Metabolomic correlates of central adiposity and earlier life body mass index

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Abbreviations:

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AGR	android-to-gynoid fat ratio
BMI	body mass index
CVD	cardiovascular disease
HDL	high-density lipoprotein
LDL	low-density lipoprotein
NMR	nuclear magnetic resonance
WHR	waist-to-hip ratio

ABSTRACT

Body mass index (BMI) is correlated with circulating metabolites but few studies discuss other adiposity measures and little is known about metabolomic correlates of body mass index from early life. We investigated associations between different adiposity measures, BMI from childhood through adulthood, and metabolites quantified from serum using ^{1}H NMR spectroscopy in 900 British men and women aged 60-64. We assessed BMI, waist-to-hip ratio (WHR), and android-to-gynoid fat ratio (AGR) and BMI from childhood through adulthood. Linear regression with Bonferroni adjustment was performed to assess adiposity and metabolites. Of 233 metabolites, 168, 126, and 133 were associated with BMI, WHR and AGR at age 60-64, respectively. Associations were strongest for high density lipoprotein (HDL), particularly HDL particle size, e.g. there was 0.08 SD decrease in HDL diameter (95% CI: 0.07-0.10) with each unit increase in BMI. BMI-adjusted AGR or WHR were associated with 31 metabolites where there was no metabolome-wide association with BMI. We identified inverse associations between BMI at age 7 and glucose or glycoprotein at age 60-64 and relative large LDL cholesterol ester with post-adolescent BMI gains. In summary, we identified metabolomic correlates of central adiposity and earlier life BMI. These findings support opportunities to leverage metabolomics in early prevention of cardiovascular risk attributable to body fatness.

Keywords: metabolomics, obesity, nutrition, lipids, epidemiology

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Introduction

Obesity predisposes to CVD (4) but it is unclear which aetiological pathways are affected by obesity. Recently, high-throughput analysis of biological samples has allowed quantification of small-molecule metabolites (5) downstream from genome or gene, mRNA, and protein (6), reflecting an integrated metabolic profile (7–9). This metabolome-wide approach has shown aberrations in metabolic profile to be predictive of CVD (10, 11). A Mendelian randomization study suggested that higher BMI is associated with altered levels of some CVD-related metabolites including lipoprotein lipid and cholesterol contents, saturated fatty acids, branched-chain (BCAA) and aromatic amino acids, and inflammatory metabolites (14). However, adults with similar BMI may have different fat distributions (15). It is not clear whether central adiposity is associated with metabolite levels independent of BMI in late adulthood when central adiposity becomes more common. Further, there is indication that changes in BMI over up to 7 years are associated with metabolic profile (14, 16). However, it is unclear which metabolites are affected by changes in BMI across the life course, which is important given the effect of childhood BMI changes on cardiovascular function in adulthood (17, 18). Therefore, we assessed the similarities and differences between associations of BMI and central adiposity measures, as well as body size across the life course, with metabolic profile at age 60-64 in the MRC NSHD birth cohort.

Materials and methods

Study population

The Medical Research Council (MRC) National Survey of Health and Development (NSHD) has been described in details (19). From this population, we included individuals with measurements of at least one circulating metabolite, and information on BMI, waist and hip circumferences at age 60-64 (N=900). A flowchart depicting selection of final study population is shown as Supplemental Figure S1. Ethical approval was obtained from the Greater Manchester Local Research Ethics Committee and the Scotland Research Ethics Committee. Written informed consent was obtained from the study member for each component of each data collection.

Metabolite quantification

Targeted metabolomics analysis was performed on serum samples collected at age 60-64. Metabolites were quantified by an automated NMR metabolomics platform (Bruker AVANCE III 500 MHz and Bruker AVANCE III HD 600 MHz spectrometers) which have been widely used in published studies (21). A total of 233 metabolite concentrations and derived measures were obtained (Supplemental Table S1, Supplementary Information). Of 233 metabolites, 159 (72%) were measured in all included participants. The remaining metabolites had <1.5% missing values. Metabolite measures that deviated from normality were log-transformed (Supplemental Table S1, Supplementary Information) and all measures were standardised.

Assessment of adiposity

At ages 60-64, weight (kg), height (m), waist and hip circumference (cm) were measured using standardised protocols by trained nurses (19). Weight and height were also measured at ages 7, 15, 36, 43, and 53 and BMI (kg/m^2) was calculated. Measures of body composition at age 60-64 were obtained in the supine position using a QDR4500 Discovery DXA scanner (Hologic Inc, Bedford, MA, USA); and reviewed using a single operator using APEX 3.1 software (Hologic Inc., Bedford, MA, USA) (15). Measures of android and gynoid fat mass were obtained and the ratio between the two (android-to-gynoid ratio) was calculated (higher values indicating greater fat distribution in the abdomen than hips).

Other covariates

Systemic metabolites have been reported to be altered with lipid medications (22), diabetes (23), and other chronic diseases (24). Self-reported information on use of statin, diabetes diagnosis and unintentional weight loss was therefore collected. Unintentional weight loss, which may represent pre-clinical chronic disease (25), was defined as losing weight more than 10 lbs unintentionally in the past year.

Data analysis

Association between current adiposity and systemic metabolites

Ordinary least square (OLS) regression was used with the adiposity measure (BMI, WHR, or AGR) as the predictor for each metabolite, adjusting for sex, age and clinic. Models for WHR and AGR were further adjusted for BMI. A sensitivity analysis was performed by restricting the models to those without statin use (N=698), diabetes (N=849), or unintentional weight loss (N=856). To address potential correlation between metabolites (9), we repeated the analysis for each adiposity measure using a multivariate approach, orthogonal partial least square (OPLS) (26). Metabolites with a variable importance in projection (VIP)≥1 were deemed strongly correlated with adiposity (27).

Association between prior BMI and systemic metabolites

We then investigated how prior BMI was associated with levels of metabolites. OLS regression models were employed to assess BMI in childhood (age 7; N=766), adolescence (age 15; N=722), and adulthood (age 36, 43 and 53; N=836, 855, and 859, respectively) and metabolite levels at age 60-64. Significant associations for prior BMI were further adjusted for BMI at age 60-64 in order to assess whether prior BMI was associated with metabolite level over and above current BMI (28, 29). Since the numbers of participants who had data on prior BMI were smaller than the maximum sample, we performed a sensitivity analysis assessing associations between BMI at all ages and metabolites in participants who had complete data on all BMI measures (N=569).

Association between BMI gains and systemic metabolites

In the main sample, we assessed whether there were sensitive periods in life during which BMI gains are associated with metabolites in late adulthood. This was conducted for age 7 to 15, 15 to 36, 36 to 43, 43 to 53, and 53 to 60-64 by regressing each BMI measure on the BMI measured earlier for each sex. Higher residuals represented greater BMI gains than expected (30). Each set of residuals was standardised and used as predictors of metabolites to address whether there are periods when gain in BMI was associated with later life metabolites.

For each model, we used a Bonferroni-adjusted significance threshold for 233 tests (p-value<0.0002). All analyses were conducted in R statistical software version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of study members are shown in Supplemental Table S2. The majority had non-manual occupations and were overweight at age 60-64. Pairwise correlations between metabolites are presented as Supplemental Figure S2.

Association between current adiposity and systemic metabolites

In OLS (N=900), 168 metabolites were associated with BMI (Figure 1), 126 with WHR (Figure 2), and 133 with AGR (Supplemental Figure S3). After adjusting for BMI, 63 metabolites remained associated with WHR and 106 with AGR (Supplemental Figure S4-5). Associations were strongest for high density lipoprotein (HDL) particle diameter (HDL D) and concentration of very large HDL phospholipids (XL HDL PL) (Table 1). Sensitivity analyses excluding participants with statin use, diabetes or unintentional weight loss yielded similar results (data not shown). We identified 25 metabolites in men and 5 in women which were associated with WHR or AGR when adjusted for BMI, but they were not associated with BMI in the main sex-stratified analysis (Table 2).

In OPLS, six predictors or principal components explained the association between metabolites and each measure of adiposity. Taking such clustering into account, a total of 99 metabolites were shown to be associated with BMI (VIP≥1), 87 with WHR and 90 with AGR. Common metabolites identified by both the hypothesis-testing approach and OPLS are indicated in Figure 1-2, Supplemental Figure S3 for BMI, WHR and AGR, respectively. Agreement between the two methods was high for associations of BMI, WHR and AGR with metabolite markers of very large and large HDL, large and medium VLDL, lipoprotein particle size and BCAAs. HDL diameter was consistently among top ten metabolite correlates of BMI, WHR, and AGR in the OPLS analysis (Supplemental Table S3).

Association between prior BMI and systemic metabolites

Associations between prior adult BMI and metabolites showed similar albeit weaker trends to current BMI (Supplemental Figure S6; Table 2). More metabolites were associated with more recent BMI, with 125, 147 and 162 metabolite correlates seen for BMI at age 36, 43, and 53, respectively. Of

these, only 3 remained associated with BMI at age 43 and 11 with BMI at age 53 when adjusted for BMI at age 60-64 (Supplementary Figure S6). No association was observed between BMI at age 7 or 15 with systemic metabolites at age 60-64. However, upon adjustment with BMI at age 60-64, we found BMI at age 7 to be inversely related with 0.11 SD decrease in glucose (95% CI: -0.06 to -0.15) and 0.10 SD decrease in glycoprotein (-0.05 to -0.15).

The sensitivity analysis in the subset of participants who had complete data of prior BMI showed similar results (data not shown); and after adjustment with current BMI, only one metabolite, small LDL phospholipids (S LDL PL) remained associated with previous BMI, i.e. BMI at age 43.

Association between BMI gains and systemic metabolites

BMI gains from adolescence through adulthood were consistently related to very large and large HDL metabolites (Figure 3). Apart from these, BMI gains at the latter ages were also associated with larger VLDL metabolites, and adolescent gains with relative contents of smaller VLDL and LDL metabolites, medium HDL, fatty acids, and aromatic amino acids (Figure 3). One metabolite, relative large LDL cholesterol ester (L LDL CE %), decreased with greater BMI gain between age 15 and 36 but was not associated with change at other ages or BMI at age 60-64. Small HDL total lipids (S HDL L), was associated with BMI gains between age 43 and 53 but not with other periods or current BMI.

Discussion

We showed associations between current adiposity and systemic metabolites in late adulthood and BMI in earlier ages. Most consistent associations were observed for HDL metabolism. We identified 25 metabolite measures independently associated with central adiposity but not BMI. When controlling for BMI at age 60-64, greater BMI at age 7 were correlated with lower glucose and glycoprotein at age 60-64. We also revealed lower relative large LDL cholesterol ester at age 60-64 with greater adolescent-to-adulthood BMI gains.

Our cross-sectional findings for BMI and metabolites in early old age corroborate prior findings linking body size and metabolites, including causal associations observed using a Mendelian

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randomisation study of young adults (31). Similar to that study, we found associations between BMI and a number of metabolites including HDL metabolites, BCAAs, markers of glycolysis and inflammation. This may suggest that BMI influences metabolites in a similar manner throughout adulthood. Metabolite correlates of central adiposity also align with those identified in previous studies, such as lipoprotein concentration, HDL particle size, and BCAAs (32–35). We added to these findings by presenting data in late adulthood in which central obesity is common, including specific lipoprotein components, and identifying associations independent of BMI. Central adiposity may represent visceral rather than overall accumulation of fat (37). There were more metabolites specifically linked to central adiposity in men compared to women. This may support sexual dimorphism in regulation of fat depot and systemic metabolism (32, 36).

A similar pattern to current BMI was observed with greater past BMI or its increments, which is in line with a previous study assessing metabolites linked to a 7 year weight change in adults aged 62-77 (16). Although this may indicate BMI tracking, there were remaining associations between past BMI in adulthood and current metabolites after taking into account current BMI. Plausible mechanisms by which high BMI in earlier adulthood may affect metabolites in early old age may involve excess adiposity affecting systemic processes such as inflammation and oxidative stress (38) or predisposing to maladaptive lifestyles such as physical inactivity (39). Additionally, we identified specific metabolite correlates of BMI at age 7 after adjusting for BMI at age 60-64, and of BMI gains from adolescence to early adulthood which were different to late adulthood. The inverse associations of BMI at age 7 with glucose and glycoprotein, both of which are greater with higher BMI at age 60-64, may indicate that they were particularly responsive to greater gains in BMI between childhood and adulthood. These metabolites have been associated with adverse metabolic pathways including insulin resistance and advanced glycated end (AGE) products which are often activated in obesity (38, 40).

Strengths and limitations

The strength on this study lies in the longitudinal measurements of BMI from childhood through adulthood, and measurements of body fat distribution in early old age. A limitation of this study is the smaller number of those with information on prior BMI. However, findings comparing prior BMI

were similar in a sensitivity analysis limited to those with complete lifelong BMI information. Metabolites were only measured on one occasion. Individuals who had higher BMI were at higher risk of cardiovascular disease and may have died or dropped out of the study prior to metabolite quantification at age 60-64, and this may result in underestimation of the observed associations. Our NMR platform only included absolute or relative quantification of metabolites. Future studies of adiposity could investigate other characteristics of metabolites such as aggregation susceptibility since LDL aggregation has been shown to be associated with CVD and is potentially modifiable by treatment (46).

Conclusion

We found metabolite correlates of current and past measures of BMI, which imply that metabolic profiling may be valuable for interventions aiming to mitigate the impacts of excess adiposity across adult life. The suggestion of alternative mechanisms for central adiposity and childhood BMI, which are independent of current BMI at age 60-64, indicates importance of future research on body composition and longitudinal measures of adiposity.

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Conflict of interest

All authors declare no competing interest.

Authors' contributions

WW and RH conceptualised the study. AW, DK and RH provided the data and the materials for the study. PP performed data pre-processing of metabolites. WW carried out data analysis with input from all authors. WW wrote the first draft of manuscript. All authors wrote the final draft and agreed on the final manuscript for publication.

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Table 1. Ten metabolites associated with each adiposity measure at highest precision. Positive (inverse) associations were highlighted in blue (red), respectively. Significant results with adjustment for multiple testing are indicated in white. For abbreviations see Supplemental Table S1.

BMI	WHR*	AGR*	Prior BMI					Conditional BMI change					Overweight onset
			7	15	36	43	53	7 to 15	15 to 36	36 to 43	43 to 53	53 to 60-64	
L HDL FC	L HDL FC %	HDL D	S HDL C	ApoA1	L HDL FC %	ApoA1	L HDL FC	IDL TG %	XL HDL PL	ApoA1	HDL D	L HDL PL %	XL HDL FC
HDL D	LA %	XL HDL PL	SM	IDL C %	XL HDL PL	HDL C	HDL D	IDL C %	XL HDL FC	HDL C	L HDL FC	Pyr	XL HDL PL
XL HDL PL	Gp	L LDL FC %	FAw3	IDL TG %	ApoA1	L HDL FC	XL HDL PL	L HDL PL %	L HDL FC	L HDL FC	XL HDL PL	L HDL FC %	HDL D
L HDL FC %	L HDL PL %	TG PG	EstC	M HDL C %	HDL C	L HDL FC %	XL HDL FC	FAw6 %	L HDL FC	HDL2 C	M VLDL CE %	L HDL FC	L HDL FC
L HDL C	FAw6 %	XL HDL P	S HDL CE	XL HDL FC %	XL HDL FC	XL HDL FC	XL HDL P	S LDL TG %	HDL D	L HDL FC %	M VLDL TG %	PUFA %	XL HDL L
XL HDL P	L HDL C %	XL HDL L	PUFA	HDL2 C	L HDL FC	XL HDL PL	XL HDL L	L HDL FC %	HDL C	HDL D	L VLDL TG	XL VLDL TG	XL HDL P
XL HDL L	XL HDL PL	Ile	Serum C	Alb	HDL2 C	HDL2 C	L HDL FC %	XL HDL FC	XL HDL L	L HDL C	TG PG	L HDL C	L HDL FC %
XL HDL FC	PUFA %	L HDL FC	S LDL PL	HDL C	HDL D	HDL D	L HDL P	S HDL TG	XL HDL P	L HDL CE	XL HDL P	L VLDL FC	XL HDL C
L HDL CE	Leu	M VLDL TG	ApoA1	L LDL TG %	XL HDL L	XL HDL L	HDL C	XL HDL C	XS VLDL FC %	L HDL L	XL HDL L	L HDL CE	HDL C
L HDL P	HDL D	VLDL TG	S LDL C	S LDL TG %	XL HDL P	XL HDL P	ApoA1	HDL2 C	L HDL C	XL HDL FC	L VLDL P	XL VLDL P	L HDL C

*Adjusted for BMI

Table 2. BMI-adjusted associations between body fat distribution and metabolites without associations with BMI at Bonferroni-adjusted significance level.

Metabolite	Android-to-gynoid ratio			Waist-to-hip ratio		
	N	Estimate	CI	N	Estimate	CI
Men						
Medium VLDL cholesterol esters	368	1.30	0.70 to 1.90			
Medium VLDL free cholesterol (%)	368	1.24	0.73 to 1.76			
Small VLDL phospholipids	368	1.59	1.06 to 2.12	465	3.56	1.88 to 524
Small VLDL total cholesterol	368	1.35	0.79 to 1.91	465	3.80	2.05 to 5.45
Small VLDL cholesterol esters				465	3.61	1.81 to 5.40
Small VLDL free cholesterol	368	1.66	1.13 to 2.19	465	3.67	1.97 to 5.36
XS VLDL concentration				465	3.48	1.68 to 5.26
XS VLDL cholesterol esters (%)	368	-1.25	-1.86 to -0.64			
Small LDL free cholesterol (%)				465	-3.86	-5.87 to -1.84
IDL triglycerides				465	3.28	1.67 to 4.89
Large LDL triglycerides				465	3.65	1.86 to 5.44
Medium LDL triglycerides				465	3.43	1.78 to 5.09
Small LDL triglycerides	368	1.24	0.63 to 1.84	465	4.42	2.49 to 6.24
XL HDL triglycerides (%)	363	1.27	0.65 to 1.90			
Medium HDL triglycerides	368	1.21	0.66 to 1.76			
VLDL cholesterol	368	1.42	0.84 to 2.00	465	3.87	2.05 to 5.68
RemNAt cholesterol				465	3.64	1.78 to 5.50
LDL triglycerides				465	3.90	2.09 to 5.71
Diacylglycerol	368	1.26	0.65 to 1.87			
Apolipoprotein B	368	1.17	0.58 to 1.76	465	3.96	2.13 to 5.78
Total fatty acids				464	3.60	1.81 to 5.38
MUFA; 16:1, 18:1	368	1.36	0.76 to 1.95	464	4.22	2.39 to 6.06
Saturated fatty acids				464	3.95	2.05 to 5.85
Citrate				465	-4.13	-6.10 to -2.16
Glycoprotein acetyls	368	1.83	1.23 to 2.43	465	5.35	3.45 to 7.25
Women						
Small HDL concentration	365	2.03	1.23 to 2.83			
Small HDL phospholipids	365	1.98	1.18 to 2.78			
Small HDL total lipids	365	1.96	1.16 to 2.76			
XXL VLDL phospholipids (%)	365	1.88	1.00 to 2.76			
Diacylglycerol	365	1.55	0.80 to 2.29			

Percentage (%) for lipoprotein lipid components refers to proportion against total lipid contents.

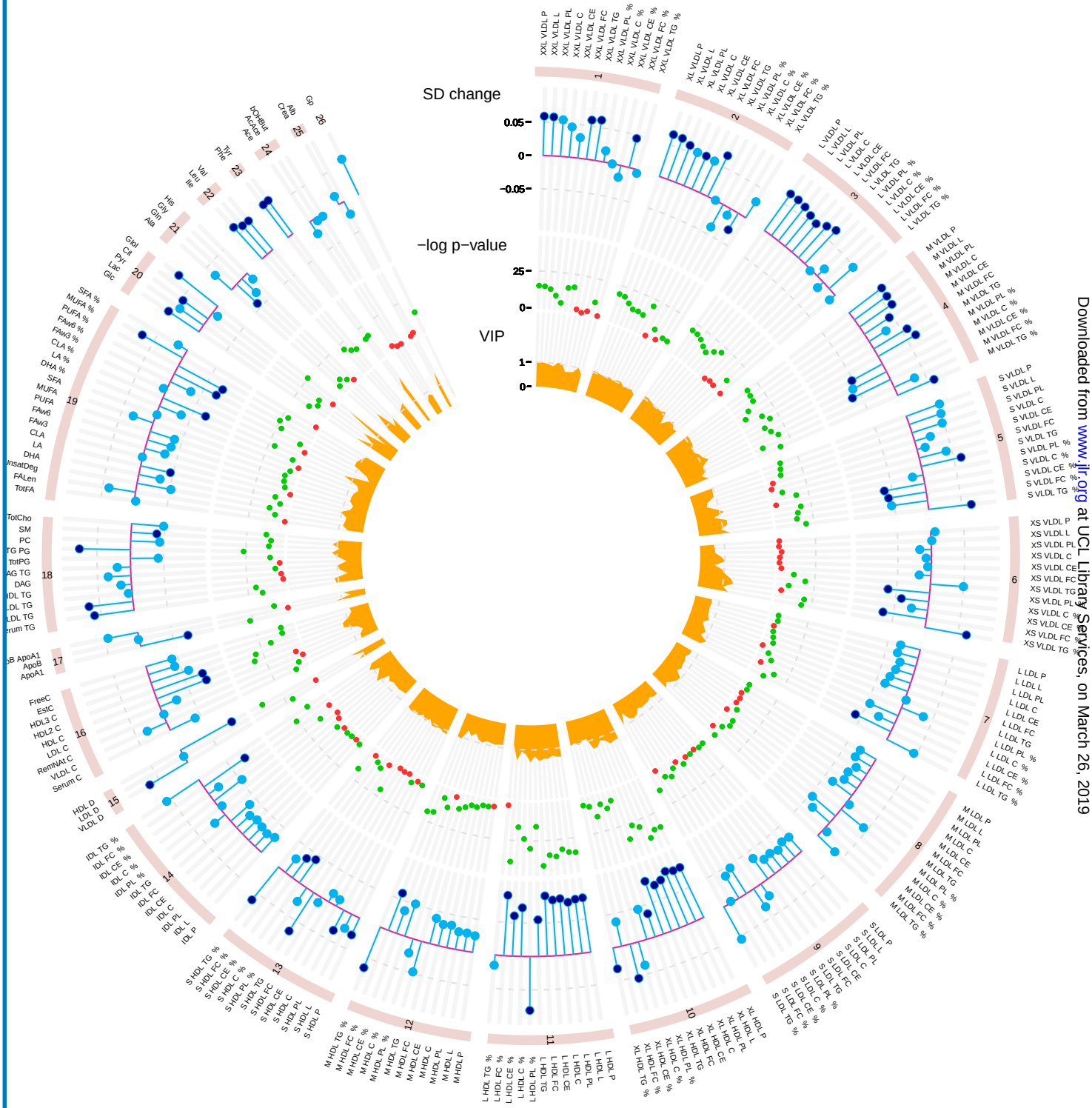
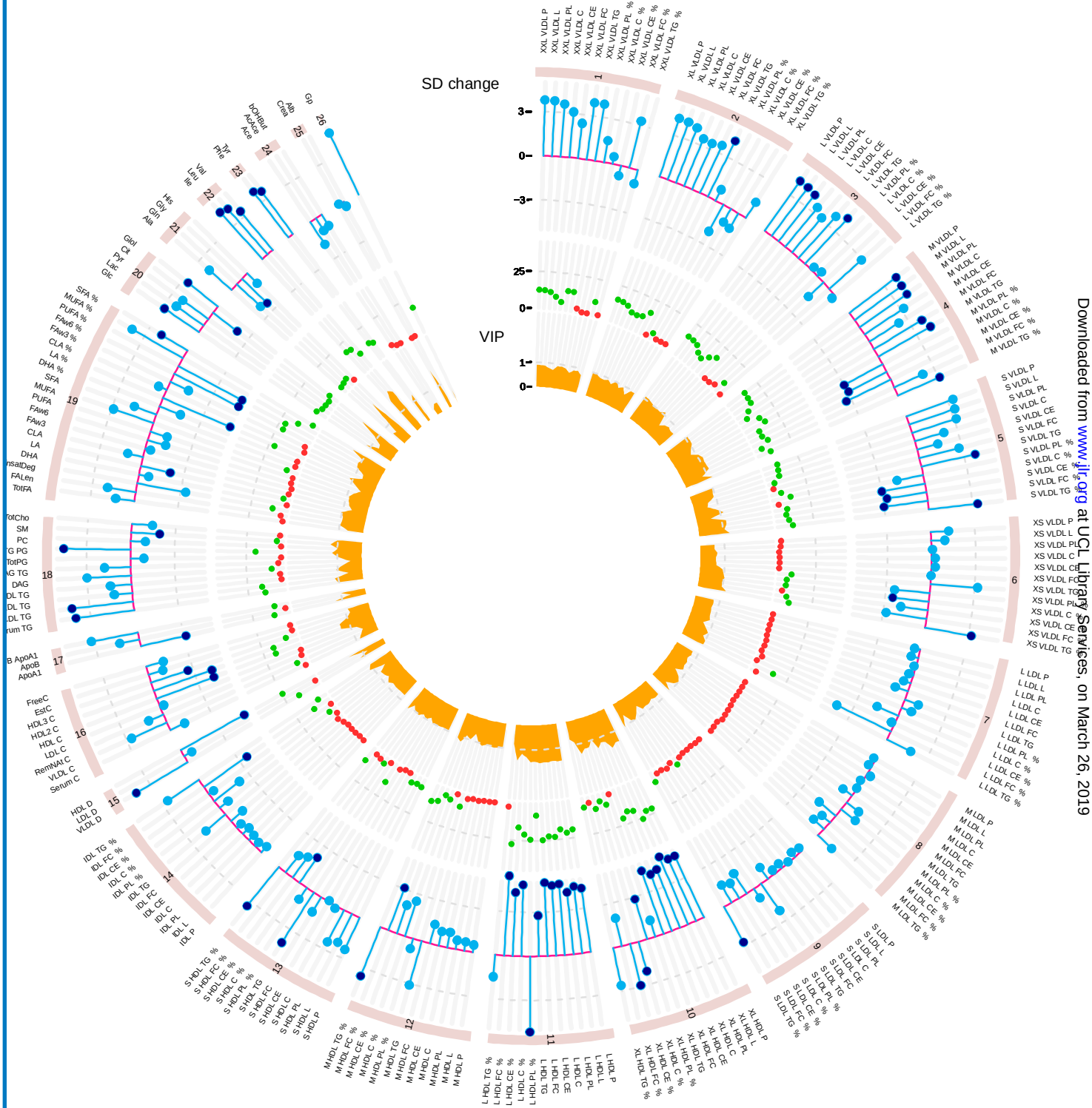


Figure 1. Cross sectional associations between BMI and systemic metabolites at age 60-64.
The outer circle shows predicted SD change in metabolite levels for every kg/m2 increase in BMI in OLS analysis.
The middle circle shows 'Manhattan plot' with green dots indicating significant p-values after Bonferroni adjustment.
Variable importance in projection (VIP) from OPLS analysis is shown in inner circle.
Metabolites identified by the two analyses were indicated with dark blue points.
See Supplemental Table S1 for abbreviations and grouping based on metabolic processes.
All models were adjusted for sex, age at NMR blood collection and NMR blood collection centre.



ure 2. Cross sectional associations between WHR and systemic metabolites at age 60-64. The outer circle shows predicted SD nge in metabolite levels for every kg/m2 increase in WHR in OLS analysis. The middle circle shows 'Manhattan plot' with green indicating significant p-values after Bonferroni adjustment. Variable importance in projection (VIP) from OPLS analysis is wn in inner circle. Metabolites identified by the two analyses were indicated with dark blue points. See Supplemental Table 1 for reviations and grouping based on metabolic processes. All models were adjusted for sex, age at NMR blood collection and R blood collection centre.