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PLASMA DIHYDROCERAMIDE SPECIES ASSOCIATE WITH WAIST CIRCUMFERENCE IN MEXICAN AMERICAN FAMILIES

Manju Mamtani[1], **Peter J. Meikle**[2], **Hemant Kulkarni**[1], **Jacquelyn M. Weir**[2], **Christopher K. Barlow**[2], **Jeremy B. Jowett**[2], **Claire Bellis**[1], **Thomas D. Dyer**[1], **Laura Almasy**[1], **Michael C. Mahaney**[1], **Ravindranath Duggirala**[1], **Anthony G. Comuzzie**[1], **John Blangero**[1], and **Joanne E. Curran**[1]

[1]Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX 78227, USA

[2]Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia

Abstract

Objective—Waist circumference (WC), the clinical marker of central obesity, is gaining popularity as a screening tool for type 2 diabetes (T2D). While there is epidemiologic evidence favoring the WC-T2D association, its biological substantiation is generally weak. Our objective was to determine the independent association of plasma lipid repertoire with WC.

Design and methods—We used samples and data from the San Antonio Family Heart Study of 1208 Mexican Americans from 42 extended families. We determined association of plasma lipidomic profiles with the cross-sectionally assessed WC. Plasma lipidomic profiling entailed liquid chromatography with mass spectrometry. Statistical analyses included multivariable polygenic regression models and bivariate trait analyses using the SOLAR software.

Results—After adjusting for age and sex interactions, body mass index, homeostasis model of assessment – insulin resistance, total cholesterol, triglycerides, high density lipoproteins and use of lipid lowering drugs, dihydroceramides as a class were associated with WC. Dihydroceramide species 18:0, 20:0, 22:0 and 24:1 were significantly associated and genetically correlated with WC. Two sphingomyelin species (31:1 and 41:1) were also associated with WC.

Conclusions—Plasma dihydroceramide levels independently associate with WC. Thus, high resolution plasma lipidomic studies can provide further credence to the biological underpinnings of the association of WC with T2D.

Keywords

waist circumference; lipidomics; central obesity; family studies; Mexican Americans

Address for correspondence: Manju Mamtani, MD, Texas Biomedical Research Institute, PO Box 760549, San Antonio, TX 78245, USA, Phone: (210) 258 9885, Fax: (210) 258 9131, mmamtani@txbiomedgenetics.org.

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INTRODUCTION

Waist circumference (WC), the established surrogate for central obesity,(1, 2, 3, 4) is continuing to gain importance as a potential screening method for Type 2 diabetes (T2D) and insulin resistance (IR).(5, 6, 7) Evidence for the association of WC with these conditions has been gleaned mostly from epidemiological studies.(8, 9, 10, 11) Recently some family based studies have also offered associations compatible with these epidemiological observations.(12) Waist circumference is strongly associated with visceral rather than subcutaneous fat (13, 14, 15, 16) as well as commonly used clinical indexes of lipemic status like total serum cholesterol, serum triglycerides and serum high-density lipoprotein (HDL) cholesterol.(17) On the other hand, reasons (18) for not favoring the use of WC for identification or stratification of T2D risk include i) WC correlates strongly with body mass index (BMI);(19) ii) WC cannot distinguish between subcutaneous and visceral fat; iii) age- and sex-adjusted models do not offer a convincing proof of association between WC and visceral fat; iv) race, age and sex can confound the association of WC with T2D; and v) WC, like BMI, is a highly heritable trait that may capture genetic similarity rather than risk of T2D.(18, 20, 21) It is noteworthy in this regard that direct or associative evidence in favor of WC that is primarily based on the biological underpinnings of T2D/IR pathogenesis is currently weak.

There is now a growing interest in the characterization of the vast repertoire of plasma lipids and their potential contributions to several complex diseases.(22, 23, 24, 25) Of special interest, plasma lipid species as measured by lipidomic profiling are being increasingly associated with the risk of T2D/IR and have been shown to be better predictors than the lipoprotein fractions that are commonly used in clinical practice.(26, 27, 28) In this context, we reasoned that associations of key plasma lipid species with WC could lend further biologically meaningful support to the candidature of WC as a crucial link between obesity and T2D/IR. In this regard, whether WC is associated with the enormous variety of plasma lipids is currently unknown. Here we investigated the potential association of a large array of plasma lipid species with WC using rich data from the ongoing San Antonio Family Heart Study (29) in large and extended Mexican American families – a high risk population for both obesity and T2D/IR.

METHODS AND PROCEDURES

Study participants

The San Antonio Family Heart Study (SAFHS) is an ongoing effort focusing on 1,431 individuals from 42 large, extended Mexican American families in San Antonio. Details of this collaborative study have been described elsewhere.(21, 29) Briefly, the SAFHS aims to quantify the relative contributions of genetic and environmental factors to the risk of developing cardiovascular diseases and metabolic syndrome. Extensive phenotypic assessment for a number of traits related to metabolic syndrome has been performed in these individuals. This project involving the Texas Biomedical Research Institute and the University of Texas Health Science Center at San Antonio was initiated in 1991. Informed consent was obtained from all participants before collection of samples. The Institutional Review Board of the University of Texas Health Sciences Center at San Antonio approved

the study. Using this data, we aimed to determine the association of the plasma lipid species with concurrently and cross-sectionally measured WC.

Lipidomic studies

Samples were analysed in the Metabolomics Laboratory at the Baker IDI Heart and Diabetes Institute, Melbourne, Australia. Analytical methods have been detailed elsewhere.(30) Briefly, a 10μL aliquot of plasma was combined with 200μL $CHCl_3$/MeOH (2:1) and 15μL of internal standard mix and then briefly vortexed. Samples were mixed (rotary mixer, 10 min), sonicated (water bath, 30 min) then allowed to stand (20 min) at room temperature. Samples were centrifuged (16,000×g, 10 min) and the supernatant was dried under a stream of nitrogen at 40°C. The extracted lipids were resuspended in 50μL H_2O saturated BuOH with sonication (10 min), followed by 50μL of 10 mM NH_4COOH in MeOH. Extracts were centrifuged (3,350×g, 5 min) and the supernatant transferred into 0.2mL glass vials with teflon insert caps. Mass spectrometric analysis was performed using 5μL and 1μL [for diacylglycerol and triacylgrlycerol (DG and TG, respectively) species] injections of the lipid extracts.

Identification and quantitation of lipid species was performed by liquid chromatography electrospray ionisation-tandem mass spectrometry using an Applied Biosystems 4000 QTRAP. Liquid chromatography was performed on a Zorbax C18, 1.8 μm, 50 × 2.1 mm column at 300 μL/min using the following gradient conditions; 0% B to 100% B over 8.0 min, 2.5 min at 100% B, a return to 0% B over 0.5 min then 3.0 min at 0% B prior to the next injection. DGs and TGs were separated using the same solvent system with an isocratic flow (100μL/min) of 85% B. Solvent A and B consisted of tetrahydrofuran:methanol:water in the ratios (30:20:50) and (75:20:5) respectively, both containing 10mM NH_4COOH. Precursor ion scans and neutral loss scans were used to identify the lipid species present in human plasma. Quantification of individual lipid species was then performed using scheduled multiple-reaction monitoring (MRM) in positive ion mode.(31, 32) Lipid concentrations were calculated by relating the peak area of each species to the peak area of the corresponding internal standard. CE species were corrected for response factors determined for each species. Total measured lipids of each class were calculated by summing the individual lipid species.

Statistical analysis

Our analyses were designed with the aim to minimize the number of statistical tests. For this, we conducted the statistical analyses at two levels: lipid classes and lipid species. To assess the association of each lipid class with WC, we used a three-step procedure (Fig 1). First, we ran a polygenic regression model in which we included all the lipid species within a given class as covariates (base model, denoted as M_1 in Fig 1). These regression models included age, age^2, sex, age × sex interaction and age^2 × sex interaction, BMI, homeostasis model of assessment – insulin resistance (HOMA-IR), use of lipid lowering drugs, total plasma cholesterol, plasma triglycerides and plasma high-density (HDL) lipoproteins as additional covariates. Second, we ran a model by constraining the regression coefficients of all the lipid species within the class to be equal (alternative model, denoted as M_2 in Fig 1) and third, we ran another model by constraining these regression coefficients to be zero (null

model, denoted as M_3 in Fig 1). The alternative model provided the regression coefficient for the mean of all lipid species within a class while comparison of the log-likelihoods of the alternative model and the null model gave a test for the significance of this mean regression coefficient. Further, comparison of the log-likelihoods from the base model and the alternative model provided a test for heterogeneity of associations within the given lipid class.

We next examined the association of lipid species with WC. In these analyses, we selected the lipid species that represented one of the following two types of lipid classes: i) a lipid class showing statistically significant association of the mean of all lipid species within that class and statistically non-significant within-class heterogeneity; or ii) a lipid class showing statistically significant within-class heterogeneity. For this, we ran polygenic models with the given lipid species as the covariate and tested the statistical significance by constraining this regression coefficient to zero and then estimating χ^2 as $-2(LL_{\text{unconstrained model}} - LL_{\text{constrained model}})$, where LL represents the log-likelihood.

Next, we conducted bivariate trait analyses in which we used each lipid species in a separate bivariate polygenic model along with WC as the two traits. Using this series of models and variance components methods we estimated the genetic correlation (ρ_g) and the environmental correlation (ρ_e) coefficients as described elsewhere.(33) The statistical significance of these correlation coefficients was tested by constraining the respective parameters to zero and estimating the χ^2 statistics as mentioned above. All genetic analyses were conducted using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software.(33) Statistical significance was assessed at a global type I error rate of 0.05 and, where appropriate, the false discovery rate (FDR) approach using the method of Benjamini and Hochberg was used to correct for multiple comparisons.

RESULTS

Study subjects

Cross-sectional data on lipidomic profiles and WC was available on 1208 subjects representing 42 extended families. The mean age of the study sample was 37.0 (SD 14.39) years and there were 292 (36.1%) males. Prevalence of IR was 74.6% based on a HOMA-IR cut-off of 2.6 (the commonly used clinical cut-point for IR) and 56.1% using a cut-off of 3.8 (as specifically recommended(34) for Mexican-American populations). The prevalence of T2D and obesity in this sample was 14.8% and 38.3%, respectively.

Association of plasma lipid classes with WC

To optimize the number of statistical tests being performed, we first conducted our analyses at the level of lipid classes. We studied the association of 23 lipid classes listed in Table 1 with WC. In multivariable polygenic regression models including the aforementioned covariates, we found (Fig 2) that the standardized regression coefficient (class-specific regression coefficient divided by its standard error) for the class of dihydroceramides was the only statistically significant (FDR-corrected $p = 0.0003$) class-level regression coefficient. Interestingly, the FDR-corrected heterogeneity around this mean class effect for

dihydroceramides was not statistically significant (p = 0.5489, Table 1) indicating that the six species constituting this class were likely associated with WC in a homogeneous fashion. No other class of plasma lipids was significantly associated with WC. It is noteworthy in this regard that ceramides have been previously implicated in the pathogenesis of T2D and IR, but we found no significant association of this class with WC (mean class effect = 0.0024, SE = 0.0039, p = 0.5398).

Association of lipid species with WC

We next examined the association of lipid species with WC. Based on the results shown in Table 1, we selected 60 lipid species belonging to the following five classes: dihydroceramides (6 species), dihexocylceramides (6 species), sphingomyelins (19 species), plasmalogen phosphatidylcholines (8 species) and lysophosphatidylcholines (21 species). Consistent with the results at the level of lipid class, we found (Table 2) that four (dhCer 24:1, dhCer 18:0, dhCer 20:0 and dhCer 22:0) of the six dihydroceramide species were statistically significantly associated with WC. In addition, two sphingomyelin species (SM 31:1 and SM 41:1) were also significantly associated with WC. Interestingly however, most of the sphingomyelin species were inversely associated with WC.

Genetic correlations of lipid species with WC

To glean further biologically meaningful support for the clinical utility of WC, we conducted bivariate trait analyses in SOLAR and estimated the genetic and environmental correlations of each significantly associated lipid species with WC. We observed (Table 3) that the dihydroceramide 22:0 and dihydroceramide 24:1 species were significantly genetically correlated with WC. In contrast, the dihydroceramide 18:0 and dihydroceramide 20:0 species were significantly environmentally but not genetically correlated with WC. Of note, both the sphingomyelin species were significantly environmentally but not genetically correlated with WC.

DISCUSSION

Our results demonstrate that dihydroceramides as a lipid class are consistently associated with WC in Mexican Americans. Of the six dihydroceramide species investigated in this study, four species showed a strong association with WC and were significantly genetically as well as environmentally correlated with WC. Notably, we have recently found dihydroceramides to be crucial determinants of future risk of T2D also.(35) In that study (data not shown), we identified 210 lipid species that correlated with diabetes status (FDR ≤ 0.05); of which 128 also predicted progression to diabetes in non-diabetics followed for ~10 years. The single best predictor of progression to diabetes was dhCer 18:0 which was significantly heritable ($h^2 = 0.247$; $p = 1.6\times10^{-9}$) and was markedly increased in diabetics ($p = 2.5\times10^{-7}$) compared to non-diabetics. Those non-diabetics who progressed to diabetes during follow-up also showed higher dhCer 18:0 levels at baseline than non-progressors ($p = 2.2\times10^{-8}$). This predictive relationship was maintained ($p = 1.2\times10^{-4}$) even when baseline fasting glucose and insulin levels were taken into account, indicating that this lipid component appears to be an independent predictor of diabetes risk. Considering these

observations in totality, our results are novel, important and informative in support of the use of WC as a screening tool for T2D/IR risk in Mexican Americans.

The exact mechanistic basis of the observed association between dihydroceramides and waist circumference is currently unknown. It is also unclear whether the plasma dihydroceramide concentrations are better representative of the visceral fat or the subcutaneous fat – a point of debate in the value of WC as a predictor of T2D.(18) It has been long recognized that dihydroceramides are the biosynthetic precursors of ceramides – a lipid class that partakes in the release of cytochrome C from mitochondria.(36) Moreover, there is evidence (37) to suggest that de novo accumulation of ceramides can be detrimental to pancreatic beta cells and can thus initiate the pathogenesis of T2D. Ceramides are also known to participate in the DES1 pathway and thereby modify the risk of T2D/IR. Conceivably, the plasma concentrations of dihydroceramides may be indicative of raised intracellular levels of ceramides especially since the DES2 pathway is involved in the conversion of dihydroceramides to ceramides, (38) but data in this regard are currently lacking. It is also interesting to note from our results that plasma levels of ceramides and dihydroceramides have differential strengths of association with WC and it is unclear which of these classes might more faithfully represent pathways that implicate intracellular ceramide levels. In the absence of direct evidence however, the potential involvement of the DES1/DES2 should be considered only conjectural at this point in time. Future studies therefore need to identify the genetic and biological bases for the strong association of dihydroceramides with waist circumference observed in this study.

An interesting observation made in this study relates to the inverse association of two sphingomyelin species (SM 31:1 and SM 41:1) with waist circumference. This is somewhat surprising considering the general understanding that sphingomyelins are associated with increased risk of obesity, atherosclerosis and metabolic syndrome. However, Cantrell Stanford et al (39) have recently shown a potentially beneficial role of sphingolipids in the plasma membrane of pancreatic beta cells such that higher levels of these sphingolipids can facilitate glucose-stimulated insulin secretion and thereby improve glycemic control. Whether plasma levels of sphingomyelins faithfully capture the sphingolipid levels in beta cells is currently not known and needs to be investigated in future studies.

An important methodological implication of our results relates to the trade-off between simplicity and accuracy in the reporting of lipidomic studies. It is intuitively appealing to summarize and present the results at the level of lipid classes. However, results shown in Table 1 clearly demonstrate that such a class-based synopsis may be an oversimplification of the underlying spectrum of species-specific associations. The within-class heterogeneity of associations should not be neglected. These results also concur with earlier observations that some (but not all) TGs are more significantly associated with HOMA-IR. For example, Kotrenon et al (27) found that only TG 16:0 16:0 18:1 was a significant predictor of HOMA-IR. Together, these results indicate that the enhanced resolution offered by lipidomic studies can be used advantageously to characterize lipidomic associations with various disease states.

In addition to the fact that we did not have information on visceral and subcutaneous lipid profiles, some other limitations of the study also need to be considered. First, ethnicity, age and sex are known to confound the association of WC with the risk of T2D and insulin resistance.(18) Whether these confounders are also operative in the context of the association of WC with the plasma lipid profile remains unknown. In our analytical protocol we included age, sex and interactions thereof as covariates in all the polygenic models. Therefore, our results are unlikely to be affected by the age and sex composition of this cohort. However, this study was conducted only in Mexican-Americans and therefore cannot be generalized to other ethnic populations. Second, WC is a heritable trait (20, 21, 40) and therefore beckons a need for appropriate statistical models in the family settings. Our use of polygenic models isolates the heritable and modifiable components of WC. Our results are thus unlikely to be affected by the heritability of WC.

On the other hand, our study has several strengths. To our knowledge, this is the first study providing associative evidence for the relationship between WC and dihydroceramides. Second, large-scale plasma lipid studies with epidemiologic overtones are still in the stage of infancy. To that end we believe that our observations are both novel and important. Lastly, our results proffer additional and indirect biological credence to the established role of WC in the pathogenesis of T2D and IR in high prevalence scenarios.

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REFERENCES

1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. 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. [PubMed: 19805654]
2. Appel SJ, Harrell JS, Davenport ML. Central obesity, the metabolic syndrome, and plasminogen activator inhibitor-1 in young adults. Journal of the American Academy of Nurse Practitioners. 2005; 17:535–541. [PubMed: 16293162]
3. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. Nutrition research reviews. 2010; 23:247–269. [PubMed: 20819243]
4. Mamtani MR, Kulkarni HR. Predictive performance of anthropometric indexes of central obesity for the risk of type 2 diabetes. Archives of medical research. 2005; 36:581–589. [PubMed: 16099342]
5. Li JK, Ng MC, So WY, Chiu CK, Ozaki R, Tong PC, et al. Phenotypic and genetic clustering of diabetes and metabolic syndrome in Chinese families with type 2 diabetes mellitus. Diabetes/metabolism research and reviews. 2006; 22:46–52. [PubMed: 16021651]

6. Urrutia-Rojas X, Menchaca J, Wadley W, Ahmad N, Lacko A, Bae S, et al. Cardiovascular risk factors in Mexican-American children at risk for type 2 diabetes mellitus (T2DM). The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2004; 34:290–299. [PubMed: 15040998]
7. Ness-Abramof R, Apovian CM. Waist circumference measurement in clinical practice. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2008; 23:397–404. [PubMed: 18682591]
8. Schulze MB, Thorand B, Fritsche A, Haring HU, Schick F, Zierer A, et al. Body adiposity index, body fat content and incidence of type 2 diabetes. Diabetologia. 2012; 55:1660–1667. [PubMed: 22349074]
9. Stevens J, Couper D, Pankow J, Folsom AR, Duncan BB, Nieto FJ, et al. Sensitivity and specificity of anthropometrics for the prediction of diabetes in a biracial cohort. Obesity research. 2001; 9:696–705. [PubMed: 11707536]
10. Warren TY, Wilcox S, Dowda M, Baruth M. Independent association of waist circumference with hypertension and diabetes in African American women, South Carolina, 2007–2009. Preventing chronic disease. 2012; 9:E105. [PubMed: 22632742]
11. Wei M, Gaskill SP, Haffner SM, Stern MP. Waist circumference as the best predictor of noninsulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist/hip ratio and other anthropometric measurements in Mexican Americans--a 7-year prospective study. Obesity research. 1997; 5:16–23. [PubMed: 9061711]
12. Gao JB, Cheng JL, Ding HP, Shen MY. [The disease characteristics and risk factors of type 2 diabetes mellitus in pedigrees]. Zhonghua nei ke za zhi [Chinese journal of internal medicine]. 2011; 50:474–477.
13. Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, et al. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. Obesity (Silver Spring). 2011; 19:402–408. [PubMed: 20948514]
14. Sekikawa A, Kadowaki T, Curb JD, Evans RW, Maegawa H, Abbott RD, et al. Circulating levels of 8 cytokines and marine n-3 fatty acids and indices of obesity in Japanese, white, and Japanese American middle-aged men. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2010; 30:541–548. [PubMed: 20626294]
15. Hayashi T, Boyko EJ, McNeely MJ, Leonetti DL, Kahn SE, Fujimoto WY. Visceral adiposity, not abdominal subcutaneous fat area, is associated with an increase in future insulin resistance in Japanese Americans. Diabetes. 2008; 57:1269–1275. [PubMed: 18299316]
16. Carroll JF, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring). 2008; 16:600–607. [PubMed: 18239557]
17. Sharp DB, Santos LA, Cruz ML. Fatty liver in adolescents on the U.S.-Mexico border. Journal of the American Academy of Nurse Practitioners. 2009; 21:225–230. [PubMed: 19366381]
18. Vazquez G, Duval S, Jacobs DR Jr, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. Epidemiologic reviews. 2007; 29:115–128. [PubMed: 17494056]
19. Ford ES, Mokdad AH, Giles WH. Trends in waist circumference among U.S. adults. Obesity research. 2003; 11:1223–1231. [PubMed: 14569048]
20. Bastarrachea RA, Kent J Jr, Comuzzie AG. Study of the genetic component of cardiovascular risk phenotypes in a Mexican population. Medicina clinica. 2007; 129:11–13. [PubMed: 17570180]
21. Voruganti VS, Lopez-Alvarenga JC, Nath SD, Rainwater DL, Bauer R, Cole SA, et al. Genetics of variation in HOMA-IR and cardiovascular risk factors in Mexican-Americans. J Mol Med (Berl). 2008; 86:303–311. [PubMed: 18204828]
22. Pietilainen KH, Sysi-Aho M, Rissanen A, Seppanen-Laakso T, Yki-Jarvinen H, Kaprio J, et al. Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects--a monozygotic twin study. PloS one. 2007; 2:e218. [PubMed: 17299598]
23. Stevens T, Berk MP, Lopez R, Chung YM, Zhang R, Parsi MA, et al. Lipidomic profiling of serum and pancreatic fluid in chronic pancreatitis. Pancreas. 2012; 41:518–522. [PubMed: 22504378]

24. Del Boccio P, Pieragostino D, Di Ioia M, Petrucci F, Lugaresi A, De Luca G, et al. Lipidomic investigations for the characterization of circulating serum lipids in multiple sclerosis. Journal of proteomics. 2011; 74:2826–2836. [PubMed: 21757039]
25. Lankinen M, Schwab U, Gopalacharyulu PV, Seppanen-Laakso T, Yetukuri L, Sysi-Aho M, et al. Dietary carbohydrate modification alters serum metabolic profiles in individuals with the metabolic syndrome. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2010; 20:249–257.
26. Kotronen A, Seppanen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepaa AL, et al. Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum. Obesity (Silver Spring). 2010; 18:937–944. [PubMed: 19798063]
27. Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, et al. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia. 2009; 52:684–690. [PubMed: 19214471]
28. Zhao C, Mao J, Ai J, Shenwu M, Shi T, Zhang D, et al. Integrated lipidomics and transcriptomic analysis of peripheral blood reveals significantly enriched pathways in type 2 diabetes mellitus. BMC medical genomics. 2013; 6(Suppl 1):S12. [PubMed: 23369247]
29. MacCluer JW, Stern MP, Almasy L, Atwood LA, Blangero J, Comuzzie AG, et al. Genetics of atherosclerosis risk factors in Mexican Americans. Nutrition reviews. 1999; 57:S59–S65. [PubMed: 10391028]
30. Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, et al. Plasma lipidomic analysis of stable and unstable coronary artery disease. Arteriosclerosis, thrombosis, and vascular biology. 2011; 31:2723–2732.
31. Murphy RC, James PF, McAnoy AM, Krank J, Duchoslav E, Barkley RM. Detection of the abundance of diacylglycerol and triacylglycerol molecular species in cells using neutral loss mass spectrometry. Analytical biochemistry. 2007; 366:59–70. [PubMed: 17442253]
32. Smyth I, Hacking DF, Hilton AA, Mukhamedova N, Meikle PJ, Ellis S, et al. A mouse model of harlequin ichthyosis delineates a key role for Abca12 in lipid homeostasis. PLoS genetics. 2008; 4:e1000192. [PubMed: 18802465]
33. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. American journal of human genetics. 1998; 62:1198–1211. [PubMed: 9545414]
34. Qu HQ, Li Q, Rentfro AR, Fisher-Hoch SP, McCormick JB. The definition of insulin resistance using HOMA-IR for Americans of Mexican descent using machine learning. PloS one. 2011; 6:e21041. [PubMed: 21695082]
35. Curran JE, Weir JM, Bellis C, Carless MA, Jowett J, Mahaney MC, et al. Lipidomic profiles as endophenotypes for predicting diabetes progression in Mexican Americans. European Human Genetics Conference. 2011:25.
36. Samad F, Badeanlou L, Shah C, Yang G. Adipose tissue and ceramide biosynthesis in the pathogenesis of obesity. Advances in experimental medicine and biology. 2011; 721:67–86. [PubMed: 21910083]
37. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology. 2003; 144:5159–5165. [PubMed: 12960011]
38. Enomoto A, Omae F, Miyazaki M, Kozutsumi Y, Yubisui T, Suzuki A. Dihydroceramide:sphinganine C-4-hydroxylation requires Des2 hydroxylase and the membrane form of cytochrome b5. The Biochemical journal. 2006; 397:289–295. [PubMed: 16571104]
39. Cantrell Stanford J, Morris AJ, Sunkara M, Popa GJ, Larson KL, Ozcan S. Sphingosine 1-phosphate (S1P) regulates glucose-stimulated insulin secretion in pancreatic beta cells. The Journal of biological chemistry. 2012; 287:13457–13464. [PubMed: 22389505]
40. Bayoumi RA, Al-Yahyaee SA, Albarwani SA, Rizvi SG, Al-Hadabi S, Al-Ubaidi FF, et al. Heritability of determinants of the metabolic syndrome among healthy Arabs of the Oman family study. Obesity (Silver Spring). 2007; 15:551–556. [PubMed: 17372303]

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

- Central obesity is a pathophysiological predeterminant of insulin resistance and type 2 diabetes
- Waist circumference, the clinical marker of central obesity, is a useful screening tool for the prediction of type 2 diabetes risk

WHAT THIS STUDY ADDS?

- This study investigates the independent association of plasma lipidomic repertoire with waist circumference
- Dihydroceramides are independently associated as well as genetically correlated with waist circumference in Mexican Americans

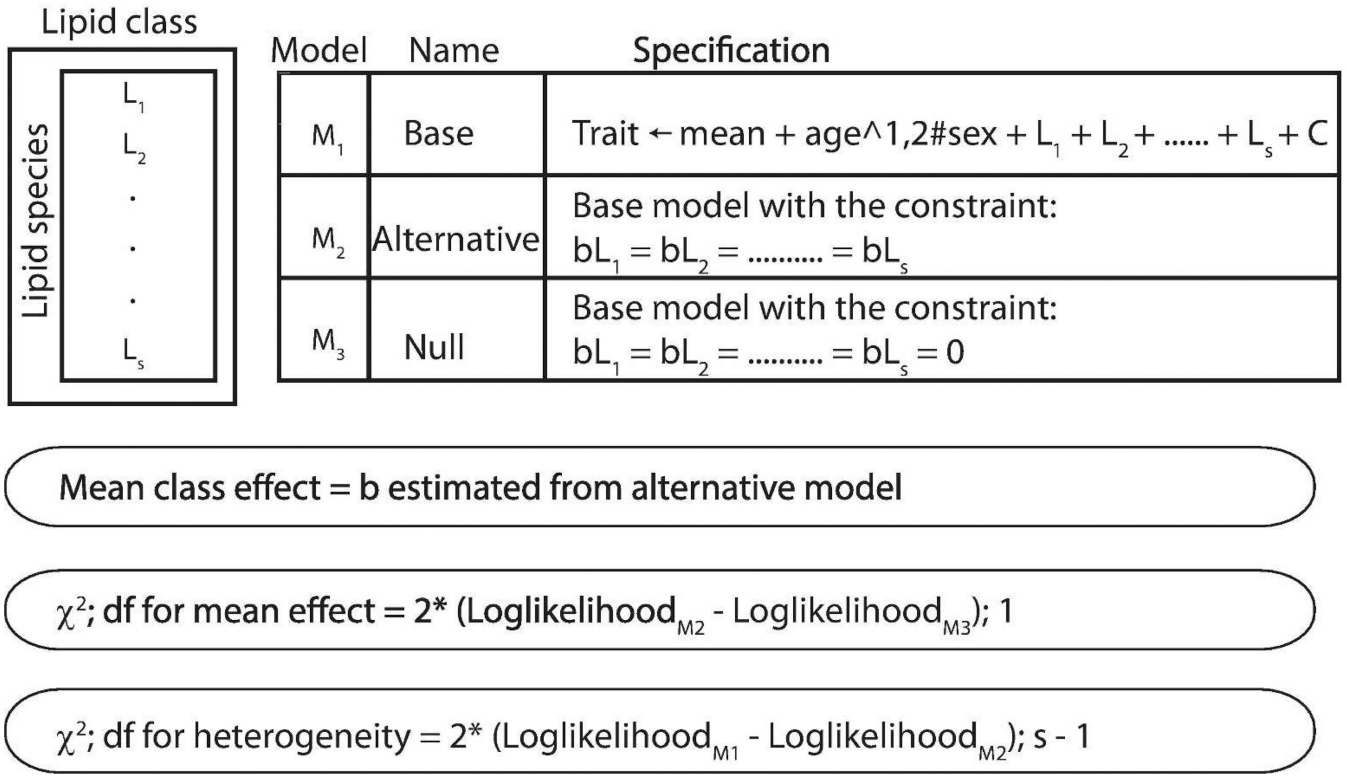

Figure 1. Statistical method used to study the association of a class of lipids with a phenotypic trait

A lipid class is a collection of s lipid species (rectangle on the left). For a given lipid class, three models (M_1, M_2 and M_3 described in a tabular format on the right) were run and log-likelihoods from these three models were used as shown at the bottom to estimate the mean effect, its statistical significance and heterogeneity of within-class associations. For details, refer to text.

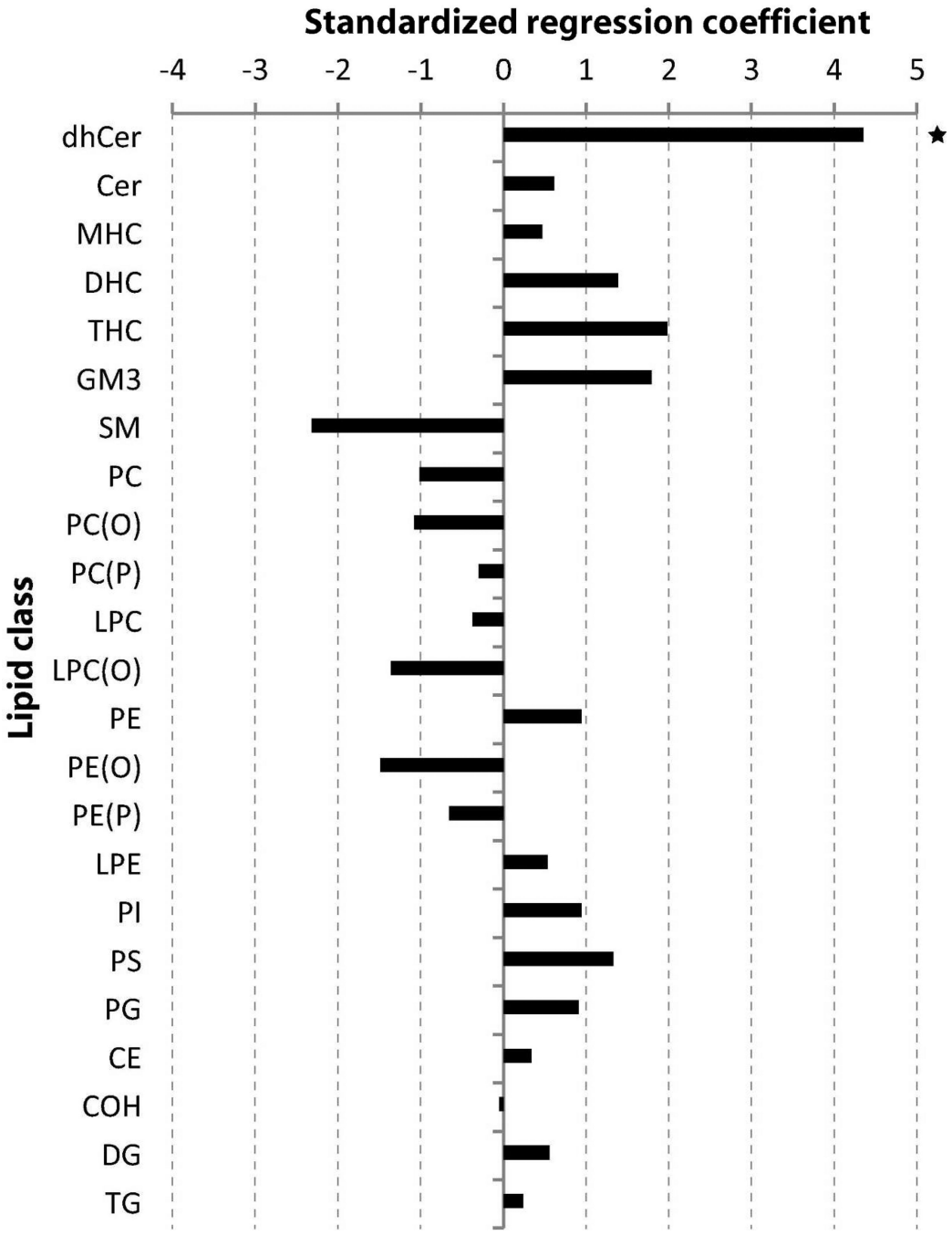

Figure 2. Associations of the lipid classes with WC

The strength of the association of each lipid class is shown as z-score (the class-specific regression coefficient divided by its standard error) estimated from polygenic regression models and the statistical significance of this association is indicated by the asterisk on the right.

Table 1

Within-lipid class heterogeneity of association with WC

Lipid class	Class	Lipid species	Nominal within-class Heterogeneity	FDR adjusted within-class Heterogeneity
Dihydroceramide	dhCer	6	3.92×10^{-2}	0.5489
Ceramide	Cer	6	2.12×10^{-2}	0.3392
Monohexosylceramide	MHC	6	9.49×10^{-2}	0.6090
Dihexosylceramide	DHC	6	1.27×10^{-4}	0.0027
Trihexosylceramide	THC	6	1.24×10^{-1}	0.6090
GM3 ganglioside	GM3	6	7.20×10^{-3}	0.1296
Sphingomyelin	SM	19	9.22×10^{-5}	0.0020
Phosphatidylcholine	PC	45	4.43×10^{-2}	0.5489
Ether-linked phosphatidylcholines	PC(O)	18	4.68×10^{-2}	0.5489
Plasmalogen phosphatidylcholines	PC(P)	8	1.85×10^{-3}	0.0370
Lysophosphatidylcholine	LPC	21	2.31×10^{-3}	0.0439
Ether-linked lysophosphatidylcholines	LPC(O)	6	9.68×10^{-1}	0.9680
Phosphatidylethanolamine	PE	18	4.99×10^{-2}	0.5489
Ether-linked phosphatidylethanolamines	PE(O)	12	1.38×10^{-1}	0.6090
Plasmalogen phosphatidylethanolamines	PE(P)	9	1.82×10^{-1}	0.6090
Lysophosphatidylethanolamine	LPE	6	3.95×10^{-1}	0.7900
Phosphatidylinositol	PI	17	1.20×10^{-1}	0.6090
Phosphatidylserine	PS	7	1.75×10^{-1}	0.6090
Phosphatidylglycerol	PG	4	2.03×10^{-1}	0.6090
Cholesterol ester	CE	26	1.53×10^{-1}	0.6090
Cholesterol	COH	1	---	---
Diacylglycerol	DG	22	7.64×10^{-3}	0.1299
Triaclyglycerol	TG	43	4.86×10^{-2}	0.5489

Table 2

Multivariable association* of plasma dihydroceramide species with WC

Lipid species	β	Nominal p	FDR-p
dhCer 16:0	0.0354	1.19×10^{-2}	4.89×10^{-1}
dhCer 18:0	0.0706	1.82×10^{-5}	**1.06×10^{-3}**
dhCer 20:0	0.0687	1.42×10^{-5}	**8.38×10^{-4}**
dhCer 22:0	0.0615	4.84×10^{-4}	**2.66×10^{-2}**
dhCer 24:0	0.0456	4.98×10^{-3}	2.49×10^{-1}
dhCer 24:1	0.0835	3.51×10^{-7}	**2.11×10^{-5}**
Cer 16:0	0.0244	1.06×10^{-01}	4.89×10^{-1}
Cer 18:0	0.0310	4.15×10^{-02}	4.89×10^{-1}
Cer 20:0	0.0093	3.07×10^{-01}	4.89×10^{-1}
Cer 22:0	−0.0183	1.77×10^{-01}	4.89×10^{-1}
Cer 24:0	−0.0330	4.95×10^{-02}	4.89×10^{-1}
Cer 24:1	0.0351	3.23×10^{-02}	4.89×10^{-1}
DHC 16:0	0.0007	4.83×10^{-1}	4.89×10^{-1}
DHC 18:0	0.0052	3.67×10^{-1}	4.89×10^{-1}
DHC 20:0	0.0413	1.70×10^{-3}	9.18×10^{-2}
DHC 22:0	0.0096	2.57×10^{-1}	4.89×10^{-1}
DHC 24:1	0.0298	2.50×10^{-2}	4.89×10^{-1}
DHC 24:0	0.0091	2.78×10^{-1}	4.89×10^{-1}
SM 31:1	−0.0652	1.26×10^{-4}	**7.18×10^{-3}**
SM 32:1	−0.0196	1.58×10^{-1}	4.89×10^{-1}
SM 32:2	−0.0180	2.00×10^{-1}	4.89×10^{-1}
SM 33:1	−0.0496	3.55×10^{-3}	1.81×10^{-1}
SM 34:1	−0.0034	4.29×10^{-1}	4.89×10^{-1}
SM 34:2	0.0141	2.65×10^{-1}	4.89×10^{-1}
SM 34:3	−0.0217	1.18×10^{-1}	4.89×10^{-1}
SM 35:1	−0.0368	1.56×10^{-2}	4.89×10^{-1}
SM 35:2	−0.0433	5.83×10^{-3}	2.86×10^{-1}
SM 36:1	0.0191	1.45×10^{-1}	4.89×10^{-1}
SM 36:2	−0.0085	3.24×10^{-1}	4.89×10^{-1}
SM 36:3	−0.0320	3.15×10^{-2}	4.89×10^{-1}
SM 37:2	−0.0332	2.43×10^{-2}	4.89×10^{-1}
SM 38:1	−0.0179	1.20×10^{-1}	4.89×10^{-1}
SM 38:2	−0.0207	9.77×10^{-2}	4.89×10^{-1}
SM 39:1	−0.0226	6.47×10^{-2}	4.89×10^{-1}
SM 41:1	−0.0673	3.58×10^{-4}	**2.00×10^{-2}**

Lipid species	β	Nominal p	FDR-p
SM 41:2	−0.0396	7.96×10^{-3}	3.82×10^{-1}
SM 42:1	−0.0362	3.35×10^{-2}	4.89×10^{-1}
LPC 14:0	0.0069	3.28×10^{-1}	4.89×10^{-1}
LPC 15:0	−0.0423	2.45×10^{-3}	1.30×10^{-1}
LPC 16:0	−0.0073	3.10×10^{-1}	4.89×10^{-1}
LPC 16:1	0.0214	7.56×10^{-2}	4.89×10^{-1}
LPC 17:0	−0.0270	2.81×10^{-2}	4.89×10^{-1}
LPC 17:1	−0.0134	1.86×10^{-1}	4.89×10^{-1}
LPC 18:0	0.0134	1.95×10^{-1}	4.89×10^{-1}
LPC 18:1	−0.0137	1.72×10^{-1}	4.89×10^{-1}
LPC 18:2	−0.0237	6.78×10^{-2}	4.89×10^{-1}
LPC 18:3	0.0214	7.85×10^{-2}	4.89×10^{-1}
LPC 20:0	−0.0084	3.06×10^{-1}	4.89×10^{-1}
LPC 20:1	−0.0042	3.98×10^{-1}	4.89×10^{-1}
LPC 20:2	−0.0035	4.04×10^{-1}	4.89×10^{-1}
LPC 20:3	−0.0044	3.88×10^{-1}	4.89×10^{-1}
LPC 20:4	0.0079	3.05×10^{-1}	4.89×10^{-1}
LPC 20:5	0.0198	9.77×10^{-2}	4.89×10^{-1}
LPC 22:0	−0.0083	2.98×10^{-1}	4.89×10^{-1}
LPC 22:1	0.0106	2.48×10^{-1}	4.89×10^{-1}
LPC 22:6	−0.0048	3.68×10^{-1}	4.89×10^{-1}
LPC 24:0	−0.0101	2.68×10^{-1}	4.89×10^{-1}
LPC 26:0	−0.0004	4.89×10^{-1}	4.89×10^{-1}
PC(P-32:0)	0.0237	6.05×10^{-2}	4.89×10^{-1}
PC(P-32:1)	0.0220	7.65×10^{-2}	4.89×10^{-1}
PC(P-34:1)	−0.0115	2.49×10^{-1}	4.89×10^{-1}
PC(P-34:2)	−0.0426	2.91×10^{-3}	1.51×10^{-1}
PC(P-36:2)	−0.0242	5.56×10^{-2}	4.89×10^{-1}
PC(P-36:5)	−0.0026	4.31×10^{-1}	4.89×10^{-1}
PC(P-38:5)	−0.0052	3.66×10^{-1}	4.89×10^{-1}
PC(P-40:5)	0.0105	2.32×10^{-1}	4.89×10^{-1}

*All models are adjusted for age, age^2, sex, age × sex, age^2 × sex, use of lipid lowering medications, body mass index, HOMA-IR, total serum cholesterol, serum triglycerides and serum high density lipoproteins.

FDR-p, Corrected significance value using Benjamini and Hochberg's method of controlling false discovery rate

Table 3

Bivariate trait analyses* of lipid species with WC

Lipid species	ρ_g	P_g	ρ_e	P_e
dhCer 18:0	0.1972	0.2228	0.1053	0.0001
dhCer 20:0	0.2779	0.0889	0.0801	0.0001
dhCer 22:0	0.4221	0.0038	−0.0297	0.0056
dhCer 24:1	0.4557	0.0017	0.0338	<0.0001
SM 31:1	−0.2193	0.0829	−0.0640	0.0008
SM 41:1	−0.2660	0.0727	−0.0423	0.0022

*All models are adjusted for age, age^2, sex, age × sex, age^2 × sex, use of lipid lowering medications, body mass index, HOMA-IR, total serum cholesterol, serum triglycerides and serum high density lipoproteins.

ρ_g, genetic correlation coefficient; P_g, significance value for genetic correlation coefficient; ρ_e, environmental correlation coefficient; P_e, significance value for environmental correlation coefficient