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**PLASMA LIPIDOME INDEPENDENTLY EXPLAINS VARIABILITY IN METABOLIC SYNDROME IN
MEXICAN AMERICAN FAMILIES**

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ABSTRACT

Plasma lipidome is now increasingly recognized as potentially important marker of chronic diseases but the exact extent of its contribution to the inter-individual phenotypic variability in family studies is unknown. Here, we used the rich data from the ongoing San Antonio Family Heart Study (SAFHS) and developed a novel statistical approach to quantify the independent and additive value of the plasma lipidome in explaining metabolic syndrome variability in Mexican American families recruited in the SAFHS. Our analytical approach included two pre-processing steps – principal components analysis of the high resolution plasma lipidomics data and construction of a subject-subject lipidomic similarity matrix. We then used the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software to model the complex family relationships, lipidomic similarities and other important covariates in a variance components framework. Our results suggested that even after accounting for the shared genetic influences, indicators of lipemic status (total serum cholesterol, triglycerides and high-density lipoprotein cholesterol) and obesity the plasma lipidome independently explained 22% variability in the homeostatic model of assessment – insulin resistance (HOMA-IR) trait and 16% – 22% variability in glucose, insulin and waist circumference. Our results demonstrate that plasma lipidomic studies can additively contribute to an understanding of the inter-individual variability in metabolic syndrome.

Supplementary Keywords: lipidomics; obesity; lipids; genetics; insulin resistance; plasma lipidomics; metabolic syndrome; variance components; Mexican Americans

INTRODUCTION

The full spectrum of lipid molecules in human plasma and its potential role in human health and disease are areas of intense interest.(1-9) This interest owes substantially to the sophisticated technologies which make it possible to accurately capture and quantify the human lipidome.(8, 10) Associative evidence gleaned from plasma lipidomic studies promises vital contributions to biomarker research – an important mainstay of the continued efforts for chronic disease prevention. The plasma lipidomic profile of humans exhibits a wide range of diversity (11, 12) and is associated with several conditions including obesity (13, 14), hypertension (14-16), disorders of glucose metabolism (13, 17), metabolic syndrome (MS) (10, 18), cardiovascular diseases (19), cystic fibrosis (20), nicotine consumption (21) and response to anti-lipid treatment (22).

Lipidomic association studies conducted in the context of families have an important consideration that the concentrations of the plasma lipid species might be phenotypically as well as genotypically correlated with each other(23) especially in related individuals. In that case, it is important to dissect out the genetic basis of the phenotypic traits and the potential contribution of the lipidome over and beyond the genetic basis. In this regard, though the diversity of the human lipidome is established (11, 12) the extent to which the lipidome can independently explain the inter-individual variability in the phenotypic expression of disease states is currently unknown. In family studies the contribution of polygenes to a trait is usually quantified as heritability (24-26) and provides a reasonable first-pass estimate of the likelihood of finding genetic variants that may contribute to the trait in question. Similarly, identification

and quantification of a lipidomic variance component can provide a clue to the likelihood of unveiling potential lipidomic biomarkers without being confounded by the genetic basis of the trait.

In this study our aim was to determine the proportion of variability of MS traits that is explained by the plasma lipidome independently of the known confounders. For these analyses we used data from the ongoing San Antonio Family Heart Study (SAFHS) in Mexican Americans. (27) These data and samples provide an appropriate opportunity for the aforementioned investigation for the following reasons: i) MS is very common in Mexican Americans;(28) ii) the SAFHS recruited 42 large and extended pedigrees that help delineate the potential contribution of genetics to MS;(29, 30) iii) high resolution plasma lipidomic studies have been conducted on a large number of SAFHS participants (15); and iv) using these data we have previously demonstrated that specific lipid species are associated with hypertension,(15) central obesity (31) and type 2 diabetes (32).

Here, we used novel variance components (VC) methods to measure the variability in phenotypic traits related to MS that is explained by the plasma lipidome. Using these methods, we addressed the following two research questions. First, what is the degree of variability in MS-related traits that is explained by the plasma lipidome in Mexican American families? Second, is this association independent of and additive to the known association of metabolic syndrome with clinically used measures of lipemic status like total serum cholesterol, serum triglycerides and serum high density lipoprotein (HDL) cholesterol?

METHODS

Study subjects

Data for this study came from Mexican American families recruited in the ongoing San Antonio Family Heart Study (SAFHS). The recruitment and ascertainment procedures used in the SAFHS have been described in details elsewhere.(27, 29, 30) Briefly, the study has now recruited over 1600 subjects from 42 large and extended families with a majority of these subjects having completed up to three additional follow-up visits spaced ~5 years apart. For this study we used the data and samples collected during the first visit only. This study therefore only uses cross-sectional data from the SAFHS cohort. Complete lipidomic and other phenotypic data were available for 1,206 subjects (from 42 families). 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.

Lipidomic studies

We analysed the plasma samples at the Metabolomics Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Australia. The lipid extraction and liquid chromatography/mass spectrometry methods used in these analyses have recently been described in detail.(23) We included a total of 319 lipid species representing 23 lipid classes.

Phenotypic traits

We used data on a total of 14 phenotypic traits related to various components of MS. These included fasting and post-glucose load plasma levels of glucose and insulin (assessed through 2-hour oral glucose tolerance test); two homeostasis model of assessment (HOMA) measures – HOMA-IR representing insulin resistance and HOMA- β representing β -cell function; three measures of obesity – body mass index (BMI), waist circumference and waist-hip ratio (WHR); systolic and diastolic blood pressure; and three serum lipid measures – total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C). The methods of assessment for these traits in the SAFHS participants have been extensively described previously.(27, 30)

Variance Components approach

In the VC approach, the total phenotypic variance (Ω) of a trait is analytically decomposed into components that reflect different characteristics.(33) The three VC models most relevant to the analyses of the lipidomics data are shown in Table 1. The models are the polygenic (P), lipidomic (L) and polygenic-lipidomic (PL) models. In these models, Ω is represented as a sum of components each of which is a multiplication of a similarity matrix and its corresponding VC. In a polygenic model the VC are σ^2_G and σ^2_E which represent the genetic and environmental components, respectively. The corresponding similarity matrices (dimension $n \times n$, where n represents the sample size) for these two components are Φ and I – the kinship and identity matrix, respectively. The elements of a kinship matrix (Φ) indicate the genetic similarity (kinship coefficient) denoted by relationships between each pair of the study subjects. For example, the routinely used kinship coefficients for different relationships are as follows: identical twins, 1; parent-offspring or sibling, 0.5; and grandparent-grandchild, avuncular, half-siblings or double

first cousins, 0.25. Further, the kinship coefficients for 3rd, 4th, 5th and 6th degree relatives are 0.0078, 0.0020, 0.0005 and 0.0001, respectively. The elements of the identity matrix (**I**) are 1 for diagonals and 0 for off-diagonals.

The flexibility of the VC approach to analyses of complex pedigrees permits additional and independent VCs by designing and defining similarity matrices based on various other measures of interest. We exploited this feature of VC by including a term based on the plasma lipidomic similarity between pairs of individuals and the corresponding lipidomic VC. The inclusion of this term alone or in addition to the polygenic component described above was referred to as the L or the PL model (Table 1). Detailed below are the methods used to generate the lipidomic similarity matrix essential for these analyses.

Lipidomic similarity matrix (λ)

Our goal was to express the similarity between a pair of individuals based on the concentrations of 319 plasma lipid species (Figure 1). To reduce the dimensionality of the plasma lipidomic data and to ensure that the reduced dimensions are orthogonal to each other, we conducted principal components analyses and extracted all the factors with an eigenvalue exceeding unity (e=referred hereafter as significant factors). We then rotated this factor solution using a varimax rotation and obtained factor scores for all the significant factors for each individual. Next, we estimated the Euclidean distance between a pair of individuals i and j as follows:

$$d_{ij} = \sqrt{\sum_{k=1}^f (l_{ik} - l_{jk})^2}, \text{ where } l \text{ indicates the score for the } k^{\text{th}} \text{ of the } f \text{ significant factors. We then}$$

scaled this Euclidean distance, as shown in Figure 1, for two reasons: first, this distance conceptually reflects the dissimilarity between two individuals whereas the elements of λ need to quantify the similarity; and second, the elements of the λ matrix are expected to lie in the range (0, 1).

Statistical analyses

Principal components analyses were conducted using Stata 12.0 (Stata Corp, College Station, TX) software package. Contribution of the factors to the explanation of the between-subject variability was assessed by analysis of variance (ANOVA). All regression models additionally included age, age², sex, age x sex interaction, age² x sex interaction and use of anti-diabetic, anti-hypertensive or anti-lipid drugs as additional covariates for adjustment. For running the polygenic, lipidomic and polygenic-lipidomic models we used the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software package.⁽³⁴⁾ In these models, the phenotypic traits were first inverse-normalized before subjecting to analyses. Statistical significance of the estimated parameters (shown in Table 1) was determined by constraining the parameter of interest to zero and then estimating χ^2 (1 degree of freedom) as $-2(LL_{\text{unconstrained model}} - LL_{\text{constrained model}})$, where LL represents the log-likelihood. Correction for multiple tests was done using Bonferroni's method.

RESULTS

Study participants

The mean age of the study participants was approximately 40 years and the study sample was 60% female. The clinical characteristics of the study subjects are detailed in Table 2. Our study subjects had a high prevalence of type 2 diabetes (~15%), central obesity (~48%) and hypertriglyceridemia (~41%). The prevalence of hypertension (SBP>140 mmHg or DBP>90 mmHg or history of anti-hypertensive treatment) was only 13.44%. Over 40% of the study participants had MS indicating that the families of Mexican Americans included in this study represented a high-risk population for MS in the United States.

Principal components analysis of the plasma lipidome

The results of principal components analysis of the 319 lipid species are shown in Figure 2. Using the criterion of a minimum eigenvalue of unity, we retained 35 orthogonal factors that were further optimized using a varimax rotation. Together these 35 factors explained 92.05% variability in the plasma lipidome of study participants. We next considered the possibility that the retained factors may be representative of the lipid classes. For this, we estimated the mean factor score for each factor-lipid class combination and then tested the significance of this potential association using analysis of variance. Our results showed (Figure 3) that for most of the factor-lipid class combinations the mean factor score was near zero. This was supported by the results of analysis of variance ($F = 0.46$, $p = 0.9853$) indicating that the retained factors reflected novel correlations among the lipid species that are not likely to be captured by the lipid classes.

Contribution of polygenes and plasma lipidomics to variability in MS traits

We studied the inter-individual variability in 14 traits related to MS (Table 3) and quantified the proportion of variability explained by the polygenic and lipidomic components. Our results indicated that traits related to glycemia, lipemia, anthropometry and blood pressure were all significantly associated with the polygenic as well as lipidomic VC. For the continuous traits the polygenic contribution ranged from a minimum of 13.88% (for WHR) to a maximum of 35.26% (for systolic blood pressure). In contrast, the contribution of the lipidomic component was minimum (9.06%) for systolic blood pressure and maximum (30.92%) for total serum cholesterol. Not surprisingly, the strongest evidence and strength of the contribution of lipidomic VC was found for the three traits related with lipemic status – total serum cholesterol, serum triglycerides and serum HDL cholesterol levels. Interestingly, statistical significance ($H_0: L^2 = 0$; $H_a: L^2 > 0$) at an alpha of 0.0036 (corrected for 14 tests using Bonferroni method) was obtained for all the traits except systolic blood pressure.

Independent contribution of the lipidomic component to MS traits

We examined the independence of the observed association in two steps. First, since the strongest association of the lipidomic component was with the routinely used indicators of lipemic status (total serum cholesterol, triglycerides and HDL cholesterol) we reasoned that the statistical association between MS traits and the plasma lipidome may have limited clinical use. To explore this, we conducted additional analyses in which we included total serum cholesterol, serum triglycerides and HDL cholesterol as additional covariates in the PL model and reran the analyses shown in Table 3. The results of these analyses are shown in Table 4. We observed (column titled “Before adjusting for BMI”) that except for the blood pressure traits, the

variability in all other traits was significantly and substantially explained by the lipidomic VC. Again, the lipidomic component significantly explained variability in the HOMA-IR and HOMA- β traits but its most significant contribution was to the anthropometric traits capturing obesity and central obesity. Considering that obesity is a major component of MS, we repeated these analyses by additionally accounting for BMI. Our results (Table 4, column titled “After adjusting for BMI”) showed that even after accounting for clinical covariates (age, sex and their linear and non-linear interactions), indicators of lipemic status (total serum cholesterol, serum triglycerides and high-density lipoprotein cholesterol) and obesity (BMI) the lipidomic VC continued to be an independent predictor of other MS-related traits (Bonferroni corrected $p < 0.0036$).

DISCUSSION

Using a novel modification of the VC approach to analysis of complex pedigrees and the rich data from a high-risk sample of Mexican American families recruited in the SAFHS, we found that phenotypic traits reflecting glycemia, insulin resistance, central obesity and general obesity were substantially and significantly determined by the plasma lipidomic profile (results shown in Table 4). This contribution of the plasma lipidome was independent of both the polygenic contribution, routinely used measures of lipemic status and general obesity. Our results therefore underscore the additive value of the plasma lipidomic profile in MS.

Novelty

An important novelty of this study is the method of analysis. We used the VC approach to quantify the explained variability due to plasma lipidome (detailed in (35)) but to successfully capture the variability in the plasma lipidome we resorted to two pre-processing steps – principal components analysis and construction of the lipidomic similarity matrix. This approach had three advantages. Firstly, the validity of using principal components was indirectly indicated by the observation that 92.05% of variability in the plasma lipidome was explained by the retained factors and that these factors were characteristically different from the lipid classes. The other advantage of using principal components was that the solution is, by design, orthogonal and therefore yields to estimation of Euclidean distances in an n-dimensional hyperspace. Secondly, the scaling and representation of the pair-wise Euclidean distances were useful in the construction of the λ matrix. This matrix was then readily used in the VC framework. Methodological variations in this approach that are based on weighting of factors and pre-minimization of correlations is also possible but would not lead to a meaningful improvement since the factor solution used in these analyses already explains most of the variability. Thirdly, the SOLAR software is a flexible modeling environment that permits custom representation of improvised models such as the one used here thereby facilitating the estimation of all related parameters and their statistical significance.(34, 35)

Limitations

There are three limitations to the use of our analytical approach. First, VC approaches used in a cross-sectional study setting can only provide an associative estimate of the explained variability. The interpretations cannot be viewed as causal. With regard to the potential

contribution of plasma lipidomics to MS, there exists a tautological complexity such that plasma lipid species may be proximal, concomitant or distal to the initiation of the disease.(36-38) Also, since obesity is a major component of MS, it can be easily argued that our observations demonstrate the changes in lipidome consequent to rather than leading to obesity. However, the fact that the observed associations continued to hold even after adjusting for obesity indicates that obesity was an unlikely confounder in our analyses. Moreover, both MS and the plasma lipidomic profile can be expected to be, at least, partially controlled by a genetic predisposition. Nevertheless, the continued search for biomarkers of MS invokes the need to quantify the degree of variability in MS that is attributable to the diversity of plasma lipid species. Our methodological approach suited for family studies represents an important first step in that direction.

Second, the VC related to the plasma lipidome is a sum and total of all the lipid species and does not represent any single lipid class or species. Consequently, the explained variability is a complex function of all the lipid species levels as well as their correlation structure. This is both an advantage and a limitation. It is an advantage because it reduces the complexity of the lipidome and offers a first pass screen for the potential use of lipidomic biomarkers in a specified setting. On the other hand, this approach does not permit identification of single (or a combination of) species most contributory to the observed association.

Third, a corollary to the abovementioned limitation here is that even if the proportion of trait variability explained by the plasma lipidome is non-significant or zero, it does not negate the

possibility that one or more lipid species may be significantly associated with the trait. The pre-processing steps used in this study can mask such potential associations at the level of lipid-species. For example, diacylglycerol species and ether lipid deficiencies have been shown to be significantly associated with the risk of incident or prevalent hypertension (14-16) but in this study we found that the plasma lipidome per se was not contributory to the variability in blood pressure. We therefore recommend that use of the analytical approach outlined in this study should not preclude more detailed and lipid species-specific analyses.

Implications

Our observations have two implications. First, we demonstrated 22%, 22%, 20% and 16% independent contribution of the lipidomic VC to variability in HOMA-IR, fasting insulin, waist circumference and fasting glucose, respectively (Table 4). These results imply that the plasma lipidome may have an independent and additive utility in detection of insulin resistance and central obesity – conditions common in Mexican Americans.(28, 39) Lipidomic studies conducted in the past (14, 40-42) have been able to detect some key associations of specific lipid species with insulin resistance but our results indicate that the entire plasma lipidome may be associated with substantial changes in these traits. In the context of VC models as used in this study, it is important to note that these contributions of the plasma lipidome are independent of the polygenic component indicating that the plasma lipidome may be partially tracking the environmental aspects of obesity and insulin resistance. In unison with the potentially contributory genetic pathways,(43) the plasma lipidome can therefore be anticipated to act as a biomarker of obesity and insulin resistance.

Second, we showed that the significant principal components derived from the plasma lipidome were independent of the chemically defined lipid classes (Figure 3). This result indicates that the prevalent concentrations of lipid species in human plasma are likely a result of complex biological pathways that need to be considered rather than the more limited vision restricted to lipid classes. Indeed, future studies need to consider if biologically meaningful information can be gleaned from the correlations among all the plasma lipid species. It is interesting in this respect that only 35 principal components accounted for 92% of overall variability of the plasma lipidome indicating that it may be possible to reduce the redundancy of the lipidome in order to better delineate the biological pathways involved in health and disease.(44)

Conclusions

Using a novel analytical approach and rich data from Mexican American families recruited in the SAFHS, we have demonstrated that high-resolution plasma lipidomic studies can provide substantial and significant improvement in our understanding of inter-individual variability associated with MS. Specifically, the plasma lipidome contributed to 22% variability in HOMA-IR and 16% – 22% variability in glucose, insulin and waist circumference independent of obesity and measures of lipemic status. Future studies need to evaluate the potential role of the plasma lipidome as a biomarker of MS.

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FIGURE LEGENDS

Figure 1. Analytical approach. For details, see text.

Figure 2. Results of principal components analyses. The red curve associates with left ordinate (eigenvalues) while the green curve associates with the right ordinate (explained variability). The abscissa represents the top 50 most significant factors in order of their ability to explain variability. All factors ($n = 35$) with an eigenvalue exceeding unity were retained.

Figure 3. Heat map representing the mean factor score for each retained factor and the lipid class. The mean factor score is represented as shown in the color index at the bottom. ANOVA p , statistical significance tested using analysis of variance

Table 1: Models used for estimating polygenic and lipidomic variance components

No	Model	Specification	Parameters estimated
1	Polygenic (P)	$\Omega = 2\phi\sigma_G^2 + I\sigma_R^2$	$h^2r = \sigma_G^2/\Omega, e^2 = \sigma_R^2/\Omega$
2	Lipidomic (L)	$\Omega = \lambda\sigma_L^2 + I\sigma_R^2$	$L^2 = \sigma_L^2/\Omega, r^2 = \sigma_R^2/\Omega$
3	Polygenic-Lipidomic (PL)	$\Omega = 2\phi\sigma_G^2 + \lambda\sigma_L^2 + I\sigma_R^2$	$h^2r = \sigma_G^2/\Omega, L^2 = \sigma_L^2/\Omega, e^2 = \sigma_R^2/\Omega$

Ω , total phenotypic variance; ϕ , kinship coefficient matrix; I , identity matrix; λ , lipidomic similarity matrix; σ_G^2 , polygenic variance component; σ_R^2 , residual variance component; σ_L^2 , lipidomic variance component

Table 2: Clinical characteristics of study participants

Characteristic	Value
Age [mean (SD)] y	39.55 (16.93)
Females [n (%)]	729 (60.45)
Fasting glucose [mean (SD)] mmol/l	5.58 (2.48)
2-hour post-glucose load glucose [mean (SD)] mmol/l	7.31 (5.03)
Fasting insulin [mean (SD)] μ U/ml	16.21 (20.22)
2-hour post-glucose load insulin [mean (SD)] μ U/ml	76.37 (73.35)
Homeostasis Model of Assessment – Insulin Resistance [mean (SD)]	4.44 (7.49)
Homeostasis Model of Assessment – beta [mean (SD)]	17.19 (56.67)
Waist circumference [mean (SD)] cm	95.08 (17.31)
Body mass index [mean (SD)] Kg/m ²	29.27 (6.63)
Waist-hip ratio [mean (SD)]	0.90 (0.10)
Systolic blood pressure [mean (SD)] mmHg	120.57 (18.79)
Diastolic blood pressure [mean (SD)] mmHg	70.67 (10.21)
Total serum cholesterol [mean (SD)] mg/dl	189.63 (38.93)
Serum triglycerides [mean (SD)] mg/dl	145.62 (103.09)
HDL cholesterol [mean (SD)] mg/dl	50.08 (12.89)
Subjects with Type 2 Diabetes [n (%)]	180 (14.92)
Subjects with central obesity [n (%)]	577 (47.84)
Subjects with raised serum triglycerides [n (%)]	495 (41.04)
Subjects with low serum HDL cholesterol [n (%)]	260 (21.56)
Subjects with high blood pressure [n (%)]	988 (81.92)
Subjects with Metabolic syndrome [n (%)]	490 (40.63)

Table 3: Estimated polygenic and lipidomic variance components for various traits related to metabolic syndrome ^a

Trait	h ² r	SE	p	L ²	SE	p
Fasting glucose	0.2542	0.0417	8.44X10 ⁻¹⁸	0.2005	0.0263	3.65X10 ⁻¹⁰
2-hour glucose	0.2670	0.0449	4.18X10 ⁻¹⁷	0.1814	0.0255	5.06X10 ⁻¹⁰
Fasting insulin	0.2434	0.0408	3.50X10 ⁻¹⁶	0.2625	0.0179	4.30X10 ⁻³²
2-hour insulin	0.2027	0.0406	2.65X10 ⁻¹²	0.2428	0.0211	1.84X10 ⁻²⁰
HOMA-IR	0.2409	0.0399	9.29X10 ⁻¹⁷	0.2666	0.0173	8.18X10 ⁻³⁴
HOMA-β	0.1709	0.0416	2.35X10 ⁻⁰⁸	0.1765	0.0324	5.40X10 ⁻⁰⁶
Waist circumference	0.3007	0.0409	8.27X10 ⁻²⁵	0.2647	0.0162	1.17X10 ⁻⁴⁰
BMI	0.3575	0.0439	1.16X10 ⁻³⁰	0.2495	0.0158	3.85X10 ⁻⁴¹
Waist -hip ratio	0.1388	0.0358	8.38X10 ⁻⁰⁸	0.2467	0.0252	2.11X10 ⁻¹⁵
Systolic blood pressure	0.3526	0.0538	2.38X10 ⁻¹⁹	0.0906	0.0369	0.0315
Diastolic blood pressure	0.3037	0.0498	5.38X10 ⁻¹⁷	0.1537	0.0357	0.0002
Serum cholesterol	0.2049	0.0347	2.69X10 ⁻¹⁷	0.3092	0.0113	1.21X10 ⁻⁹⁹
Serum triglycerides	0.2303	0.0383	3.16X10 ⁻¹⁷	0.2875	0.0121	6.99X10 ⁻⁸²
HDL cholesterol	0.3212	0.0429	3.27X10 ⁻²⁶	0.2587	0.0151	7.92X10 ⁻⁴⁹

SE, standard error; p, significance using likelihood ratio test

^a, All models are adjusted for age, age², sex, age x sex interaction, age² x sex interaction, and use of anti-diabetic, anti-lipid and anti-hypertensive drugs

Table 4: Independent contribution of the lipidomic variance component to variability in traits related to type 2 diabetes, blood pressure and obesity ^a

Trait	Before adjusting for BMI			After adjusting for BMI		
	L ²	SE	p	L ²	SE	p
Fasting glucose	0.1846	0.0298	3.1X10 ⁻⁷	0.1550	0.0353	0.0003
2-hour glucose	0.1404	0.0255	8.5X10 ⁻⁵	0.1186	0.0356	0.0023
Fasting insulin	0.2521	0.0204	6.2X10 ⁻²³	0.2181	0.0279	1.0x10 ⁻⁹
2-hour insulin	0.2280	0.0243	1.7X10 ⁻¹⁴	0.1869	0.0311	1.1x10 ⁻⁶
HOMA-IR	0.2509	0.0203	9.0X10 ⁻²³	0.2215	0.0270	1.3x10 ⁻¹⁰
HOMA-β	0.1518	0.0389	0.0012	0.1330	0.0448	0.0199
Waist circumference	0.2608	0.0174	1.0X10 ⁻³⁴	0.1984	0.0390	3.66x10 ⁻⁶
BMI	0.2438	0.0169	3.8X10 ⁻³⁵	---	---	---
Waist -hip ratio	0.2233	0.0309	1.2X10 ⁻⁹	0.1724	0.0438	0.0002
Systolic blood pressure	0.0000	0.0000	---	0.0000	0.0000	---
Diastolic blood pressure	0.0931	0.0466	0.0726	0.0000	0.0000	---

SE, standard error; p, significance using likelihood ratio test

^a, All models are adjusted for age, age², sex, age x sex interaction, age² x sex interaction, total serum cholesterol, serum triglycerides, serum HDL cholesterol and use of anti-diabetic, anti-lipid and anti-hypertensive drugs

Figure 1.

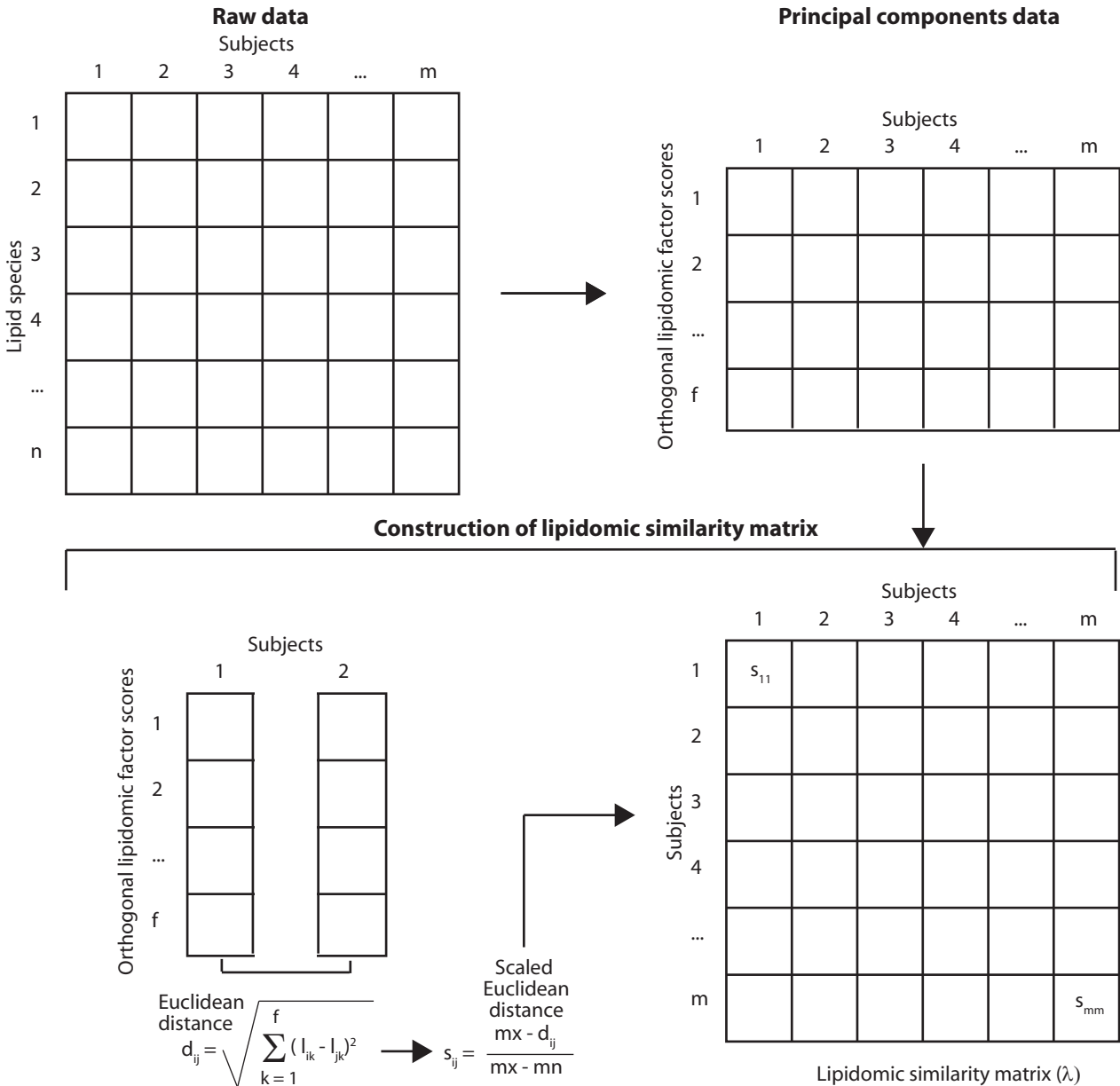


Figure 2

