, MacIntosh GL, Goudey B, Stern L, Kowalczyk A, Haviv I, White AJ, Dart AM, Duffy SJ, Jennings GL, Kingwell BA. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2011;31:2723–2732. doi: 10.1161/ATVBAHA.111.234096.
20. Jové M, Mauri-Capdevila G, Suárez I, Cambray S, Sanahuja J, Quílez A, Farré J, Benabdelhak I, Pamplona R, Portero-Otín M, Purroy F. Metabolomics predicts stroke recurrence after transient ischemic attack. *Neurology*. 2015;84:36–45. doi: 10.1212/WNL.0000000000001093.
21. Ojala PJ, Hirvonen TE, Hermansson M, Somerharju P, Parkkinen J. Acyl chain-dependent effect of lysophosphatidylcholine on human neutrophils. *J Leukoc Biol*. 2007;82:1501–1509. doi: 10.1189/jlb.0507292.
22. Yan JJ, Jung JS, Lee JE, Lee J, Huh SO, Kim HS, Jung KC, Cho JY, Nam JS, Suh HW, Kim YH, Song DK. Therapeutic effects

of lysophosphatidylcholine in experimental sepsis. *Nat Med*. 2004;10:161–167. doi: 10.1038/nm989.
23. Marathe GK, Pandit C, Lakshmikanth CL, Chaithra VH, Jacob SP, D'Souza CJ. To hydrolyze or not to hydrolyze: the dilemma of platelet-activating factor acetylhydrolase. *J Lipid Res*. 2014;55:1847–1854. doi: 10.1194/jlr.R045492.
24. Fahy E, Cotter D, Sud M, Subramaniam S. Lipid classification, structures and tools. *Biochim Biophys Acta*. 2011;1811:637–647. doi: 10.1016/j.bbalip.2011.06.009.
25. Pausova Z, Paus T, Abrahamowicz M, Bernard M, Gaudet D, Leonard G, Peron M, Pike GB, Richer L, Seguin JR and Veillette S. Cohort profile: the Saguenay Youth Study (SYS). *Int J Epidemiol*. doi: 10.1093/ije/dyw023.
26. Syme C, Abrahamowicz M, Leonard GT, Perron M, Pitiot A, Qiu X, Richer L, Totman J, Veillette S, Xiao Y, Gaudet D, Paus T, Pausova Z. Intra-abdominal adiposity and individual components of the metabolic syndrome in adolescence: sex differences and underlying mechanisms. *Arch Pediatr Adolesc Med*. 2008;162:453–461. doi: 10.1001/archpedi.162.5.453.
27. Tanaka H, Thulesius O, Yamaguchi H, Mino M, Konishi K. Continuous non-invasive finger blood pressure monitoring in children. *Acta Paediatr*. 1994;83:646–652.
28. Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension*. 1989;13(6 pt 1):647–655.
29. Sorof J, Daniels S. Obesity hypertension in children: a problem of epidemic proportions. *Hypertension*. 2002;40:441–447.
30. Grebla RC, Rodriguez CJ, Borrell LN, Pickering TG. Prevalence and determinants of isolated systolic hypertension among young adults: the 1999–2004 US National Health And Nutrition Examination Survey. *J Hypertens*. 2010;28:15–23. doi: 10.1097/HJH.0b013e328331b7ff.
31. Griffith TF, Klassen PS, Franklin SS. Systolic hypertension: an overview. *Am Heart J*. 2005;149:769–775. doi: 10.1016/j.ahj.2005.01.037.
32. Thompson FE, Subar AF. Dietary assessment methodology. In: Bousky C, ed. *Nutrition in the Prevention and Treatment of Disease*. Waltham, MA: Academic Press; 2013: 5–46.
33. Buzzard IM, Faucett CL, Jeffery RW, McBane L, McGovern P, Baxter JS, Shapiro AC, Blackburn GL, Chlebowski RT, Elashoff RM, Wynder EL. Monitoring dietary change in a low-fat diet intervention study: advantages of using 24-hour dietary recalls vs food records. *J Am Diet Assoc*. 1996;96:574–579. doi: 10.1016/S0002-8223(96)00158-7.
34. Nutrific. https://nutrific.fsaa.ulaval.ca/. Accessed June 21, 2013.
35. Berthiaume P, Lavalee C, Villeneuve M, Vigneaut M. Enquête sociale et de santé auprès des enfants et des adolescents québécois [in French]. In: *Volet Nutrition Québec, Canada: Les Publications du Québec*. 2004:19–33.
36. Demidenko E. *Mixed Models: Theory and Applications with R*. Hoboken, NJ: John Wiley & Sons; 2013.
37. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67:1–48.
38. RCoreTeam. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. https://www.R-project.org/.
39. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. *Sociol Methodol*. 1982;13:290–312.
40. Mackinnon DP, Lockwood CM, Williams J. Confidence limits for the indirect effect: distribution of the product and resampling methods. *Multivariate Behav Res*. 2004;39:99. doi: 10.1207/s15327906mbr3901_4.
41. Selig JP, Preacher KJ: Monte Carlo method for assessing mediation: an interactive tool for creating confidence intervals for indirect effects [computer program]. 2008. http://quantpsy.org/. Accessed July 6, 2016.
42. Roberts KC, Shields M, de Groh M, Aziz A, Gilbert J-A. Overweight and obesity in children and adolescents: results from the 2009 to 2011 Canadian Health Measures Survey. *Health Rep*. 2012; Statistics Canada Catalogue no. 82-003. http://www.statcan.gc.ca/pub/82-003-x/2012003/article/11706-eng.pdf. Accessed July 6, 2016.
43. Sigruener A, Kleber ME, Heimerl S, Liebisch G, Schmitz G, Maerz W. Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *PLoS One*. 2014;9:e85724. doi: 10.1371/journal.pone.0085724.
44. Heimerl S, Fischer M, Baessler A, Liebisch G, Sigruener A, Wallner S, Schmitz G. Alterations of plasma lysophosphatidylcholine species in obesity and weight loss. *PLoS One*. 2014;9:e111348. doi: 10.1371/journal.pone.0111348.
45. Pausova Z. From big fat cells to high blood pressure: a pathway to obesity-associated hypertension. *Curr Opin Nephrol Hypertens*. 2006;15:173–178. doi: 10.1097/01.mnh.0000214775.42103.a5.
46. Sevastou I, Kaffe E, Mouratis MA, Aidinis V. Lysoglycerophospholipids in chronic inflammatory disorders: the PLA(2)/LPC and ATX/LPA axes. *Biochim Biophys Acta*. 2013;1831:42–60. doi: 10.1016/j.bbalip.2012.07.019.
47. Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. *Annu Rev Biochem*. 2000;69:419–445. doi: 10.1146/annurev.biochem.69.1.419.
48. Shai I, Spence JD, Schwarzfuchs D, Henkin Y, Parraga G, Rudich A, Fenster A, Mallett C, Liel-Cohen N, Tirosh A, Bolotin A, Thiery J, Fiedler GM, Blüher M, Stumvoll M, Stampfer MJ; DIRECT Group. Dietary intervention to reverse carotid atherosclerosis. *Circulation*. 2010;121:1200–1208. doi: 10.1161/CIRCULATIONAHA.109.879254.

Glycerophosphocholine Metabolites and Cardiovascular Disease Risk Factors in Adolescents: A Cohort Study

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SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1: Serum concentrations of identified glycerophosphocholines

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
Acyl-PAF	538.3a	PC(16:0/2:0)	9.55E-02	(6.85E-02 - 1.26E-01)	15.39	0.06
Acyl-PAF	536.4b	PC(16:1/2:0)	7.11E-02	(5.05E-02 - 1.03E-01)	11.47	0.05
Acyl-PAF	566.4a	PC(18:0/2:0)	1.99E-02	(1.01E-02 - 4.27E-02)	3.20	0.01
Acyl-PAF	508.3d	PC(14:1/2:0)	1.48E-01	(1.12E-01 - 2.03E-01)	23.80	0.10
Acyl-PAF	594.3a	PC(20:0/2:0)	2.17E-01	(1.48E-01 - 3.42E-01)	34.96	0.15
Acyl-PAF	610.3a	PC(22:6/2:0)	6.94E-02	(4.87E-02 - 1.11E-01)	11.18	0.05
TOTAL Acyl-PAF			**6.20E-01**		**100.00**	**0.42**
Diacyl-GPC	570.3a	PC(20:5/1:0)	4.03E-02	(2.38E-02 - 7.65E-02)	3.63	0.03
Diacyl-GPC	580.4a	PC(20:0/1:0)	1.32E-01	(8.97E-02 - 2.07E-01)	11.89	0.09
Diacyl-GPC	572.3a	PC(20:4/1:0)	3.98E-01	(2.52E-01 - 6.09E-01)	35.84	0.27
Diacyl-GPC	548.4a	PC(18:2/1:0)	4.36E-01	(2.93E-01 - 6.59E-01)	39.30	0.30
Diacyl-GPC	550.4a	PC(18:1/1:0)	1.04E-01	(6.60E-02 - 1.62E-01)	9.34	0.07

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
TOTAL Diacyl-GPC			**1.11E+00**		**100.00**	**0.75**
LPC	512.4b	PC(18:6/0:0)	1.19E+00	(9.30E-01 - 1.54E+00)	1.08	0.80
LPC	540.4b	PC(20:6/0:0)	2.16E-01	(1.55E-01 - 2.78E-01)	0.20	0.15
LPC	536.4c	PC(19:1/0:0)	1.23E-01	(5.38E-02 - 1.95E-01)	0.11	0.08
LPC	440.4b	PC(12:0/0:0)	5.64E-02	(3.86E-02 - 8.52E-02)	0.05	0.04
LPC	480.4a	PC(15:1/0:0)	8.62E-03	(5.79E-03 - 1.26E-02)	0.01	0.01
LPC	466.3a	PC(14:1/0:0)	3.60E-02	(2.51E-02 - 5.16E-02)	0.03	0.02
LPC	468.3b	PC(14:0/0:0)	1.81E+00	(1.36E+00 - 2.41E+00)	1.64	1.23
LPC	542.3c	PC(20:5/0:0)	7.66E-01	(5.15E-01 - 1.08E+00)	0.70	0.52
LPC	518.4c	PC(18:3/0:0)	1.32E+00	(9.38E-01 - 1.85E+00)	1.20	0.90
LPC	552.4f	PC(20:0/0:0)	3.52E-01	(2.53E-01 - 5.19E-01)	0.32	0.24
LPC	550.4c	PC(20:1/0:0)	4.12E-01	(3.00E-01 - 5.59E-01)	0.37	0.28
LPC	538.3c	PC(19:0/0:0)	1.68E-01	(1.21E-01 - 2.29E-01)	0.15	0.11
LPC	510.4a	PC(17:0/0:0)	1.93E+00	(1.44E+00 - 2.57E+00)	1.75	1.31

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
LPC	522.4b	PC(18:1/0:0)	1.75E+01	(1.37E+01 - 2.25E+01)	15.87	11.85
LPC	572.3c	PC(22:4/0:0)	1.41E-01	(1.01E-01 - 2.01E-01)	0.13	0.10
LPC	548.4c	PC(20:2/0:0)	3.37E-01	(2.54E-01 - 4.63E-01)	0.31	0.23
LPC	580.4c	PC(22:0/0:0)	1.08E-01	(7.61E-02 - 1.60E-01)	0.10	0.07
LPC	608.5c	PC(24:0/0:0)	4.01E-01	(2.79E-01 - 5.70E-01)	0.36	0.27
LPC	578.3b	PC(22:1/0:0)	4.71E-02	(3.26E-02 - 7.18E-02)	0.04	0.03
LPC	520.3b	PC(18:2/0:0)	1.97E+01	(1.55E+01 - 2.43E+01)	17.86	13.34
LPC	524.4b	PC(18:0/0:0)	2.14E+01	(1.72E+01 - 2.80E+01)	19.49	14.56
LPC	622.4b	PC(25:0/0:0)	1.72E-01	(1.11E-01 - 3.06E-01)	0.16	0.12
LPC	496.3b	PC(16:0/0:0)	2.50E+01	(1.99E+01 - 3.04E+01)	22.69	16.95
LPC	568.4b	PC(22:6/0:0)	1.52E+00	(1.10E+00 - 2.04E+00)	1.38	1.03
LPC	544.4b	PC(20:4/0:0)	7.46E+00	(5.62E+00 - 9.79E+00)	6.78	5.06
LPC	546.3c	PC(20:3/0:0)	2.22E+00	(1.71E+00 - 2.97E+00)	2.02	1.51
LPC	570.3c	PC(22:5/0:0)	5.88E-01	(4.15E-01 - 8.16E-01)	0.53	0.40
LPC	482.4b	PC(15:0/0:0)	9.25E-01	(7.27E-01 - 1.16E+00)	0.84	0.63

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
LPC	494.3b	PC(16:1/0:0)	4.17E+00	(3.21E+00 - 5.35E+00)	3.79	2.83
LPC	508.3b	PC(17:1/0:0)	3.44E-02	(2.49E-02 - 4.72E-02)	0.03	0.02
TOTAL LPC			**1.10E+02**		**100.00**	**74.70**
Lyso-PAF	554.4a	PC(O-22:6/0:0)	8.10E-02	(2.44E-02 - 1.46E-01)	16.65	0.06
Lyso-PAF	582.4a	PC(O-24:6/0:0)	1.60E-02	(9.62E-03 - 3.05E-02)	3.30	0.01
Lyso-PAF	480.4b	PC(O-16:1/0:0)	1.86E-02	(1.32E-02 - 2.52E-02)	3.82	0.01
Lyso-PAF	510.4b	PC(O-18:0/0:0)	9.18E-02	(6.80E-02 - 1.31E-01)	18.87	0.06
Lyso-PAF	482.4c	PC(O-16:0/0:0)	1.82E-01	(1.41E-01 - 2.43E-01)	37.44	0.12
Lyso-PAF	536.4d	PC(O-20:1/0:0)	9.69E-02	(7.11E-02 - 1.35E-01)	19.92	0.07
TOTAL Lyso-PAF			**4.86E-01**		**100.00**	**0.33**
Lyso-Plasmalogen	480.4c	PC(P-16:0/0:0)	9.54E-02	(6.40E-02 - 1.31E-01)	100.00	0.06
TOTAL Lyso-Plasmalogen			**9.54E-02**		**100**	**0.06**
PAF	512.4a	PC(O-16:6/2:0)	3.38E-01	(2.50E-01 - 4.55E-01)	0.98	0.23
PAF	578.3a	PC(O-20:1/2:0)	4.06E-02	(1.69E-02 - 7.26E-02)	0.12	0.03

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
PAF	598.4a	PC(O-22:5/2:0)	1.05E-02	(6.68E-03 - 1.95E-02)	0.03	0.01
PAF	542.3b	PC(O-18:5/2:0)	2.49E-01	(1.66E-01 - 3.71E-01)	0.72	0.17
PAF	518.4b	PC(O-16:3/2:0)	1.82E-01	(1.23E-01 - 2.59E-01)	0.52	0.12
PAF	468.3a	PC(O-12:0/2:0)	3.68E-01	(2.52E-01 - 5.43E-01)	1.06	0.25
PAF	494.3a	PC(O-14:1/2:0)	5.82E-01	(4.40E-01 - 7.73E-01)	1.68	0.40
PAF	524.4a	PC(O-16:0/2:0)	4.10E+00	(2.94E+00 - 5.56E+00)	11.82	2.78
PAF	552.4e	PC(O-18:0/2:0)	4.49E-02	(3.32E-02 - 6.31E-02)	0.13	0.03
PAF	496.3a	PC(O-14:0/2:0)	1.01E+01	(8.43E+00 - 1.23E+01)	29.22	6.88
PAF	580.4b	PC(O-20:0/2:0)	2.67E-02	(1.72E-02 - 4.37E-02)	0.08	0.02
PAF	622.4c	PC(O-26:0/0:0)	6.35E-02	(4.26E-02 - 9.48E-02)	0.18	0.04
PAF	568.4a	PC(O-20:6/2:0)	6.02E-01	(4.03E-01 - 9.28E-01)	1.74	0.41
PAF	544.4a	PC(O-18:4/2:0)	3.51E+00	(2.43E+00 - 5.10E+00)	10.13	2.39
PAF	570.3b	PC(O-20:5/2:0)	3.08E-01	(2.13E-01 - 4.63E-01)	0.89	0.21
PAF	572.3b	PC(O-20:4/2:0)	1.28E-01	(7.80E-02 - 2.19E-01)	0.37	0.09
PAF	546.3b	PC(O-18:3/2:0)	9.42E-01	(6.86E-01 - 1.33E+00)	2.71	0.64

Type	ID by mass	ID by composition	Median	IQR	% Type	% Total
PAF	522.4a	PC(O-16:1/2:0)	4.09E+00	(3.02E+00 - 5.92E+00)	11.79	2.78
PAF	520.3a	PC(O-16:2/2:0)	8.63E+00	(6.72E+00 - 1.09E+01)	24.87	5.86
PAF	482.4a	PC(O-13:0/2:0)	3.33E-01	(2.48E-01 - 4.43E-01)	0.96	0.23
TOTAL PAF			**3.47E+01**		**100.00**	**23.55**
Plasmenyl-PAF	512.4d	PC(P-16:5/2:0)	2.69E-01	(2.09E-01 - 3.55E-01)	100.00	0.18
TOTAL Plasmenyl-PAF			**2.69E-01**		**100.00**	**0.18**

Identified GPCs are grouped by type of GPC. Median and inter-quartile ranges of concentrations are reported, along with the % of the GPC class and the % of the total GPC concentration that each lipid species contributed. Concentrations expressed as pmol-equivalents of PC 13:0/0:0.

Table S2: Associations of CVD-risk factors with visceral fat

CVD-risk factor	Estimate (S.E.)	P-value
Systolic blood pressure	4.3 (1.4)	2.4×10^{-3}
Insulin	0.32 (0.02)	3.3×10^{-47}
Triacylglycerols	0.21 (0.02)	2.7×10^{-23}
HDL-cholesterol	-0.07 (0.01)	3.8×10^{-12}

The associations of cardiometabolic risk factors with visceral fat were assessed using linear mixed effects modeling that adjusted for age, sex, height, and family relatedness.

Table S3: Associations of PC 16:0/2:0 with CVD-risk factors: additional adjusting for diet and physical activity

CVD-risk factor	Variables added to model	Estimate (S.E.)	P-value
Visceral fat	Basic Model	-0.20 (0.02)	$1.4x10^{-19}$
	Dietary total fat	-0.19 (0.02)	$2.0x10^{-18}$
	Dietary polyunsaturated fat	-0.19 (0.02)	$1.6x10^{-18}$
	Dietary saturated fat	-0.19 (0.02)	$5.0x10^{-19}$
	Dietary fiber	-0.19 (0.02)	$2.0x10^{-18}$
	Dietary total carbohydrates	-0.18 (0.02)	$2.9x10^{-16}$
	Physical activity	-0.19 (0.02)	$2.4x10^{-18}$
Blood pressure	Basic Model	-0.0021 (0.0005)	$7.7x10^{-5}$
	Dietary fat	-0.0018 (0.0006)	$1.3x10^{-3}$
	Dietary polyunsaturated fat	-0.0018 (0.0006)	$1.3x10^{-3}$
	Dietary saturated fat	-0.0018 (0.0005)	$1.0x10^{-3}$
	Dietary fiber	-0.0020 (0.0005)	$1.4x10^{-4}$
	Dietary total carbohydrates	-0.0020 (0.0005)	$1.8x10^{-4}$
	Physical activity	-0.0022 (0.0005)	$5.4x10^{-4}$

Associations were assessed using linear mixed effects modeling that adjusted for age, sex, height, and family relatedness in a “Basic Model”, as well as for dietary intake of total fat [g], polyunsaturated fat [% of total fat] and saturated fat [% of total fat]), fiber (g), and

carbohydrates (g), and for physical activity (number of days per week that the participant engaged in an exercise session of at least 20 minutes in duration) in additional models. Blood pressure indicates systolic blood pressure.

Table S4: Associations of PC 14:1/0:0 with CVD-risk factors: additional adjusting for diet and physical activity

CVD-risk factor	Variables added to model	Estimate (S.E.)	P-value
Visceral fat	Basic Model	0.14 (0.03)	3.0×10^{-7}
	Dietary total fat	0.16 (0.03)	3.3×10^{-8}
	Dietary polyunsaturated fat	0.16 (0.03)	3.4×10^{-8}
	Dietary saturated fat	0.16 (0.03)	3.3×10^{-8}
	Dietary fiber	0.16 (0.03)	2.2×10^{-8}
	Dietary total carbohydrates	0.17 (0.03)	2.3×10^{-9}
	Physical activity	0.14 (0.03)	1.2×10^{-6}
Insulin	Basic Model	0.41 (0.03)	5.4×10^{-32}
	Dietary total fat	0.41 (0.03)	2.1×10^{-32}
	Dietary polyunsaturated fat	0.42 (0.03)	1.3×10^{-33}
	Dietary saturated fat	0.42 (0.03)	5.6×10^{-33}
	Dietary fiber	0.42 (0.04)	3.4×10^{-33}
	Dietary total carbohydrates	0.42 (0.03)	4.9×10^{-34}
	Physical activity	0.41 (0.03)	4.5×10^{-32}
Triacylglycerols	Basic Model	0.43 (0.04)	1.4×10^{-29}
	Dietary total fat	0.43 (0.04)	4.3×10^{-29}

Dietary polyunsaturated fat	0.43 (0.04)	$3.0x10^{-28}$
Dietary saturated fat	0.43 (0.04)	$5.4x10^{-29}$
Dietary fiber	0.43 (0.04)	$7.5x10^{-29}$
Dietary total carbohydrates	0.44 (0.04)	$1.2x10^{-30}$
Physical activity	0.44(0.04)	$1.8x10^{-30}$

Associations were assessed using linear mixed effects modeling that adjusted for age, sex, height, and family relatedness in a “Basic Model”, as well as for dietary intake of total fat [g], polyunsaturated fat [% of total fat] and saturated fat [% of total fat]), fiber (g), and carbohydrates (g), and for physical activity (number of days per week that the participant engaged in an exercise session of at least 20 minutes in duration) in additional models.

Table S5: Details of mediation analyses

	***c*-path: VF->outcome (total)**		***c*'-path: VF->outcome (direct)**		***b*-path: GPC->outcome**		***a*-path: VF->GPC**		**Sobel's test**		**Monte Carlo CI**
Mediation model	**Estimate (SE)**	**P**	**Estimate (SE)**	**P**	**Estimate (SE)**	**P**	**Estimate (SE)**	**P**	**z-value**	**P**	**95% CI of Indirect Effect[§]**
VF -> PC16:0/2:0 -> SBP	4.86 (1.44)	7.47E-04	3.32 (1.50)	2.68E-02	-7.35 (2.13)	5.76E-04	-0.21 (0.02)	2.39E-20	3.23	1.26E-03	1.55 (0.65 - 2.56)
VF -> PC14:1/0:0 -> Insulin	0.32 (0.02)	6.58E-47	0.28 (0.02)	5.98E-40	0.25 (0.02)	3.10E-24	0.15 (0.03)	1.98E-07	4.63	3.66E-06	0.04 (0.02 - 0.05)
VF -> PC14:1/0:0 -> Triacylglycerols	0.21 (0.02)	8.68E-23	0.18 (0.02)	9.19E-18	0.24 (0.02)	2.72E-23	0.14 (0.03)	2.23E-07	4.59	4.37E-06	0.03 (0.02 - 0.05)

For all mediation models, we used linear mixed effects models with family-level random intercept (1|fam_id).

For c-path: Outcome ~ c*logVF + age + sex + height + (1|fam_id).

For c' and b-paths: Outcome ~ c'*logVF + b*GPC + age + sex + height + (1|fam_id).

For a-path: GPC ~ a*logVF + age + sex + height + (1|fam_id)

[§]Obtained based on 20,000 simulated parameter sets (a, b), using R-based tool[1]: Selig, J. P., & Preacher, K. J. (2008, June). Monte Carlo method for assessing mediation: An interactive tool for creating confidence intervals for indirect effects. Available from http://quantpsy.org/

Supplemental Figure

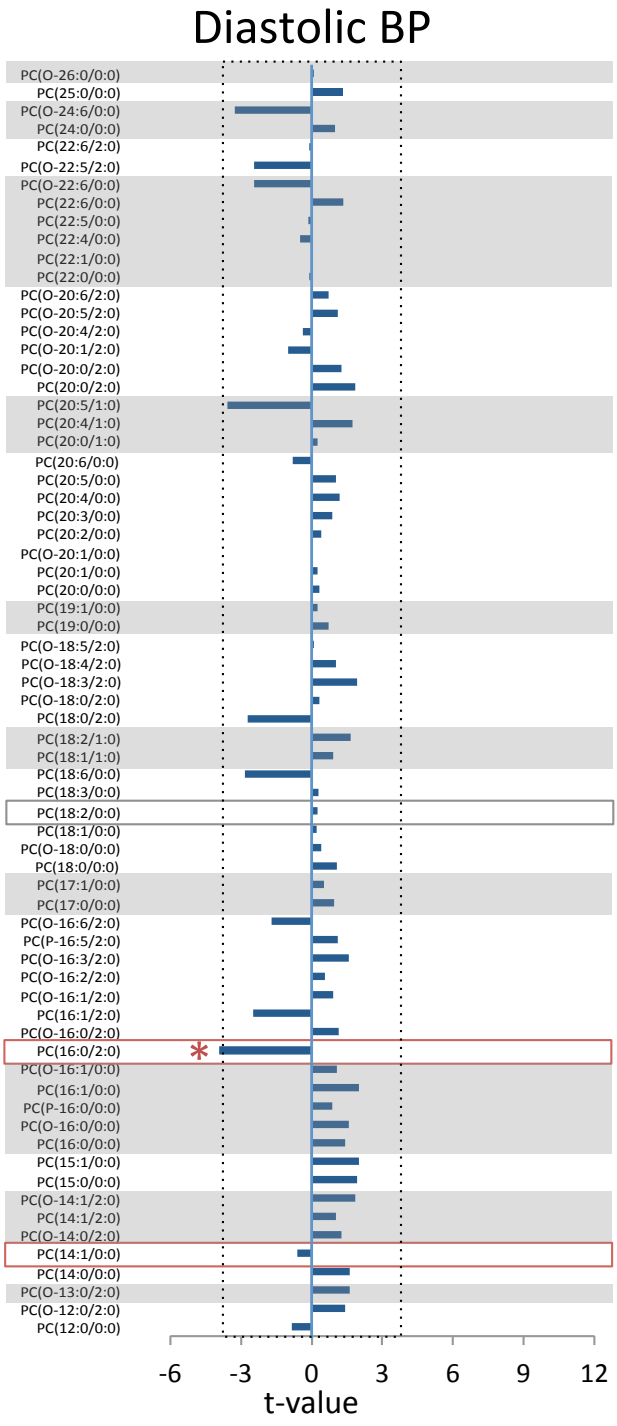

Figure S. Associations of the identified 69 GPCs with diastolic blood pressure

Statistical strengths of associations (t-values) of individual GPCs with diastolic blood pressure were assessed with linear mixed regression modeling that adjusted for sex, age, height

and family relatedness. For the ease of comparison with Figure from the main text, we indicate in this Figure S1 the boundaries of statistical significance after Bonferroni correction for multiple comparisons (dotted lines; $P<1.4x10^{-4}$, 5 primary outcomes x 69 GPC species = 345 comparisons). Star indicates the most significant negative association with diastolic blood pressure. Red boxes highlight the 2 GPCs that were associated with multiple CVD-risk factors in the present study (PC 14:1/0:0 and PC 16:0/2:0) and grey box highlights a GPC (PC 18:2/0:0) associated with CVD-risk factors in adults in a previous study[2]. GPCs are ordered according to the length and degree of saturation of fatty acid moieties at *sn-1* and *sn-2*, with grey and white bands indicating different groups of GPCs based on these features.

Supplemental References

1. Selig JP and Preacher KJ: Monte Carlo method for assessing mediation: An interactive tool for creating confidence intervals for indirect effects [computer program]. http://quantpsy.org/; 2008 [cited 2016 July 6].

2. Ganna A, Salihovic S, Sundstrom J, Broeckling CD, Hedman AK, Magnusson PK, Pedersen NL, Larsson A, Siegbahn A, Zilmer M, Prenni J, Arnlov J, Lind L, Fall T and Ingelsson E. Large-scale metabolomic profiling identifies novel biomarkers for incident coronary heart disease. *PLoS genetics*. 2014;10:e1004801.