Relationship between the lipidome, inflammatory markers and insulin resistance

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Relationship between the lipidome, inflammatory markers and insulin resistance

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Abstract

The objectives of the present study were to (1) examine the effects of the phenotypic factors age, gender and BMI on the lipidomic profile and (2) investigate the relationship between the lipidome, inflammatory markers and insulin resistance.

Specific ceramide, phosphatidylcholine and phosphatidylethanolamine lipids were increased in females relative to males and specific lysophosphatidylcholine, lysophosphatidylethanolamine , phosphatidylcholine and phosphatidylethanolamine lipids decreased as BMI increased. However, age had a minimal effect on the lipid profile with significant differences found in only two lipid species. Network analysis revealed strong negative correlations between the inflammatory markers CRP, TNF-α, resistin and MCP-1 and lipids in the LPC, PC and PE classes, whereas IL-8 formed positive correlations with lipids from the CER and SM classes. Further analysis revealed that LPC a C18:1 and PE ae C40:6 were highly associated with insulin resistance as indicated by HOMA-IR score.

The present study identified lipids that are affected by BMI and gender and identified a series of lipids which had significant relationships with inflammatory markers. LPC a C18:1 and PE ae C40:6 were found to be highly associated with insulin resistance pointing to the possibility that the alterations in these specific lipids may play a role in the development of insulin resistance.

Keywords: lipidomics/ inflammatory markers/ insulin resistance/ BMI/ gender

Introduction

Lipidomics is the systematic identification of the lipid species of a cell, tissue, biofluid or whole organism [1]. Lipids have diverse biological functions such as cellular architecture, energy storage and cell signaling [2] and deregulated lipid metabolism has been implicated in many diseases such as alzheimers disease [3], schizophrenia [4], multiple sclerosis [5] and diabetes [6]. The lipid composition of human plasma has been extensively characterised and revealed over 500 different lipid molecular species[7]. This structural diversity is mirrored by the enormous variation in lipid physiological function [8] and highlights the potential for biomarker discovery and biological insight to be gained from analysis of the plasma lipidome. As an example of this, specific plasma triglycerides implicated in insulin resistance improve diabetes risk prediction [9] and plasma levels of specific lysophospholipids have been proposed as diagnostic and prognostic markers of ovarian cancer [10].

In addition to its application in biomedical research, plasma lipidomics holds enormous potential for nutritional research [11, 12]. The health risks associated with an adverse plasma lipid and lipoprotein profile such as elevated TAGs and low levels of HDL-c and the impact of diet on these parameters are well established [8, 13-16]. Detailed analysis of the lipid composition of plasma can provide increased insight into the interaction between diet and metabolism and their contribution to health and disease. For example, lipidomic analysis of plasma or serum has been applied to investigate the effects of a wholegrain, fish and bilberry enriched diet[17], dietary carbohydrate composition[18], fatty fish intake[19], fish oil supplementation [20] coffee consumption [21], plant sterol intake [22] and probiotic supplementation [23].

In order to develop lipidomic analysis for biomarker discovery and as a means to understand the mechanistic basis of disease and nutritional effects, it is important to understand basic

physiological variation in the lipidome. Multiple studies have demonstrated that genetic variation affects the plasma and serum lipidome composition [24]. Obesity affects the plasma lipidome with changes primarily seen in TAGs, lysophosphatidylcholines and ether phospholipids, however results have varied thus the impact of BMI needs further characterisation [25-29] Other studies have shown age and gender dependent effects on the lipidome [29-32], however further characterisation in varied populations is needed

Although it is well accepted that obesity is associated with the development of insulin resistance and Type 2 Diabetes (T2DM) the precise mechanism involved is unclear. However, extensive evidence exists to suggest that dyslipidemia and inflammation play a role [33-35]. With the advancement in our ability to profile lipid classes in recent years it is has become apparent that dyslipidemia in obesity extends beyond free fatty acids and indeed a number of lipid species have been proposed as mediators of insulin resistance[9, 36]. Notwithstanding this, the relationship between the lipidome and inflammatory markers in the context of insulin resistance has not been studied in detail. The objectives of the present study were to (1) examine the effects of the phenotypic factors on the lipidomic profile and (2) investigate the relationship between the lipidome, inflammatory markers and insulin resistance.

Results

Characteristics of the subjects

The phenotypic characteristics of 19 males and 20 females aged from 18 to 60 with an average age of 34 who participated in the study are presented in Table I. Only the percentage body fat and HDL-c levels were significantly different between gender groups.

Gender and BMI significantly impact the lipidome

The lipids used in the analysis represented the following classes: 6 lysophosphatidylcholines (LPC), 7 lysophosphatidylethanolamines (LPE), 48 phosphatidylethanolamines (PE), 27 phosphatidylserines (PS), 40 phosphatidylcholines (PC), 26 sphingomyelins (SM), 58 ceramides (CER) and 3 phosphatidylglcerols (PG).

A total of 19 lipids varied due to gender (Table II), the majority of which were from the CER and PC lipid classes. All plasma gender specific lipids were elevated in females relative to males with the exception of a 2-hydroxyacyl-dihydroceramide lipid (N-C23:0-Cer(2H)). To examine gender effects further, differences in the double bond content between genders was calculated. Analysis revealed a significant difference in the total amount of lipid analytes with two double bonds (male; 530.32 ± 151.52 μM, female; 645.40 ± 99.70 μM, p=0.009). Further examination of double bond content according to lipid class revealed a significant difference in the total amount of PC lipids with two double bonds (male; 451.09 ± 135.28 μM, female; 557.29 ± 88.14 μM, p=0.008) and three double bonds (male; 113.26 ± 37.96 μM, female;145.72 ± 32.07 μM, p=0.010) and PE lipids with two double bonds (male; 4.68 ± 2.23 μM, female; 6.65 ± 2.18 μM, p=0.013) and three double bonds (male; 2.57 ± 1.04, female; 3.52 ± 1.21 μM, p=0.020). Supplementary Figure 1 depicts the mean male/female

ratio of individual lipid analytes from (a) the PC and (b) the PE lipid class organised along the x-axis to highlight the differences in acyl chain double bond content according to gender.

A total of 47 lipids were found to be influenced by BMI (Table III); these lipids decreased in concentration as BMI increased. The main lipid classes found to vary with BMI were the LPC, LPE, PC and PE classes where 100%, 71%, 50% and 23% of the total lipids in these classes respectively displayed a significant relationship with BMI. Age had a minimal effect on the lipid profile in the present cohort with significant differences found in only two lipids (PS aa C42:4; p=0.016, q= 0.242, LPE a C16:0, p=0.021 q = 0.146).

The relationship between the lipidome, inflammatory markers and insulin resistance

Regularised CCA (rCCA) was employed to investigate the relationship between the lipidome and various biochemical/inflammatory parameters; an overview of the results are presented in Supplementary Figure 2. Strong positive and negative relationships with lipids were observed for both leptin and TNF-α. The network graph (Figure 1) depicts associations between variables with a similarity score higher than 0.3 and allows more detailed interpretation of the trends noted in Supplementary Figure 2 The similarity score of each lipid-inflammatory parameter association is reported in the Supplementary Information Table I. The majority of negative correlations were between lipids in the LPC, LPE, PC and PE classes and leptin, CRP, TNF-α, resistin and MCP-1 (Supplementary. Figure 2 and Figure 1). The LPC lipid class was found to have the strongest associations, specifically LPC a C18:1 and LPC a C18:2 and their negative association with both leptin and CRP. In addition to this, the negative association between TNF-α and various lipids from the PE class is noteworthy. A striking feature of the analysis was the predominant positive associations observed for IL8 and IL10 (Figure 1). IL8 associated predominantly with the SM lipid class whereas IL10 formed associations with lipids in the PC, PE and CER classes.

Focusing on the lipids with significant relationships with the inflammatory parameters the relationship with the HOMA score was explored (Figure 2). Again, the strongest trends were seen in the LPC and LPE lipid classes. Specifically, the negative correlation between the lipids LPC a C18:1, LPC a C18:2, LPE a C18:1, LPC e C18:0 and CRP and leptin was associated with HOMA-IR score. Individuals with decreased levels of these lipids and increased leptin or CRP levels had increased HOMA scores. A similar trend was seen with PE ae C40:6 and TNF-α and resistin, wherein low levels of PE ae C40:6 with high TNF-α and/or resistin tended to have higher HOMA scores. Linear regression analysis revealed that LPC a C18:1(β = -0.441, p=0.006) and PE ae C40:6(β = -0.347, p=0.028) were the most significant predictors of HOMA score (R^2=0.461, p<0.0005). Figure 3 shows the relationship between LPC a C18:1, PE ae C40:6 and HOMA: decreasing levels of both these lipids was associated with increased HOMA score.

The lipidome-lipoprotein network

As plasma lipoprotein levels are known to vary with both gender and BMI, we examined the relationship between the lipidome and different lipoproteins to determine whether any lipid specific changes may be correlated with lipoprotein changes. Examination of the relationship between the lipidome and the lipoproteins revealed a number of positive associations with the strongest relationships observed for LDL and APO B (Supplementary Figure 3 and Figure 4). The majority of associations LDL formed were with lipids from the CER and SM lipid classes. However two of the strongest lipid-lipoprotein associations formed were between LDL and the lipids PC aa C40:5 and PE aa C40:5 (Supplementary Information Table II). Of the different lipid classes measured, the PC and PE classes tended to be the most highly associated with the lipoproteins measured. Other noteworthy relationships included the relationships between HDL-c and PC aa C34:2 and Apo C3 and both PE ae C40:6 and PE aa

C38:0. Lipids from the LPE class were primarily associated with HDL-c, specifically LPE a C18:1, LPE a C18:2 and LPE a C22:6.

Discussion

The present study investigated the influence of phenotypic factors on the glycerophospholipid and sphingolipid composition in plasma of a group of healthy subjects. Furthermore examination of the relationship between the lipidome, inflammatory markers and insulin resistance revealed some interesting patterns which support the hypothesis of lipid mediated insulin resistance. More specifically two lipids were found to be highly associated with insulin resistance.

Gender specific differences were predominantly found in the ceramide, phosphatidylcholine, and phosphatidylethanolamine lipid classes which, apart from a decrease in N-C23:0-Cer(2H), were increased in females. Moreover, females had significantly higher levels of lipids with 2 or 3 double bonds in the PC and PE lipid classes. This reflects the changes in specific lipids seen in these classes as the majority had 2 or 3 double bonds. Previous studies have also found increased levels of ceramides and PC's in females compared to males [37-40] and studies in mice have shown gender related differences in PC homeostasis [41]. The majority of the gender specific lipids identified associated positively with HDL-c, LDL-c and Apo B lipoprotein levels. Gender specific differences in lipoprotein levels have been shown previously with increased levels of HDL-c, LDL-c and total cholesterol in females whereas males have been found to have higher VLDL [42-44]. However, N-C23:0 Cer(2H), the only gender specific lipid found to be decreased in females relative to males, did not correlate with any of the lipoproteins measured.

BMI had a significant impact on the plasma lipidome where decreased levels of LPC's, LPE's and specific PC's and PE's were the main changes associated with increasing BMI. LPC lipids have been identified as important signaling molecules and have been proposed to be involved in regulating cellular proliferation, tumour cell invasion and inflammation [45-48]. Findings from studies investigating LPC levels in individuals with varying BMI's have been mixed [25-28]. In agreement with findings from the current study, both total and specific plasma LPC levels (including LPC C18:1, LPC C18:2 and LPC C18:0) have been shown to be reduced in obese individuals and LPC levels were significantly negatively associated with BMI and plasma insulin levels in recent studies [25, 29]. However, opposite effects have also been reported[26]. Interestingly, the LPC lipids showed no association with lipoproteins, agreeing with previous proposals that most of the circulating LPC is bound to albumin [49, 50]. Previous studies have found increasing BMI is associated with both increases and decreases in lipids from the PC and PE lipid classes depending on fatty acid composition [26, 27, 51]. In the present study, lipids that had a relationship with BMI from the PC and PE class had decreased levels with increasing BMI. In addition to this, the most significant changes in the PC lipid class and the majority of lipids from the PE class that changed with BMI possessed ether bonds.Ether phospholipids have been reported to have antioxidant properties [52] and are decreased in obese relative to non-obese co-twins [26]. However, a recent study of the plasma lipidome in lean and obese individuals found increased ether linked PC and PE species in obese individuals [53]. As with LPC, these differing results may be due to differences in the presence and severity of metabolic complications associated with increasing obesity such as Type 2 Diabetes in the obese group.

Examining the relationship between the lipids and inflammatory markers revealed that the sphingomyelin class primarily formed positive associations, with the strongest associations

with the pro-inflammatory cytokines TNF-alpha and IL8. Elevated plasma sphingomyelin levels have been correlated with incidence of cardiovascular disease [54] and short and medium chain fatty acid sphingomyelins positively correlate with insulin and intra-abdominal fat [26]. Cytokines increase hepatic sphingomyelin synthesis to increase plasma sphingomyelin levels [55] and induce secretory sphingomyelinase which promotes sphingolipid hydrolysis [56]. This in turn may increase sphingolipid levels such as sphingosine-1-phosphate [57]. Interestingly sphingosine-1-phosphate is involved in signal transduction and regulation of the immune system [58] modulating macrophage IL8 and TNF-α [59]. Translation of these functional relationships between specific sphingomyelins with IL-8 and TNF-α in the present study suggests a functional relevance in humans. Indeed recent studies have confirmed sphingomyelins as an independent risk factor in the development of cardiovascular disease (CVD) [60, 61]. Nevertheless establishing their potential role in the development of insulin resistance prior to overt CVD is warranted.

Lipids from the LPC class were negatively associated with a number of inflammatory markers and adipokines (including IL-8, leptin, MCP-1, CRP, TNF-α) which are associated with increasing obesity and insulin resistance [62, 63]. The novel aspect of the present work is the relationship between the lipids, the inflammatory markers/adipokines and the HOMA-IR score. Individuals with low levels of the lysophospholipids LPC a C18:1, LPC a C18:2, LPC e C18:0 and LPE a C18:1 who also had increased CRP or leptin levels had increased HOMA-IR scores. Interestingly these lipids were all lower in obesity suggesting them as a link between obesity, inflammation and insulin resistance. Furthermore LPC a C18:1 in combination with PE ae C40:6 was highly associated with HOMA-IR score. Previous studies have shown plasma LPC levels are reduced in individuals with impaired glucose tolerance [64] and LPC C16:0 was lower in insulin resistant subjects with non-alcoholic fatty liver [65]. LPC stimulates adipocyte glucose uptake, potentiates glucose stimulated insulin secretion and

lowers blood glucose levels in murine models of diabetes [66 67] . Although some studies report the opposite relationship[68], overall the emerging view is that LPC's may play an important role in glucose homeostasis. However, it remains to be determined whether the low LPC levels in obese and insulin resistant individuals is due to greater clearance or decreased production. The clear relationship between LPC's, BMI, inflammatory makers and HOMA-IR score suggests a potential role as mediators of insulin resistance: further work is needed to decipher whether this is the case.

PE ae C40:6 was also highly correlated with HOMA-IR and associated with resistin and TNF-α, which are implicated in insulin resistance [63]. In general, lipids from the PE and PC lipid classes were negatively associated with many inflammatory/biochemical markers such as leptin, MCP-1, CRP, resistin and TNF-α , however the majority of specific PE and PC lipids that formed strong associations with multiple inflammatory markers possessed ether bonds. It has been shown that ether phospholipids are decreased in obese co-twins and levels of specific ether phospholipids positively correlate with insulin sensitivity[26]. In a study investigating the lipid composition of LDL, it was found that plasmalogen PEs, a subclass of ether phospholipids, were lower in both metabolic syndrome and diabetic subjects compared to lean subjects [69]. It has been proposed that plasmalogens are targets of oxidative stress and lower levels are indicative of oxidative stress in individuals with the metabolic syndrome and/or type 2 diabetes [69]. An inflammatory phenotype may reduce plasma ether phospholipid species via concomitant oxidative stress thus explaining the negative association between many of the ether phospholipids and inflammatory markers measured in the present cohort.

Conclusion

A striking finding in the present study was the identification of a series of lipids which had significant relationships and potential synergy with inflammatory markers. Further analysis identified two lipids, LPC a C18:1 and PE ae C40:6, which were associated with insulin resistance pointing to the possibility that the alterations in these specific lipids play a role in the development of insulin resistance. Overall, the present study also identified lipids that are affected by phenotypic traits such as BMI and gender. In conclusion, the present study provides strong evidence for the link between lipid levels, inflammation and markers of insulin resistance. Future work will be needed to define the directionality of the relationship between the lipids and inflammatory markers.

Experimental

Volunteers

Volunteers were recruited as part of a larger clinical registered study (clinicaltrials.gov NCT01172951) called the Metabolic Challenge Study (MECHE). Ethical approval was obtained from the Human Research Ethics Committee of University College Dublin (UCD) and St. Vincent's University Hospital. A total of 214 subjects aged 18- 60 years with general good health were recruited and provided written informed consent [70, 71]. Exclusion criteria included BMI < 18.5 kg/m^2, iron deficiency anaemia (hemoglobin <12 g/dL), elevated fasting glucose (fasting plasma glucose ≥ 7 mM), cholesterol >7.5 mM, TAG > 3.8 mM, raised liver or kidney enzymes, pregnant or lactating females and the presence of any chronic or infectious disease and any prescribed medication for such. Body weight was measured following an overnight fast, to within 0.1 kg on a flatbed-weighing scale. Height was measured using a wall-mounted stadiometer to within 0.1 cm.

Fasting blood samples were collected following a 12 hour overnight fast into EDTA and lithium-heparin coated tubes for plasma and vacutainer tubes for serum isolation. Serum samples were allowed to clot for 30 minutes at room temperature while EDTA and lithium heparin tubes were placed directly on ice. All blood samples were centrifuged at 1800 g for 15 minutes at 4 ºC and 500 µl aliquots were stored at -80 ºC until further analysis.

Biochemical/Inflammatory analysis

Clinical chemistry analysis was performed using an RxDaytona™ analyser (Randox Laboratories, Crumlin, UK) and Randox reagents. Details of the analytes and methods are previously reported [60, 71]. Cytokines and hormones were measured using a biochip array system (Evidence Investigator™, Randox Laboratories, Crumlin, Northern Ireland). The metabolic array I kit was used for the measurement of the cytokines and hormones as

previously described [71]. The HOMA index was used as an estimate of insulin sensitivity and calculated as (fasting insulin (μU/ml) x fasting glucose mM)/22.5. LDL was calculated using the Friedwald equation: LDL = Total Cholesterol - HDL - TAG/5.0 (mg/dL)[61].

Lipid profiling

Lipidomic analysis was performed on a total of 39 subjects. The analysis was performed by BIOCRATES Life Sciences AG (Innsbruck, Austria). The biologically most abundant members of (lyso-) glycerophospholipids, i.e. (lyso-) glycerolphosphocholines, -ethanolamines, -serines, -glycerols, as well as sphingolipids, i.e. sphingomyelins, ceramides, dihydroceramides, and 2-hydroxyacyl ceramides, were quantitatively analysed by a high throughput flow injection ESI-MS/MS screening method. A total of 325 lipids were detected and quantified in the plasma.. Five internal standards were used to compensate for matrix effects, and 43 external standards for a multipoint calibration. Quantitative data analysis was performed using the in-house software Met***IDQ***™ which enables isotope correction. Individual lipid analytes which had ≥ 80% of values below the limit of detection were excluded from analysis [29] leaving a total of 215 lipids for the final analysis.

Statistical Analysis

Data was imported into PASW version 18.0 for statistical analysis. General linear model analysis was used to assess the impact of BMI and phenotypic factors. Linear regression analysis was employed to examine associations in data. An estimate of the false discovery rate (q-value) was calculated to take into account multiple comparisons, it was estimated on a family wide basis using the QVALUE software package [72] in R (version 2.15.1).

Regularized canonical correlation analysis (rCCA) is a multivariate statistical method used to assess correlations between two multivariate datasets [73] and was employed to assess the

298 relationship between the lipidome and biochemical parameters or lipoproteins. This was
299 performed using the mixOMICS software package [74] in R version 2.15.1. Regularization
300 parameters were estimated using the *estim.regul* function and the *rcc* function was used to
301 define the canonical correlations and the canonical variates. The rCCA approach was applied
302 to the lipidome – biochemical parameter and lipidome – lipoprotein datasets with
303 regularization parameters λ_1 0.042, λ_2 0.116 and λ_1 0.1, λ_2 0.421 respectively (based on leave
304 one out cross validation). The first 5 dimensions (canonical correlation values of 0.992,
305 0.989, 0.983, 0.981 and 0.976) and the first 4 dimensions (canonical correlation values of
306 0.934, 0.918, 0.875 and 0.837) were retained for the lipidome – biochemical parameter and
307 the lipidome – lipoprotein rCCA analysis respectively after which values dropped for the
308 following dimensions.

309 In order to visualise pair-wise associations highlighted by rCCA, the *cim* function was used
310 to generate clustered heatmaps and the *network* function to produce relevance networks from
311 the similarity matrix derived from rCCA [75]. The values in the similarity matrix are computed
312 as the correlation between the two types of projected variables onto the space spanned by the
313 components/dimensions retained in the analysis and can be seen as a robust approximation of
314 pearson correlation [32].

315 The relevance network graph is a bipartite graph derived from the similarity matrix where
316 nodes represent variables and edge colour represents the strength of the association. Only
317 associations exceeding a specified threshold are shown. These threshold values were
318 arbitrarily chosen in order to obtain biologically interpretable networks that were neither too
319 sparse nor too dense. Data was exported to Gephi (0.8.1 beta) [76] where the layout algorithm
320 Yifan Hu was applied to create the network figure and aid in the visualisation and exploration
321 of the network for biological interpretation.

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Figure Legends

Figure 1. Relevance network graph depicting correlations derived from rCCA between various biochemical parameters and the lipidome. Only associations where the correlation coefficient exceeds the threshold of 0.3 are shown. Nodes (circles) represent variables and are sized according to number of connections. Edges are coloured according to association score with red showing positive correlations and blue showing negative correlations. Biochemical parameters are shown in grey. Lipids are coloured according to lipid class; lysophosphatidylcholine, yellow; phosphatidylserine, blue; ceramides, orange; phosphatidylcholine, light green; phosphatidylethanolamine, dark green; sphingomyelin, pink.

Figure 2. Bubble plots of specific lipids with inflammatory markers. The circle size is proportionate to HOMA-IR score. Each data point represents a volunteer.

Figure 3. Bubble plot of PE ae C40:6 and LPC a C18:1. Dot size reflects HOMA-IR score. Each data point represents a volunteer.

505

506 **Figure 4.** Relevance network graph depicting correlations derived from rCCA between the
507 lipoprotein and lipidome. Only associations where the correlation coefficient exceeds the
508 threshold of 0.4 are shown. Nodes (circles) represent variables and are sized according to
509 number of connections. Edges are coloured according to association score with increased
510 intensity reflecting higher correlation scores. Lipoprotein variables are in grey. Lipids are
511 coloured according to lipid class; lysophosphatidylcholine and
512 lysophosphatidylethanolamine, yellow; phosphatidylserine, blue; ceramides, orange;
513 phosphatidylcholine, light green; phosphatidylethanolamine, dark green; sphingomyelin,
514 pink.

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Table I. Anthropometric and biochemical parameters.

	Male (n=19)	Female (n=20)	Total	Total (range)
Age (years)	33 ± 12	36 ± 14	34 ± 13	18 – 60
BMI (kg/m^2)	28 ± 5	26 ± 7	26 ± 6	19 – 50
Body fat (%)	21 ± 12*	35 ±12*	28 ± 14	9 – 59
HOMA-IR	2.68 ±2.61	1.87 ±1.60	2.28 ± 2.17	0.31 – 9.19
Fasting plasma glucose (mM)	5.29 ±0.66	5.24 ±0.43	5.27 ± 0.55	3.64 – 6.65
Fasting plasma insulin (μIU/ml)	10.69 ± 9.33	7.94 ± 6.42	9.32 ± 8.01	1.57 – 34.32
Triglycerides (mM)	1.14 ±0.68	1.14 ±0.55	1.09 ± 0.50	0.37 – 2.61
NEFA (mM)	0.51 ± 0.31	0.69 ± 0.38	0.60 ± 0.35	0.15 – 1.7
Total cholesterol (mM)	4.55 ±1.09	4.90 ±1.04	4.76 ± 1.07	3.01 – 7.66
HDL-c (mM)	1.19 ± 0.27*	1.67 ± 0.48*	1.44 ± 0.46	0.69 – 2.55
LDL-c (mM)	2.69 ± 1.12	2.72 ± 0.83	2.70 ± 0.97	0.13 – 4.83
Apo B (mg/dl)	73.33 ± 25.57	82.11 ± 23.32	77.84 ± 24.50	32 – 136
Apo C3 (mg/dl)	6.63 ± 2.68	7.93 ± 2.04	4.00 ± 1.89	0.61 – 13.27
Apo C2 (mg/dl)	3.95 ± 2.28	4.05 ± 1.51	7.29 ± 2.43	1.10 – 8.41
Apo A1 (mg/dl)	111.61 ± 25.79	135.28 ± 47.95	123.44 ± 39.79	12 – 219
Apo E (mg/dl)	2.05 ± 0.80	2.57 ± 1.58	2.32 ± 1.27	0.67 – 8.15

Data presented as mean ± standard deviation unless otherwise stated. BMI, body mass index; HOMA, homeostasis model assessment; NEFA, non-esterified fatty acid.* $p<0.05$ when comparing males and female.

Table II. Significantly different lipids between males and females.

Lipid class	Lipid	Male µM (n=19)	Female µM (n=20)	p - value	q - value
Phosphatidylcholine	PC aa C32:1	10.739 ± 6.142	15.405 ± 6.426	0.033	0.169
	PC aa C34:2	291.299 ± 83.598	369.514 ± 65.985	0.003	0.065
	PC aa C34:3	9.264 ± 4.325	13.452 ± 5.654	0.019	0.137
	PC aa C36:2	134.078 ± 45.760	161.891 ± 30.128	0.043	0.169
	PC aa C36:3	76.034 ± 24.782	97.812 ± 20.575	0.007	0.072
	PC aa C38:3	17.758 ± 8.298	22.625 ± 5.516	0.047	0.169
Phosphatidylethanolamine	PE aa C34:2	0.991 ± 0.517	1.556 ± 0.703	0.010	0.232
	PE aa C36:2	2.829 ± 1.502	4.094 ± 1.479	0.017	0.232
	PE aa C36:3	1.202 ± 0.600	1.834 ± 0.915	0.023	0.232
Sphingomyelin	SM C16:1	16.256 ± 5.611	19.832 ± 4.356	0.038	0.047
Ceramide	N-C21:0-Cer(2H)	0.001 ± 0.001	0.002 ± 0.001	0.000	0.002
	N-C23:0-Cer(2H)	0.723 ± 0.035	0.105 ± 0.056	0.048	0.029
	N-C24:0-Cer(2H)	0.188 ± 0.093	0.272 ± 0.138	0.039	0.029
	N-C22:0(OH)-Cer	0.021 ± 0.014	0.035 ± 0.019	0.007	0.017
	N-C23:0(OH)-Cer	0.026 ± 0.013	0.044 ± 0.026	0.007	0.017
	N-C24:0(OH)-Cer	0.096 ± 0.052	0.129 ± 0.056	0.044	0.029
	N-C24:0-Cer	2.009 ± 0.763	2.568 ± 0.731	0.035	0.029
	N-C16:0-Cer	0.232 ± 0.079	0.281 ± 0.056	0.044	0.029
	N-C22:1-Cer	0.019 ± 0.009	0.025 ± 0.007	0.046	0.029

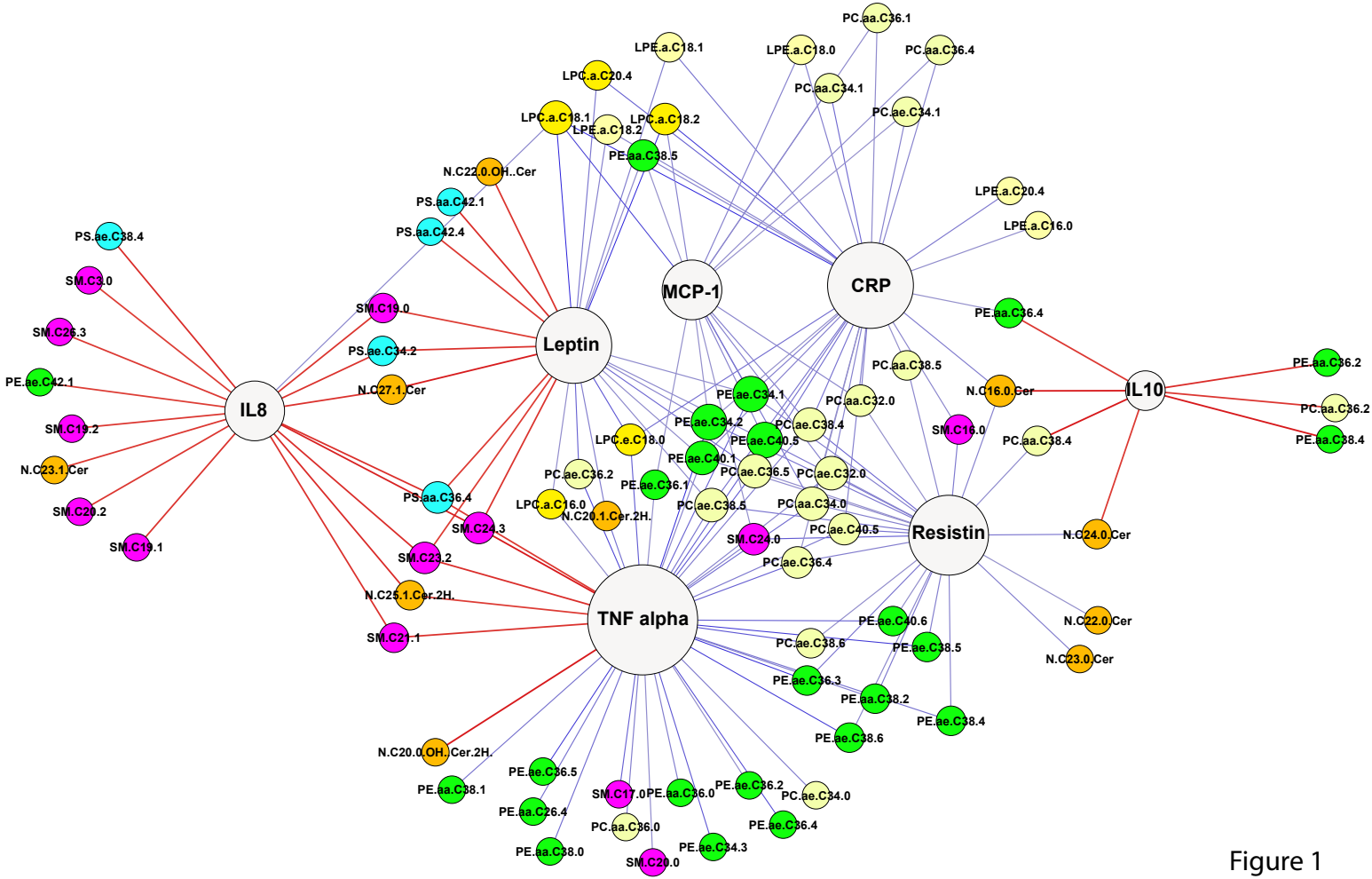
Lipids with significant gender effects were determined by general linear model controlling for BMI. Data presented as mean ± standard deviation.

Table III. Lipids with a significant relationship with BMI.

Lipid class	Lipid	p - value	q – value
Lysophosphatidylcholines	LPC a C16:0	0.010	0.002
	LPC a C18:0	0.025	0.003
	LPC a C18:1	0.000	0.000
	LPC a C18:2	0.001	0.000
	LPC a C20:4	0.019	0.003
	LPC e C18:0	0.002	0.000
Lysophosphatidylethanolamine	LPE a C16:0	0.011	0.002
	LPE a C18:0	0.028	0.003
	LPE a C18:1	0.011	0.002
	LPE a C18:2	0.021	0.003
	LPE a C22:6	0.028	0.003
Phosphatidylcholine	PC aa C32:0	0.009	0.008
	PC aa C34:0	0.042	0.019
	PC aa C34:1	0.043	0.019
	PC aa C34:2	0.009	0.008
	PC aa C36:0	0.009	0.008
	PC aa C36:2	0.018	0.011
	PC aa C36:3	0.020	0.011
	PC aa C36:4	0.049	0.019
	PC aa C38:6	0.036	0.018
	PC aa C40:7	0.033	0.018
	PC ae C32:0	0.006	0.008
	PC ae C34:0	0.045	0.019
	PC ae C34:1	0.005	0.008
	PC ae C36:2	0.020	0.012
	PC ae C36:4	0.007	0.008
	PC ae C36:5	0.008	0.008
	PC ae C38:4	0.006	0.008
	PC ae C38:5	0.006	0.008
	PC ae C38:6	0.016	0.012
	PC ae C40:5	0.007	0.008
Phosphatidylethanolamine	PE aa C38:0	0.043	0.084
	PE ae C34:1	0.020	0.084
	PE ae C34:2	0.014	0.084
	PE ae C34:3	0.017	0.084
	PE ae C36:2	0.013	0.084
	PE ae C36:3	0.009	0.084
	PE ae C36:5	0.041	0.084
	PE ae C38:6	0.043	0.084
	PE ae C40:1	0.036	0.084
	PE ae C40:5	0.028	0.084
	PE ae C40:6	0.034	0.084
Phosphatidylserine	PS aa C42:1	0.048	0.216

Sphingomyelin	SM C16:0	0.017	0.239
	SM C24:0	0.031	0.239

Lipids with significant BMI effects were determined by general linear model controlling for gender.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Leptin
MCP-1
CRP
IL10
Resistin
TNF alpha
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SM.C3.0
SM.C26.3
PE.ae.C42.1
SM.C19.2
N.C23.1.Cer
SM.C20.2
SM.C19.1
SM.C19.0
PS.ae.C34.2
N.C27.1.Cer
PS.aa.C42.1
PS.aa.C42.4
N.C22.0.OH..Cer
LPC.a.C18.1
LPE.a.C18.2
LPC.a.C18.2
PE.aa.C38.5
LPC.a.C20.4
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LPE.a.C18.0
PC.aa.C36.1
PC.aa.C36.4
PC.aa.C34.1
PC.ae.C34.1
LPE.a.C20.4
LPE.a.C16.0
PE.aa.C36.4
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SM.C16.0
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PC.aa.C36.2
PE.aa.C38.4
PE.ae.C34.1
PC.aa.C32.0
PE.ae.C34.2
PC.ae.C38.4
PE.ae.C40.5
PE.ae.C40.1
PC.ae.C36.5
PC.ae.C32.0
LPC.e.C18.0
PC.ae.C36.2
PE.ae.C36.1
LPC.a.C16.0
N.C20.1.Cer.2H.
PC.ae.C36.5
PC.aa.C34.0
PC.ae.C40.5
SM.C24.0
PC.ae.C36.4
PS.aa.C36.4
SM.C24.3
SM.C23.2
N.C25.1.Cer.2H.
SM.C21.1
N.C24.0.Cer
PE.ae.C40.6
PC.ae.C38.6
PE.ae.C38.5
PE.ae.C36.3
PE.aa.C38.2
PE.ae.C38.4
PE.ae.C38.6
N.C22.0.Cer
N.C23.0.Cer
N.C20.0.OH..Cer.2H.
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SM.C17.0
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PC.ae.C34.0
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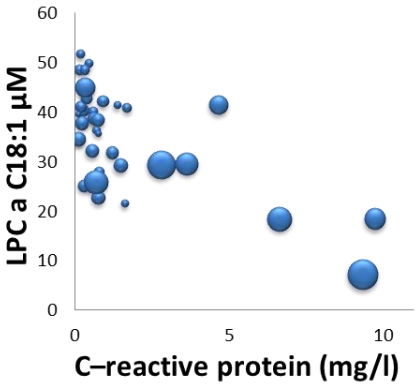
PE.ae.C34.3
SM.C20.0

Figure 1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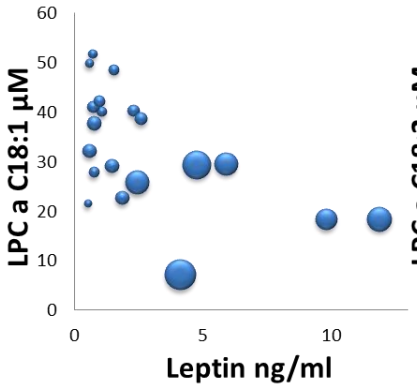
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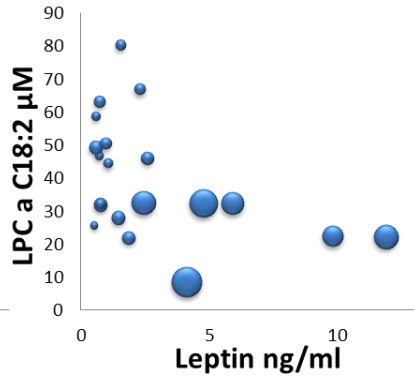
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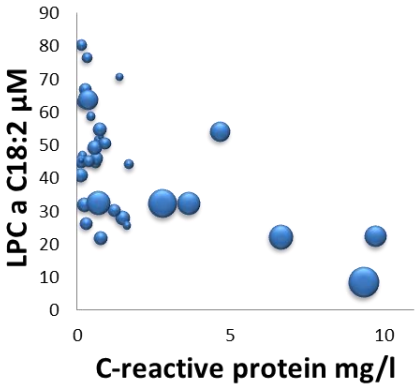
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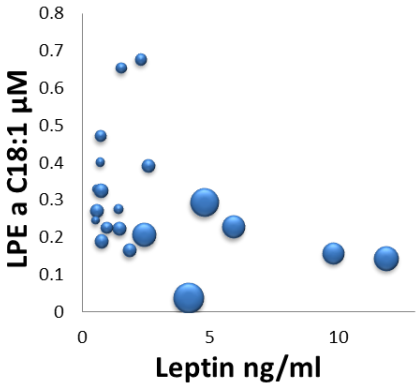
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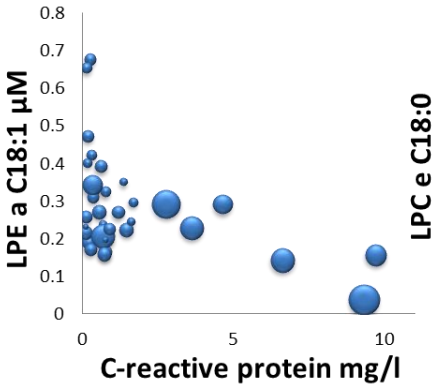
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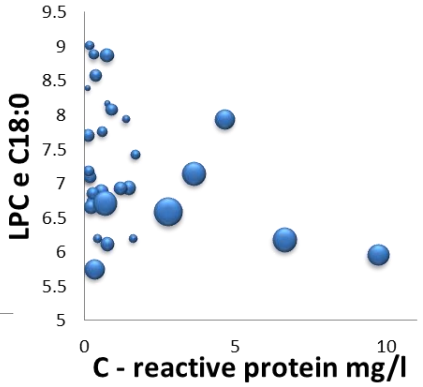
C-reactive protein mg/l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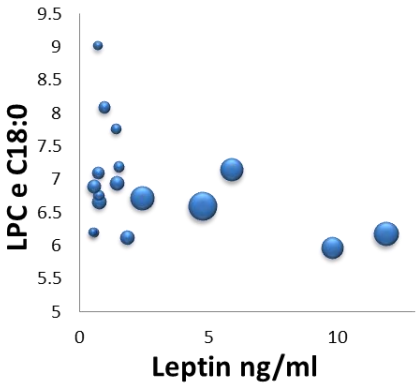
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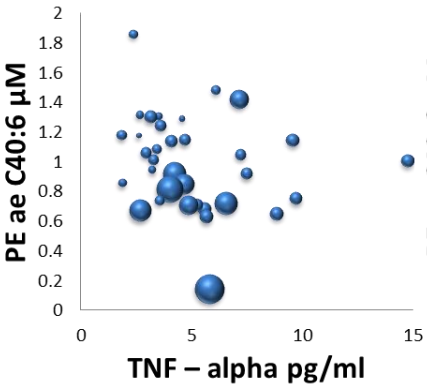
PE ae C40:6 µM
TNF – alpha pg/ml

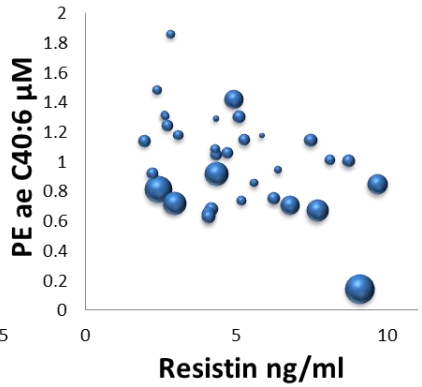
PE ae C40:6 µM
Resistin ng/ml

Figure 2

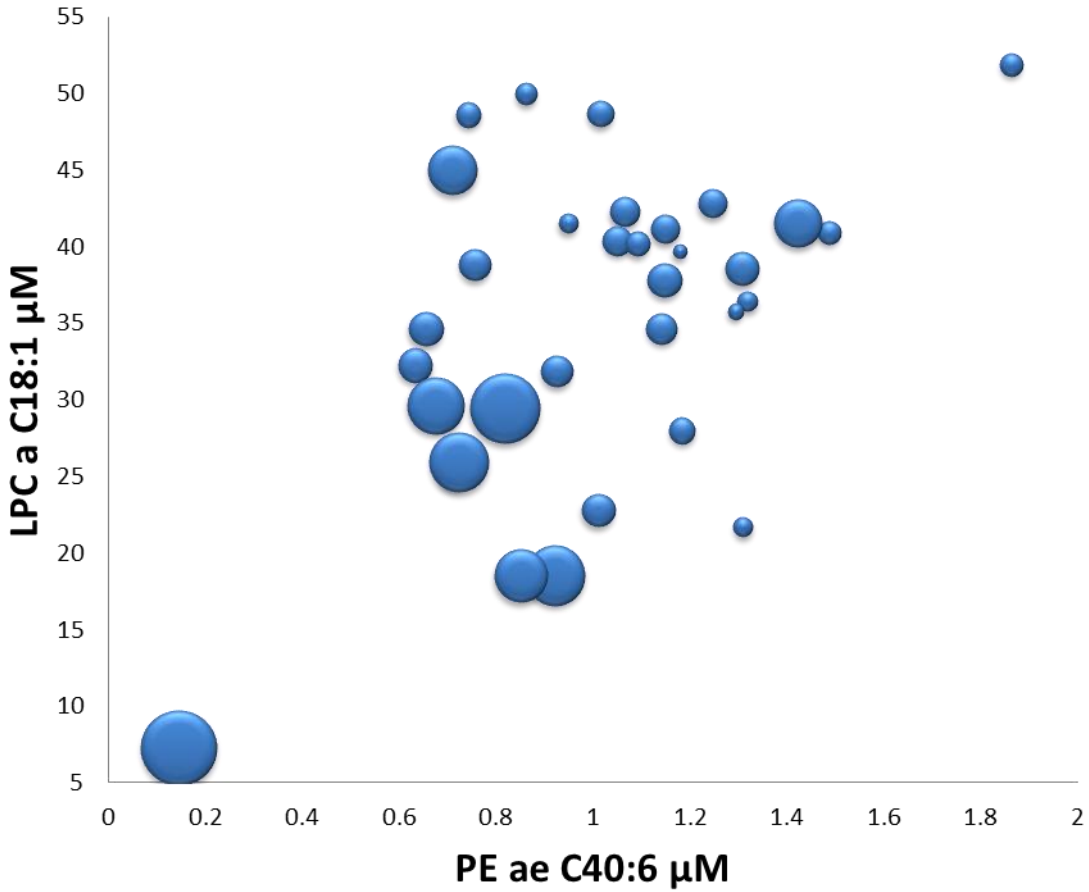
LPC a C18:1 µM
PE ae C40:6 µM
55
50
45
40
35
30
25
20
15
10
5
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

Figure 3

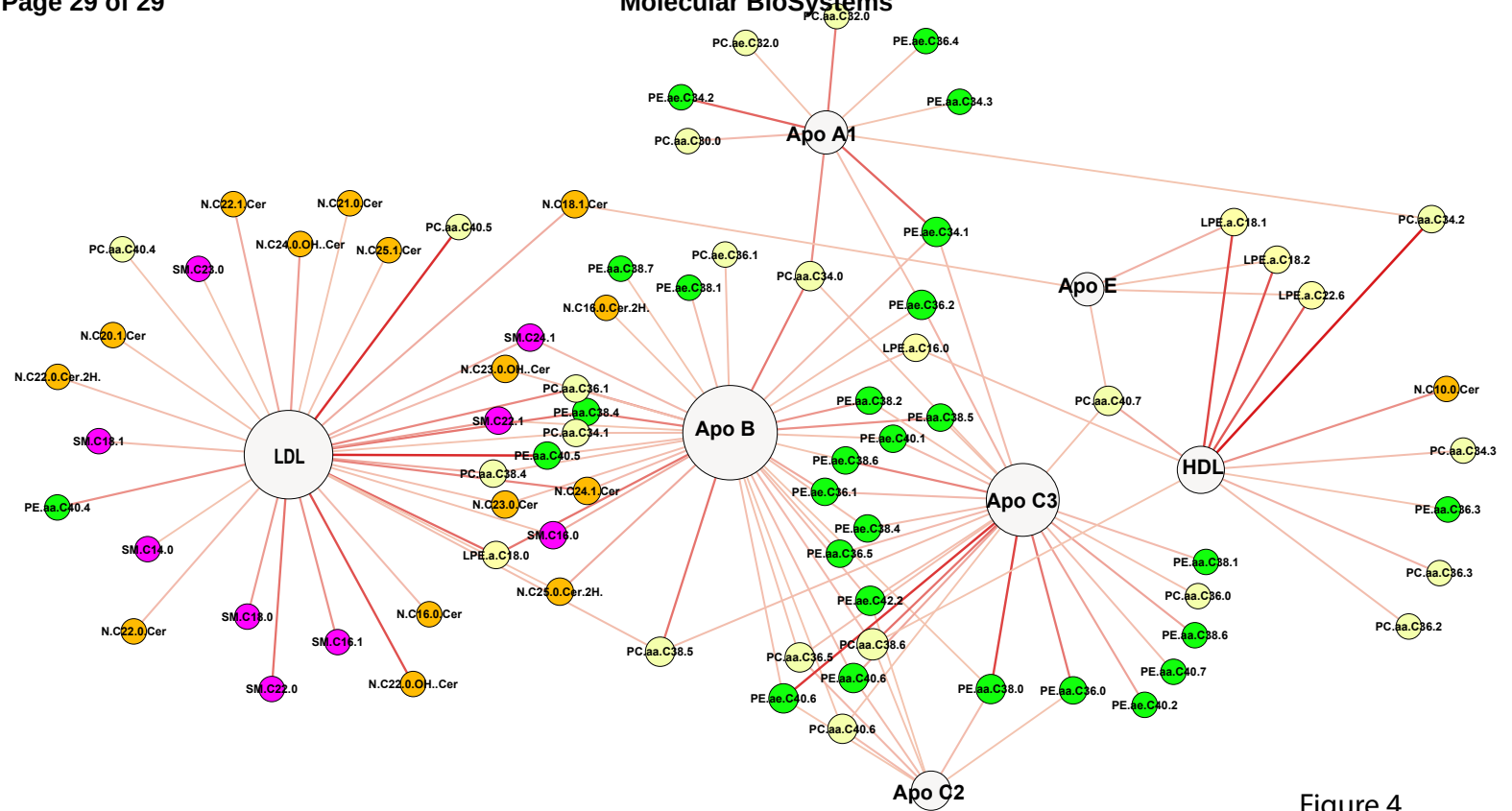
PC.aa.C32.0
PC.ae.C32.0
PE.ae.C36.4
PE.ae.C34.2
PE.aa.C34.3
PC.aa.C30.0
Apo A1
N.C22.1.Cer
N.C21.0.Cer
N.C18.1.Cer
PC.aa.C40.5
PC.aa.C40.4
N.C24.0.OH..Cer
N.C25.1.Cer
SM.C23.0
PE.ae.C34.1
PC.ae.C36.1
PE.aa.C38.7
PC.aa.C34.0
PE.ae.C38.1
Apo E
N.C16.0.Cer.2H.
PE.ae.C36.2
N.C20.1.Cer
SM.C24.1
LPE.a.C16.0
N.C23.0.OH..Cer
N.C22.0.Cer.2H.
PC.aa.C36.1
PE.aa.C38.4
SM.C22.1
PC.aa.C34.1
PE.aa.C40.5
SM.C18.1
PC.aa.C38.4
N.C24.1.Cer
N.C23.0.Cer
PE.aa.C40.4
SM.C16.0
LPE.a.C18.0
SM.C14.0
N.C25.0.Cer.2H.
N.C16.0.Cer
SM.C18.0
N.C22.0.Cer
SM.C16.1
SM.C22.0
N.C22.0.OH..Cer
LDL
Apo B
PE.aa.C38.2
PE.aa.C38.5
PE.ae.C40.1
PE.ae.C38.6
PE.ae.C36.1
PE.ae.C38.4
PC.aa.C36.5
PE.ae.C42.2
PC.aa.C38.5
PC.aa.C38.6
PC.aa.C36.5
PE.aa.C40.6
PE.ae.C40.6
PC.aa.C40.6
Apo C3
Apo C2
PE.aa.C38.0
PE.aa.C36.0
PE.ae.C40.2
PE.aa.C40.7
PE.aa.C38.6
PC.aa.C36.0
PE.aa.C38.1
PC.aa.C36.2
PC.aa.C36.3
PE.aa.C36.3
PC.aa.C34.3
N.C10.0.Cer
PC.aa.C40.7
HDL
LPE.a.C18.1
LPE.a.C18.2
LPE.a.C22.6
PC.aa.C34.2

Figure 4