1 **Title**

2 Sex and puberty-related differences in metabolomic profiles associated with adiposity

3 measures in youth with obesity

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Abstract

Background: Specific patterns of metabolomic profiles relating to cardiometabolic disease are associated with increased weight in adults. In youth with obesity, metabolomic data are sparse and associations with adiposity measures unknown.

Objectives: Primary, to determine associations between adiposity measures and metabolomic profiles with increased cardiometabolic risks in youth with obesity. Secondary, to stratify associations by sex and puberty.

Methods: Participants were from COBRA (Childhood Overweight BioRepository of Australia; a paediatric cohort with obesity). Adiposity measures (BMI, BMI z-score, %truncal and %whole body fat, waist circumference and waist/height ratio), puberty staging and NMR metabolomic profiles from serum were assessed. Statistics included multivariate analysis (principal component analysis, PCA) and multiple linear regression models with false discovery rate (FDR) adjustment.

Results: 214 participants had metabolomic profiles analyzed, mean age 11.9y (SD+/-3.1), mean BMI z-score 2.49 (SD+/-0.24), 53% females. Unsupervised PCA identified no separable clusters of individuals. Positive associations included BMI z-score and phenylalanine, total body fat% and lipids in medium HDL, and waist circumference and tyrosine; negative associations included total body fat% and the ratio of docosahexaenoic acid/total fatty acids and histidine. Stratifying by sex and puberty, patterns of associations with BMI z-score in post-pubertal males included positive associations with lipid-, cholesterol- and triglyceride-content in VLDL lipoproteins; total fatty acids; total triglycerides; isoleucine, leucine and glycoprotein acetyls.

Conclusion: In a paediatric cohort with obesity, increased adiposity measures, especially in post-pubertal males, were associated with distinct patterns in metabolomic profiles.

48 **Tables: 2**

49 **Figures: 4**

50 **Supplementary tables: 3**

51 **Supplementary figures: 5**

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Introduction

One fifth of children in industrialised countries is overweight or obese (Ng *et al.*, 2014). Excess adiposity in childhood is associated with increased cardiovascular risk factors (Chung *et al.*, 2018). Cardiovascular risk factors are more common in those with higher body mass index (BMI) z-score (Gidding *et al.*, 2004; Norris *et al.*, 2011). They cluster in individuals with obesity (May *et al.*, 2012), track from childhood into adulthood (Bjerregaard *et al.*, 2014; Chen and Wang, 2008; Juhola *et al.*, 2011) and are associated with increased risk of cardiovascular and metabolic outcomes. Predictive markers for cardiometabolic disease in youth are inadequate and body mass index (the most widely used parameter) has poor predictive capacity. Therefore novel markers are needed to identify, and subsequently prevent, cardiometabolic disease that cause a significant and increasing burden on public health and healthcare systems (Olshansky *et al.*, 2005).

Metabolomics refers to the quantitative analysis of small metabolites within a biospecimen of interest. Nuclear magnetic resonance (NMR) spectroscopy is commonly used to generate metabolomic profiles that include measures of lipids, sugars, amino and organic acids and markers of chronic inflammation (Wishart, 2016). Cumulative data have confirmed that these are influenced by both genetic and environmental factors (Zhang *et al.*, 2013). Previous studies in adults utilising metabolomics platforms have revealed distinct metabolic patterns related to increasing body mass index, cardiometabolic risk factors (Ho *et al.*, 2016; Holmes *et al.*, 2014; Tulipani *et al.*, 2016; Wang *et al.*, 2011; Welsh *et al.*, 2018; Zhao *et al.*, 2016) and CVD events (Holmes *et al.*, 2018). *Wurtz et al.* used a cardiometabolic-risk focussed metabolomic platform in young adults (mean age 26 years) to show with Mendelian randomisation that such metabolomic patterns are causally elicited by increasing BMI (Wurtz *et al.*, 2014). In healthy and obese adults, NMR metabolomic analysis have also shown sex-

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77   dependent variation, in particular for levels of branched chain amino acids (Vignoli *et al.*, 2018;

78   Xie *et al.*, 2014). Limited analogous data are available in children and adolescents, particularly

79   in those with early-onset obesity. Pubertal development impacts substantially on body

80   composition (Loomba-Albrecht and Styne, 2009) and cardiometabolic risk factors (Reinehr *et*

81   *al.*, 2015), but the effect on metabolomic profile is unknown.

82   In the present study of a cohort of obese children and adolescents, we aimed to investigate

83   patterns of associations between different adiposity measures and metabolites that might

84   reflect an increased risk for obesity-related, adverse cardiometabolic outcomes. In addition,

85   we hypothesized that there would be sex- and puberty-related differences. We utilised high-

86   throughput, NMR-based metabolomics (Fischer *et al.*, 2014; Kettunen *et al.*, 2012; Stancakova

87   *et al.*, 2012; Wurtz *et al.*, 2012b; Wurtz *et al.*, 2014) to test the association between adiposity

88   measures (BMI, BMI z-score, truncal fat percentage, body fat percentage, waist circumference

89   and waist to height ratio) with specific metabolite measures and the possible effect of sex and

90   puberty on metabolomic profiles.

Methods

Study design

The study population was derived from the Childhood Overweight BioRepository of Australia (COBRA) cohort study (total n=412). Children and adolescents were recruited from the Royal Children's Hospital (RCH, Melbourne, Australia) Weight Management Service as previously described (Sabin *et al.*, 2010). Informed, written consent was obtained from the participant or the legally authorised representative from minors (aged less than 16 years). In brief, at the initial clinical appointment, data on demographics, medical history, and anthropometry (including adiposity measures) were collected. A fasting venous blood sample for plasma and serum was collected, immediately processed and stored at − 80˚ C in the Melbourne Children's Bioresource Centre (MCBC). The study protocol was approved by the Royal Children`s Hospital Human Research Ethics Committee, RCH, Melbourne, Australia (HREC Ref. # 28081Q, 9th of October 2017) and is in accordance with Helsinki principles.

Anthropometry measures of study participants

Height was measured without shoes and to the nearest 0.5 cm, using a fixed Harpenden stadiometer. Weight and percentages of total body and truncal fat mass were measured in light clothes with a four-point bio-impedance device (Tanita® Japan), previously validated for use in children (McCarthy *et al.*, 2006). Body mass index (BMI) was calculated according to the formula weight in kg divided by height in m^2 and then converted into BMI z-scores adjusted for age and sex using the US Centres for Disease Control (CDC) growth reference charts (Kuczmarski *et al.*, 2000). Waist circumference was measured with a non-flexible meter between iliac crest and lower end of rips to the nearest 0.5cm in expiration. BMI and BMI z-score were considered raw mass measures, while whole body and truncal fat percentage fat

measures and waist circumference and waist to height ratio served as indicators of body composition. A specialist paediatric endocrinologist or a consultant general paediatrician assessed Tanner stage for pubertal development, where Tanner 1 was considered pre-pubertal, Tanner 2-3 peri-pubertal and Tanner 4-5 post-pubertal (Marshall and Tanner, 1969, 1970).

Metabolite quantification

Metabolomic analysis from stored serum samples was performed on the Nightingale® Nuclear Magnetic Resonance (NMR) spectroscopy platform as previously described (Kettunen *et al.*, 2012; Soininen *et al.*, 2009). A total of 73 metabolites, capturing the majority of variation within the dataset, were used in analyses, including lipid subclass concentration, composition, size and ratios and concentrations of apolipoproteins, cholesterols, fatty acids, glycerides, phospholipids, amino acids, glycolysis related products, albumin, creatinine and glycoprotein acetyls (supplementary table 1).

Statistical analysis

i) Descriptive statistics:

Participant's characteristics are described in mean and standard deviation (SD) for continuous and number (%) for categorical variables. Metabolites and participant's characteristics were checked for their degree of skewness, calculated by the skew function in the e1071 (David Meyer, 2018) package for R statistics ((2018), 2018). If skewness of a specific measure was greater or equal to 2, values were log10-transformed.

ii) Inferential statistics

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139   Adiposity measures (BMI, total body fat percentage, truncal fat percentage, waist

140   circumference and waist/height ratio but not BMI z-scores) and metabolites were scaled to

141   SD units to facilitate the comparison of estimates across metabolite measures. Principal

142   component analysis (PCA), a common approach in multivariate data analysis, was used to

143   investigate the dataset`s variability and to uncover clusters within the dataset (Worley and

144   Powers, 2013). The principle of PCA is to reduce the dataset`s variability into principal

145   components (PC`s) containing most (PC1), second most (PC2) and so forth of the variability.

146   For illustration, PCA plots with PC1 and PC2 further characterised for age and sex and a table

147   providing the percentage contribution of variation from the most important five principal

148   components were provided.

149   Multiple linear regression was applied using a change of 1 standard deviation for each

150   adiposity measure as predictor and the metabolite measure as outcome, adjusted for age and

151   sex. A Benjamini-Hochberg (Benjamini and Hochberg, 1995) false discovery rate of 0.1 was

152   used to adjust for multiple testing. In addition, linear modelling was applied to sex and

153   puberty-specific datasets to illustrate trends for metabolites. R® version 3.5 was used for all

154   statistical analysis. For graphics, R® version 3.5 or MS Office Powerpoint® was used.

Results

Blood samples were available for 269 participants, of which 50 individuals were excluded due to age (<6 years) and/or a BMI z-score below 2.0. Additional 5 individuals were excluded due to analytical errors. A total of 214 participants with serum derived metabolomic data were included. Descriptive characteristics of participants are shown in Table 1.

Multivariate analysis

Principal component analysis did not reveal separable clusters, and age (figure 1a) and sex (figure 1b) were not major confounders, i.e. these characteristics did not result in separable clusters within our dataset. The top 5 principal components contained 73% of the dataset`s variability (see table in figure 1c).

Adiposity and metabolomic patterns

Adiposity measures were associated with metabolites following adjustment for age, sex and false discovery rate (FDR). The associations were broadly similar across the different mass and body composition measures, although the strength of specific associations with individual metabolites showed some variation (see figure 2 and supplementary figures 1-5). For example, a 1-SD increase in BMI z-score was positively associated with phenylalanine ($p<0.001$) and negatively with log acetate concentrations ($p<0.001$). For total percentage of body fat there was a positive association with total lipids in medium-sized HDL lipoproteins ($p<0.01$) and negative associations with the ratio of docosahexaenoic fatty acids to total fatty acids (%), with histidine, log lactate (all $p<0.01$) and creatinine ($p<0.001$). Percentage of truncal fat was negatively associated with creatinine ($p<0.01$), whereas waist circumference was positively associated with tyrosine ($p<0.001$) and negatively with log lactate ($p<0.001$). Table 2 illustrates a complete list of associations between adiposity measures and metabolites

178   including adjustment for FDR. Supplementary table 2 shows characteristics from multiple

179   linear regression with BMI z-score as the exposure variable, adjusted for age and sex.

180

181   *Impact of sex on metabolites*

182   Figure 3 illustrates the changes in estimates of metabolites for every 1-SD increase in BMI z-

183   score categorized for sex. In females, positive associations were found with total lipids in small

184   HDL lipoproteins ($p<0.05$), phenylalanine ($p<0.05$) and tyrosine ($p<0.05$). Negative

185   associations were found with the estimated degree of unsaturation ($p<0.05$), 22:6

186   docosahexaenoic acid ($p<0.05$), ratio of 22:6 docosahexaenoic acid to total fatty acids

187   ($p<0.01$), histidine ($p<0.05$) and log acetate ($p<0.05$). Each of these attenuated towards the

188   null following FDR correction. In males, an increase in BMI z-score was negatively associated

189   with log acetate ($p<0.001$) even with FDR correction.

190

191   *Impact of pubertal development on metabolites*

192   Figure 4 illustrates the association of BMI z-score with metabolites by sex and puberty.

193   Supplementary table 3 lists the associations between all investigated adiposity measures and

194   metabolites in post-pubertal individuals. In post-pubertal females, the only metabolite

195   associated with BMI z-score after multiple comparison was tyrosine (positively associated). In

196   contrast in post-pubertal males, several associations were found with BMI z-score after

197   adjustment for multiple comparisons. These included concentration of total lipids,

198   cholesterols and triglycerides in VLDL lipoproteins, the ratio of apolipoprotein B/A1 and the

199   mean diameter for VLDL and LDL particles. Associations were also seen with concentrations

200   of total fatty acids, linoleic acid, omega-6 fatty acids, and polyunsaturated, monounsaturated

201   and saturated fatty acids (all positively associated) and the estimated degree of unsaturation

202   (negatively associated). Further associations were found with triglycerides in HDL, total

203   phosphoglycerides, isoleucine and leucine, glycoprotein acetyls (all positively associated) and

204   log acetoacetate (negatively associated) (see figure 4).

205

Discussion

Studies in adults have revealed distinct metabolomic patterns with weight gain and cardiometabolic risk and disease. Here we investigated the relationship between clinical adiposity measures and an NMR-based metabolomic profile in a cohort of children and adolescents with obesity, aged 6-18 years. We observed that an increase in a range of adiposity measures (BMI, BMI z-score, whole body and truncal fat percentage, waist circumference and waist to height ratio) was associated with changes in the concentration of several metabolites and lipid subclass size. The strongest evidence for associations was observed in post-pubertal males.

Increasing BMI z-score in the study cohort was positively associated with elevated concentrations for phenylalanine and negatively related to log acetate after correction for FDR. Changes in lipid content in very large HDL lipoproteins, the estimated degree of unsaturation, the ratio of 22:6 docosahexaenoic acid and omega-3 fatty acids to total fatty acids were all negatively associated with BMI z-score after linear regression modelling, but these associations did not remain significant after adjustment for FDR. Due to growth-related changes in BMI z-scores during childhood, we cannot directly compare effect sizes with available data from adulthood. However, the direction of associations we observed between increasing BMI z-scores and metabolites was comparable to a previous study in young adults (Wurtz *et al.*, 2014), where a similar metabolomic platform was investigated with increasing body mass index measures. With a longitudinal study design over 6 years using Mendelian randomisation, this study showed that increased adiposity had causal effects on multiple cardiometabolic risk markers. Risk markers included elevated levels of triglyceride- and cholesterol-carrying VLDL and LDL particles, lower levels of larger HDL particles, increased levels of fatty acids, higher levels of branched-chain (e.g. leucine, isoleucine and valine) and

230 aromatic amino acids (e.g. phenylalanine, tyrosine, histidine) and elevated levels for a marker

231 of chronic inflammation - glycoprotein acetyls (Wurtz *et al.*, 2014). We observed an additional

232 increase in effect size of this metabolomic risk profile for an increase in BMI z-score in post-

233 pubertal males (see figure 2 & 4 for comparison). In contrast to previous adult studies however

234 we found no distinct sex-related effects as per the unsupervised PCA analysis of the

235 metabolome (see figure 1b).

236 Similar metabolomic profiles have previously been related to cardiovascular disease outcomes

237 including myocardial infarction (MI) and ischaemic stroke (IS) in the China Kadoorie Biobank,

238 a cohort of more than half a million Chinese adults aged 30-79 years (Holmes *et al.*, 2018).

239 Increased levels of VLDL lipoprotein particles were positively associated with MI and IS (OR

240 per SD increase of 1.18 to 1.30). Similar, triglycerides levels in all lipoprotein subclasses

241 containing apoplipoprotein B were positively associated with MI and triglycerides in VLDL

242 lipoproteins were positively associated with IS. The mean VLDL diameter was positively

243 associated with MI (OR per SD 1.03-1.25) and IS (OR per SD 1.07-1.28) and HDL particle size

244 was inversely associated with MI (OR per SD 0.73-0.89) and IS (OR per SD 0.80-0.96).

245 Cholesterol concentrations in VLDL and IDL particles were positively associated with MI and IS

246 (OR per SD 1.15-1.39 and 1.09-1.32), whereas cholesterol in HDL2 (larger HDL particles) was

247 inversely associated with MI (OR per SD 0.72-0.87). The ratio of apolipoprotein B /

248 apolipoprotein A1 was positively associated with MI (OR per SD 1.18-1.43) and IS (OR per SD

249 (1.14-1.38) (Holmes *et al.*, 2018).

250 Many metabolites associated with cardiovascular endpoints in the China Kadoorie Biobank

251 were likewise associated with increasing BMI z-score in this study, mainly in post-pubertal

252 males. Of these, several are critically involved in the process of atherosclerosis: i) elevated

253 levels of VLDL lipoproteins, their cholesterol- and triglyceride-content and the ratio of

apolipoprotein B/AI are key to initial steps of atherosclerosis, from endothelial penetration and subendothelial retention of modified lipoprotein particles to the initiation of an inflammatory cascade (Back *et al.*, 2019). ii) decreased ratios of docosahexaenoic acid/total fatty acids and omega-6 fatty acids/total fatty acids have recently been related to a decreased inhibition of the NLRP3 inflammasome, a core inflammatory pathway involved in atherosclerosis (Lopategi *et al.*, 2019). iii) decreasing levels of larger and increasing levels of small HDL particles. Recent studies have shown that NMR based differentiation of HDL particles revealed superior cardiovascular risk assessment as compared to the classical HDL-cholesterol concentration (Santos-Gallego, 2015).

Another previous study using an *untargeted* metabolomic approach in a paediatric cohort (*Viva La Familia Study,* n=803, 56% with obesity, mean age 11y), reported positive associations between increasing weight and circulating branched chain amino acids and insulin resistance (Butte *et al.*, 2015). In adults, increased branched-chain amino acids (BCAA) and aromatic amino acids (AAA) have been positively associated with insulin resistance and metabolic syndrome (Wiklund *et al.*, 2014; Wurtz *et al.*, 2012a). In our study, we did not observe any relationship between increasing adiposity measures and BCAA, but there was evidence of an association between BMI and BMI z-score with phenylalanine, BMI and waist circumference with tyrosine and a negative association between total body fat percentages and histidine (see supplementary table 3). In females, we found negative associations for histidine and positive associations for tyrosine and phenylalanine that attenuated towards the null following FDR correction. In post-pubertal males, associations between increasing BMI z-score and BCAA (leucine and isoleucine) were significant, adjusted for FDR. Our results for associations with adiposity measures for the non-essential amino acid alanine, glutamine and glycine most likely

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277 reflect endogenous turnover in a fasting condition, however, none of these associations were

278 significant after adjustment for FDR.

279 Glycoprotein acetyls (represented by GlycA in this metabolomic profile) are of particular

280 interest in the context of cardiometabolic disease risk. GlycA reflect chronic inflammation, a

281 major mechanism underlying obesity-related comorbidities. In a recent study, GlycA was

282 associated with increased cardiovascular (MI, IS and intracranial haemorrhage) and all-cause

283 mortality (Akinkuolie *et al.*, 2014; Lawler *et al.*, 2016; Wurtz *et al.*, 2015). In 27,491 initially

284 healthy women followed up for 17.2 years, the hazard ratio of the upper quartile of GlycA was

285 1.23 (95%CI 1.04-1.46) for an incident cardiovascular event (MI, IS, coronary revascularization,

286 and CVD death) (Akinkuolie *et al.*, 2014). Interestingly, weight loss over 12 months after

287 bariatric surgery (Roux-en-Y gastric bypass or Sleeve gastrectomy) was associated with a

288 significant reduction in GlycA (Manmadhan *et al.*, 2019). In our study, GlycA was positively

289 associated with increasing BMI, total and truncal body fat percentages, although not after FDR

290 correction (see table 2). In sex-specific analyses, we found some evidence of an association

291 with higher GlycA in males in association with higher BMI, BMI z-score or waist to height ratio,

292 primarily among post-pubertal adolescents.

293 The strengths of this study included the availability of comprehensively assessed clinical data

294 from a large cohort of children and adolescents with obesity. The investigated metabolomic

295 platform has been widely used in epidemiologic studies in adults with outcome data available

296 for cardiovascular disease and type 2 diabetes mellitus. Limitations include the lack of a

297 normal weight control group, which would be an interesting comparator. In addition, the

298 cross-sectional study design does not allow us to assess the direction of causality and given

299 this is a child and adolescent cohort, data on cardiovascular events and type 2 diabetes

300 mellitus would only be available with long-term follow up.

301 Our findings illustrate metabolomic evidence of increased cardiometabolic risk with increasing

302 severity of adiposity in children and adolescents with obesity, revealing the adverse effects of

303 weight gain, in particular in post-pubertal males with obesity. These data highlight the

304 importance of efforts to reduce the increasing prevalence of obesity in youth. Our findings

305 support commencing weight management prior to puberty to avoid the development of an

306 adverse cardiometabolic profile observed in post-pubertal males. Longitudinal studies are

307 warranted to investigate whether these metabolomic changes in childhood and adolescence

308 predict definite cardiovascular and metabolic outcomes and to understand mechanisms

309 underlying the sex- and puberty-related associations.

310 **Table 1**

Variable	n	Mean (SD)	Range (min-max)	%
Age (years)	214	11.9 (3.1)	6.0 - 18.1	
Sex (female)	113			53
Pubertal stage				
Pre-pubertal	83			39
Peri-pubertal	58			27
Post-pubertal	73			34
Weight (kg)	214	85.9 (30.2)	30.7 - 157.9	
Height (m)	214	1.55 (0.16)	1.10 - 1.94	
BMI (kg/m^2)	214	34.5 (7.1)	22.0 - 58.5	
BMI z-score	214	2.49 (0.24)	2.00 - 3.10	
Body fat %	182	44.3 (7.8)	24.6 - 64.5	
Truncal fat %	175	38.2 (8.7)	17.3 - 70.8	
Waist Circumference (m)	169	1.06 (0.19)	0.63 - 1.54	
Waist to height ratio	169	0.69 (0.08)	0.50 - 0.93	

311

312 **Table 2**

		BMI	BMI z -score	Total body fat %	Truncal fat %	WC	WtH ratio
Metabolites & direction of association							
Total lipids in very large HDL (mmol/L)	neg	ns.	$p < 0.05$	ns.	ns.	ns.	ns.
Total lipids in medium HDL (mmol/L)	pos	ns.	ns.	**$p < 0.01$**	$p < 0.01$	ns.	ns.
Total lipids in small HDL (mmol/L)	pos	$p < 0.05$	ns.	$p < 0.05$	$p < 0.05$	ns.	ns.
Estimated degree of unsaturation	neg	$p < 0.05$	$p < 0.01$	$p < 0.05$	ns.	ns.	ns.
22:6, docosahexaenoic acid(mmol/L)	neg	ns.	ns.	ns.	$p < 0.05$	ns.	ns.
Ratio of docosahexaenoic acid to total fatty acids (%)	neg	$p < 0.05$	$P < 0.05$	**$p < 0.01$**	$p < 0.01$	ns.	ns.
Ratio of omega3 fatty acids to total fatty acids (%)	neg	ns.	$p < 0.05$	ns.	ns.	ns.	ns.
Glutamine (mmol/l)	neg	ns.	ns.	$p < 0.05$	$p < 0.05$	ns.	ns.
Histidine (mmol/l)	neg	ns.	$p < 0.05$	**$p < 0.01$**	$p < 0.01$	ns.	$p < 0.05$
Phenylalanine (mmol/l)	pos	**$p < 0.001$**	**$p < 0.01$**	ns.	$p < 0.05$	$p < 0.01$	ns.
Tyrosine (mmol/l)	pos	**$p < 0.001$**	$p < 0.05$	$p < 0.05$	ns.	**$p < 0.001$**	$p < 0.05$
Log acetate (mmol/l)	neg	**$p < 0.001$**	**$p < 0.001$**	**$p < 0.01$**	$p < 0.05$	**$p < 0.001$**	$p < 0.05$
Log acetoacetate (mmol/l)	neg	ns.	ns.	$p < 0.05$	ns.	ns.	ns.
Albumin (mmol/l)	neg	ns.	ns.	ns.	ns.	$p < 0.05$	$p < 0.05$
Creatinine (umol/l)	neg	ns.	ns.	**$p < 0.001$**	**$p < 0.01$**	$p < 0.05$	$p < 0.05$
Glycoprotein acetyls (mmol/L)	pos	$p < 0.05$	ns.	$p < 0.05$	$p < 0.05$	ns.	ns.

313

314 **Supplementary**

315 Supplementary table 1

Metabolite name	Metabolite subgroup
Log Total lipids in chylomicrons and extr. large VLDL (mmol/L)	Lipoprotein subclass
Log Total lipids in very large VLDL (mmol/L)	Lipoprotein subclass
Log Total lipids in large VLDL (mmol/L)	Lipoprotein subclass
Total lipids in medium VLDL (mmol/L)	Lipoprotein subclass
Total lipids in small VLDL (mmol/L)	Lipoprotein subclass
Total lipids in very small VLDL (mmol/L)	Lipoprotein subclass
Total lipids in IDL (mmol/L)	Lipoprotein subclass
Total lipids in large LDL (mmol/L)	Lipoprotein subclass
Total lipids in medium LDL (mmol/L)	Lipoprotein subclass
Total lipids in small LDL (mmol/L)	Lipoprotein subclass
Total lipids in very large HDL (mmol/L)	Lipoprotein subclass
Total lipids in large HDL (mmol/L)	Lipoprotein subclass
Total lipids in medium HDL (mmol/L)	Lipoprotein subclass
Total lipids in small HDL (mmol/L)	Lipoprotein subclass
Mean diameter for VLDL particles (nm)	Lipoprotein particle size
Mean diameter for LDL particles (nm)	Lipoprotein particle size
Mean diameter for HDL particles (nm)	Lipoprotein particle size
Apolipoprotein AI (g/L)	Apolipoproteins
Apolipoprotein B (g/L)	Apolipoproteins
Ratio of apolipoprotein B to apolipoprotein AI	Apolipoproteins
Serum total cholesterol (mmol/L)	Cholesterol
Total cholesterol in VLDL (mmol/L)	Cholesterol
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	Cholesterol
Total cholesterol in LDL (mmol/L)	Cholesterol
Total cholesterol in HDL (mmol/L)	Cholesterol
Total cholesterol in HDL2 (mmol/L)	Cholesterol
Total cholesterol in HDL3 (mmol/L)	Cholesterol
Esterified cholesterol (mmol/L)	Cholesterol
Free cholesterol (mmol/L)	Cholesterol
Total fatty acids (mmol/L)	Fatty acids
Estimated degree of unsaturation	Fatty acids
22:6, docosahexaenoic acid (mmol/L)	Fatty acids
18:2, linoleic acid (mmol/L)	Fatty acids
Omega3 fatty acids (mmol/L)	Fatty acids
Omega6 fatty acids (mmol/L)	Fatty acids
Polyunsaturated fatty acids (mmol/L)	Fatty acids
Monounsaturated fatty acids 16:1, 18:1 (mmol/L)	Fatty acids
Saturated fatty acids (mmol/L)	Fatty acids

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Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	Fatty acid ratios
Ratio of 18:2 linoleic acid to total fatty acids (%)	Fatty acid ratios
Ratio of omega3 fatty acids to total fatty acids (%)	Fatty acid ratios
Ratio of omega6 fatty acids to total fatty acids (%)	Fatty acid ratios
Ratio of polyunsaturated fatty acids to total fatty acids (%)	Fatty acid ratios
Ratio of monounsaturated fatty acids to total fatty acids (%)	Fatty acid ratios
Ratio of saturated fatty acids to total fatty acids (%)	Fatty acid ratios
Log Serum total triglycerides (mmol/L)	Glycerides and phospholipids
Log Triglycerides in VLDL (mmol/L)	Glycerides and phospholipids
Triglycerides in LDL (mmol/L)	Glycerides and phospholipids
Triglycerides in HDL (mmol/L)	Glycerides and phospholipids
Total phosphoglycerides (mmol/L)	Glycerides and phospholipids
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids
Phosphatidylcholine and other cholines (mmol/L)	Glycerides and phospholipids
Sphingomyelins (mmol/L)	Glycerides and phospholipids
Total cholines (mmol/L)	Glycerides and phospholipids
Pyruvate (mmol/L)	Glycolysis related
Citrate (mmol/L)	Glycolysis related
Glucose (mmol/L)	Glycolysis related
Lactate (mmol/L)	Glycolysis related
Log Acetate (mmol/L)	Ketone bodies
Log Acetoacetate (mmol/L)	Ketone bodies
Log 3hydroxybutyrate (mmol/L)	Ketone bodies
Alanine (mmol/L)	Amino acids
Glutamine (mmol/L)	Amino acids
Glycine (mmol/L)	Amino acids
Histidine (mmol/L)	Amino acids
Isoleucine (mmol/L)	Amino acids
Leucine (mmol/L)	Amino acids
Valine (mmol/L)	Amino acids
Phenylalanine (mmol/L)	Amino acids
Tyrosine (mmol/L)	Amino acids
Albumin (mmol/l)	Liver function
Creatinine (umol/L)	Renal function
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	Inflammation

316

Lipoprotein subclass lipids	Estimate	95% CI	p-Value	BH
Log Total lipids in chylomicrons and extr. large VLDL (mmol/L)	0.011	-0.021 to 0.044	0.493	ns.
Log Total lipids in very large VLDL (mmol/L)	0.015	-0.016 to 0.047	0.348	ns.
Log Total lipids in large VLDL (mmol/L)	0.017	-0.015 to 0.049	0.293	ns.
Total lipids in medium VLDL (mmol/L)	0.011	-0.02 to 0.043	0.488	ns.
Total lipids in small VLDL (mmol/L)	0.015	-0.016 to 0.047	0.339	ns.
Total lipids in very small VLDL (mmol/L)	0.013	-0.018 to 0.045	0.411	ns.
Total lipids in IDL (mmol/L)	0.001	-0.03 to 0.033	0.914	ns.
Total lipids in large LDL (mmol/L)	-0.001	-0.032 to 0.03	0.945	ns.
Total lipids in medium LDL (mmol/L)	-0.003	-0.034 to 0.028	0.845	ns.
Total lipids in small LDL (mmol/L)	-0.005	-0.037 to 0.025	0.726	ns.
Total lipids in very large HDL (mmol/L)	-0.033	-0.064 to -0.002	0.035	ns.
Total lipids in large HDL (mmol/L)	-0.023	-0.054 to 0.007	0.137	ns.
Total lipids in medium HDL (mmol/L)	0.012	-0.019 to 0.044	0.428	ns.
Total lipids in small HDL (mmol/L)	0.026	-0.005 to 0.059	0.102	ns.
Lipoprotein particle size				
Mean diameter for VLDL particles (nm)	0.014	-0.017 to 0.046	0.372	ns.
Mean diameter for LDL particles (nm)	0.013	-0.018 to 0.045	0.415	ns.
Mean diameter for HDL particles (nm)	-0.03	-0.061 to 0.001	0.058	ns.
Apolipoproteins				
Apolipoprotein AI (g/L)	-0.016	-0.047 to 0.015	0.315	ns.
Apolipoprotein B (g/L)	0.005	-0.026 to 0.037	0.718	ns.
Ratio of apolipoprotein B to apolipoprotein AI	0.015	-0.016 to 0.047	0.346	ns.
Cholesterol				
Serum total cholesterol (mmol/L)	0.002	-0.029 to 0.033	0.896	ns.
Total cholesterol in VLDL (mmol/L)	0.012	-0.019 to 0.045	0.426	ns.
Remnant cholesterol (nonHDL nonLDL cholesterol) (mmol/L)	0.008	-0.023 to 0.04	0.611	ns.
Total cholesterol in LDL (mmol/L)	-0.006	-0.037 to 0.025	0.700	ns.
Total cholesterol in HDL (mmol/L)	-0.02	-0.051 to 0.011	0.217	ns.
Total cholesterol in HDL2 (mmol/L)	-0.019	-0.051 to 0.012	0.231	ns.
Total cholesterol in HDL3 (mmol/L)	-0.018	-0.05 to 0.012	0.243	ns.
Esterified cholesterol (mmol/L)	-0.011	-0.043 to 0.019	0.464	ns.
Free cholesterol (mmol/L)	-0.01	-0.042 to 0.021	0.513	ns.
Fatty acids				
Total fatty acids (mmol/L)	0.013	-0.018 to 0.045	0.406	ns.
Estimated degree of unsaturation	-0.045	-0.076 to -0.013	0.005	ns.
22:6, docosahexaenoic acid(mmol/L)	-0.024	-0.056 to 0.007	0.127	ns.
18:2 linoleic acid (mmol/L)	0.005	-0.026 to 0.037	0.722	ns.
Omega3 fatty acids (mmol/L)	-0.009	-0.04 to 0.022	0.571	ns.
Omega6 fatty acids (mmol/L)	0.003	-0.028 to 0.034	0.850	ns.
Polyunsaturated fatty acids (mmol/L)	0.001	-0.03 to 0.032	0.943	ns.
Monounsaturated fatty acids 16:1 18:1 (mmol/L)	0.016	-0.014 to 0.048	0.296	ns.
Saturated fatty acids (mmol/L)	0.017	-0.014 to 0.049	0.278	ns.
Fatty acid ratios				

Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	-0.034	-0.066 to -0.003	0.031	ns.
Ratio of 18:2 linoleic acid to total fatty acids (%)	-0.014	-0.046 to 0.017	0.371	ns.
Ratio of omega3 fatty acids to total fatty acids (%)	-0.032	-0.063 to 0	0.047	ns.
Ratio of omega6 fatty acids to total fatty acids (%)	-0.026	-0.058 to 0.005	0.107	ns.
Ratio of polyunsaturated fatty acids to total fatty acids (%)	-0.03	-0.062 to 0.001	0.060	ns.
Ratio of monounsaturated fatty acids to total fatty acids (%)	0.02	-0.011 to 0.053	0.203	ns.
Ratio of saturated fatty acids to total fatty acids (%)	0.025	-0.006 to 0.056	0.120	ns.
Amino acids				
Alanine (mmol/L)	0.021	-0.01 to 0.053	0.178	ns.
Glutamine (mmol/L)	-0.011	-0.043 to 0.02	0.466	ns.
Glycine (mmol/L)	-0.013	-0.045 to 0.018	0.401	ns.
Histidine (mmol/L)	-0.035	-0.067 to -0.004	0.027	ns.
Isoleucine (mmol/L)	0.025	-0.007 to 0.058	0.129	ns.
Leucine (mmol/L)	0.017	-0.015 to 0.049	0.305	ns.
Valine (mmol/L)	0.014	-0.017 to 0.046	0.385	ns.
Phenylalanine (mmol/L)	0.052	0.019 to 0.085	0.002	significant
Tyrosine (mmol/L)	0.038	0.006 to 0.069	0.018	ns.
Glycerides and phopholipids				
Serum total triglycerides (mmol/L)	0.016	-0.015 to 0.048	0.308	ns.
Triglycerides in VLDL (mmol/L)	0.014	-0.017 to 0.046	0.380	ns.
Triglycerides in LDL (mmol/L)	0.025	-0.005 to 0.057	0.111	ns.
Triglycerides in HDL (mmol/L)	0.019	-0.012 to 0.051	0.232	ns.
Total phosphoglycerides (mmol/L)	0.001	-0.03 to 0.033	0.930	ns.
Ratio of triglycerides to phosphoglycerides	0.02	-0.01 to 0.052	0.195	ns.
Phosphatidylcholine and other cholines (mmol/L)	0.003	-0.028 to 0.034	0.843	ns.
Sphingomyelins (mmol/L)	0.003	-0.028 to 0.034	0.837	ns.
Total cholines (mmol/L)	0.002	-0.029 to 0.034	0.896	ns.
Glycolysis related				
Pyruvate (mmol/L)	0.017	-0.013 to 0.048	0.273	ns.
Citrate (mmol/L)	0.018	-0.012 to 0.05	0.240	ns.
Glucose (mmol/L)	-0.012	-0.044 to 0.018	0.423	ns.
Ketones				
Log Lactate (mmol/L)	-0.005	-0.036 to 0.026	0.753	ns.
Log Acetate (mmol/L)	-0.061	-0.091 to -0.03	<0.001	significant
Log Acetoacetate (mmol/L)	-0.03	-0.061 to 0.001	0.059	ns.
Log 3hydroxybutyrate (mmol/L)	0.014	-0.025 to 0.054	0.473	ns.
Liver function				
Albumin (mmol/l)	-0.014	-0.045 to 0.017	0.383	ns.
Kidney function				
Creatinine (umol/L)	-0.014	-0.055 to 0.027	0.506	ns.
Inflammation				
Glycoprotein acetyls mainly a1acid glycoprotein (mmol/L)	0.028	-0.004 to 0.06	0.086	ns.

318

319   Supplementary table 3

		BMI		BMI z -score		Total body fat %		Truncal fat %		WC		WtH ratio	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Metabolites & direction of association													
Log Total lipids in chylomicrons and extr. large VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Log Total lipids in very large VLDL (mmol/L)	pos.	ns	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.01**
Log Total lipids in large VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.01**
Total lipids in medium VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Total lipids in small VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	Ns	ns	**<0.05**
Total lipids in very small VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	Ns	ns	ns
Total lipids in small HDL (mmol/L)	neg.	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.01	ns	**<0.01**
Mean diameter for VLDL particles (nm)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Mean diameter for LDL particles (nm)	pos.	ns	**<0.05**	ns	**<0.05**	ns	<0.05	ns	<0.05	ns	<0.05	ns	**<0.05**
Total cholesterol in VLDL (mmol/L)	pos.	ns	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	pos.	ns	**<0.05**	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Serum total triglycerides (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Triglycerides in VLDL (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Triglycerides in HDL (mmol/L)	pos.	ns	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Ratio of triglycerides to phosphoglycerides	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.01**
Apolipoprotein B (g/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	<0.05
Ratio of apolipoprotein B to apolipoprotein AI	pos.	ns	**<0.05**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Total fatty acids (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Estimated degree of unsaturation	neg.	ns	**<0.05**	ns	**<0.05**	ns	<0.05	ns	ns	ns	<0.05	ns	**<0.05**
18:2, linoleic acid (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	**<0.05**
Omega3 fatty acids (mmol/L)	pos.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**<0.05**
Omega6 fatty acids (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	**<0.05**
Polyunsaturated fatty acids (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	**<0.05**
Monounsaturated fatty acids 16:1, 18:1 (mmol/L)	pos.	ns	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.01**
Saturated fatty acids (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	**<0.05**
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	neg.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	ns
Ratio of 18:2 linoleic acid to total fatty acids (%)	neg.	ns	<0.05	ns	**<0.05**	ns	ns	ns	ns	ns	ns	ns	**<0.05**

23

Ratio of omega6 fatty acids to total fatty acids (%)	neg.	ns	**<0.05**	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Ratio of polyunsaturated fatty acids to total fatty acids (%)	neg.	ns	**<0.05**	ns	**<0.05**	ns	ns	ns	ns	ns	<0.05	ns	**<0.05**
Ratio of monounsaturated fatty acids to total fatty acids (%)	pos.	ns	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	<0.05	ns	**<0.01**
Pyruvate (mmol/L)	pos.	ns	ns	ns	ns	ns	ns	ns	<0.05	ns	ns	ns	ns
Alanine (mmol/L)	pos.	ns	ns	ns	ns	ns	<0.05	ns	ns	ns	ns	ns	ns
Isoleucine (mmol/L)	pos.	ns	ns	ns	**<0.05**	ns	<0.05	ns	ns	ns	ns	ns	<0.05
Leucine (mmol/L)	pos.	ns	<0.05	ns	**<0.05**	ns	<0.05	ns	ns	ns	<0.05	ns	**<0.05**
Valine (mmol/L)	pos.	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.05	ns	ns
Phenylalanine (mmol/L)	pos.	<0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tyrosine (mmol/L)	pos.	**<0.01**	ns	**<0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Log Acetoacetate (mmol/L)	neg.	ns	**<0.01**	ns	**<0.01**	ns	<0.05	ns	ns	ns	ns	ns	ns
Albumin (mmol/l)	neg.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.05	ns
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	pos.	ns	**<0.05**	ns	**<0.01**	ns	<0.05	ns	ns	ns	<0.05	ns	**<0.01**

Table and Figure legends

Table 1:   Characteristics of the study cohort

Legend 1:   *Pubertal stage: Pre-pubertal: Tanner stage 1; Peri-pubertal: Tanner stage 2 & 3; Post-pubertal: Tanner stage 4 & 5. BMI: body mass index; BMI z-score: body mass index z-score using Centre of Disease Control (CDC) data. n: number of participants*

Table 2:   Patterns in metabolomics profile by clinical adiposity measures

Legend 2:   *Results from multiple regression modelling with each adiposity measure as explanatory variable and the metabolite as outcome, adjusted for age and sex. In bold are the ones adjusted for false discovery rate (FDR).*

Supplementary table 1:   Metabolites and subgroups

Supplementary legend 1:   *SI units are given in brackets. Metabolites with a log-prefix were log10-transformed due to skewed distribution.*

Supplementary table 2:   Model characteristics for metabolites per 1-SD increase in BMI z-score

Supplementary legend 2:   *Estimates reflect change in standard deviation per metabolite by 1-SD increase in BMI z-score. 95% CI: 95% Confidence interval. BH: Significance test according to Benjamin-Hochberg for false discovery rate*

Supplementary table 3:   Patterns in metabolomics profile in post-pubertal individuals categorised by clinical adiposity measure and sex, adjusted for age

Supplementary legend 3:   *Results from multiple regression modelling with each adiposity measure as explanatory variable and the metabolite as outcome in post-pubertal individuals, adjusted for age. In bold are the ones withstanding adjustment for multiple comparison. ns=not significant*

365   Figure 1:   Principal component analysis (PCA) plots

366   Legend 1:   PCA plot for principal component 1 (PC1) and principal component 2 (PC2),
367   graded for age (1a) and sex (1b). Section 1c lists percentage contribution of
368   the top 5 principal components.

369

370   Figure 2:   Changes in metabolites by 1 SD increase in BMI z-score

371   Legend 2:   Changes in mean and 95% confidence interval per 1-SD increase in BMI z-
372   score. * indicates association after multiple regression modelling (p <0.05).
373   Estimates and 95% CI's in **bold** illustrate significance after adjustment for false
374   discovery rate (FDR, according to Benjamini-Hochberg).

375

376   Figure 3:   Changes in metabolites by 1 SD increase in BMI z-score categorized by sex

377   Legend 3:   Changes in mean and 95% confidence interval per 1-SD increase in BMI z-
378   score categorized by sex. * indicates association after multiple regression
379   modelling (p <0.05). ** illustrate significance after adjustment for false
380   discovery rate (FDR, according to Benjamini-Hochberg).

381

382   Figure 4:   Changes in metabolites by 1 SD increase in BMI z-score, categorized by sex
383   and pubertal stage.

384   Legend 4:   Changes in mean and 95% confidence interval per 1-SD increase in BMI z-
385   score categorized by sex and pubertal stage. Pre-pubertal (red) = Tanner stage
386   1. Peri-pubertal (blue) = Tanner stage 2-3. Post-pubertal (yellow) = Tanner
387   stage 4-5). Females in the left panel, males in the right panel. In post-pubertal
388   subgroup (yellow), * indicates association after multiple regression modelling,
389   including adjustment for false discovery rate (FDR, according to Benjamini-
390   Hochberg).

391 **Supplements**
392
393 Supplementary figure 1:
394 Changes in metabolites by 1 SD increase in body mass index, adjusted for age and sex
395
396 Legend supplementary figure 1:
397 Changes in mean and 95% confidence interval per 1-SD increase in BMI.* indicate significant
398 associations after multiple regression modelling (p-value <0.05). Estimates and 95% CI's in
399 **bold** illustrate significance after adjustment for false discovery rate (FDR, according to
400 Benjamini-Hochberg).
401
402 Supplementary figure 2:
403 Changes in metabolites by 1 SD increase in total body fat %, adjusted for age and sex
404
405 Legend supplementary figure 2:
406 Changes in mean and 95% confidence interval per 1-SD increase in total body fat %.
407 *indicate significant associations after multiple regression modelling (p-value <0.05).
408 Estimates and 95% CI's in **bold** illustrate significance after adjustment for false discovery rate
409 (FDR, according to Benjamini-Hochberg).
410
411 Supplementary figure 3:
412 Changes in metabolites by 1 SD increase in truncal fat %, adjusted for age and sex
413
414 Legend supplementary figure 3:
415 Changes in mean and 95% confidence interval per 1-SD increase in truncal fat %.
416 * indicate significant associations after multiple regression modelling (p-value <0.05).
417 Estimates and 95% CI's in **bold** illustrate significance after adjustment for false discovery rate
418 (FDR, according to Benjamini-Hochberg).
419
420 Supplementary figure 4:
421 Changes in metabolites by 1 SD increase in waist circumference, adjusted for age and sex
422
423 Legend supplementary figure 4:
424 Changes in mean and 95% confidence interval per 1-SD increase in waist circumference.
425 * indicate significant associations after multiple regression modelling (p-value <0.05).
426 Estimates and 95% CI's in **bold** illustrate significance after adjustment for false discovery rate
427 (FDR, according to Benjamini-Hochberg).
428
429 Supplementary figure 5:
430 Changes in metabolites by 1 SD increase in waist to height ratio, adjusted for age and sex
431
432 Legend supplementary figure 5:
433 Changes in mean and 95% confidence interval per 1-SD increase in waist to height ratio.
434 * indicate significant associations after multiple regression modelling (p-value <0.05).
435 Estimates and 95% CI's in **bold** illustrate significance after adjustment for false discovery rate
436 (FDR, according to Benjamini-Hochberg).
437

465

466 *Ethical standards*

467 All procedures performed in studies involving human participants were in accordance with the

468 ethical standards of the institutional and/or national research committee and with the 1964

469 Helsinki declaration and its later amendments or comparable ethical standards.

470

471 *Participant's informed consent*

472 Informed consent was obtained from all individual participants included in the study.

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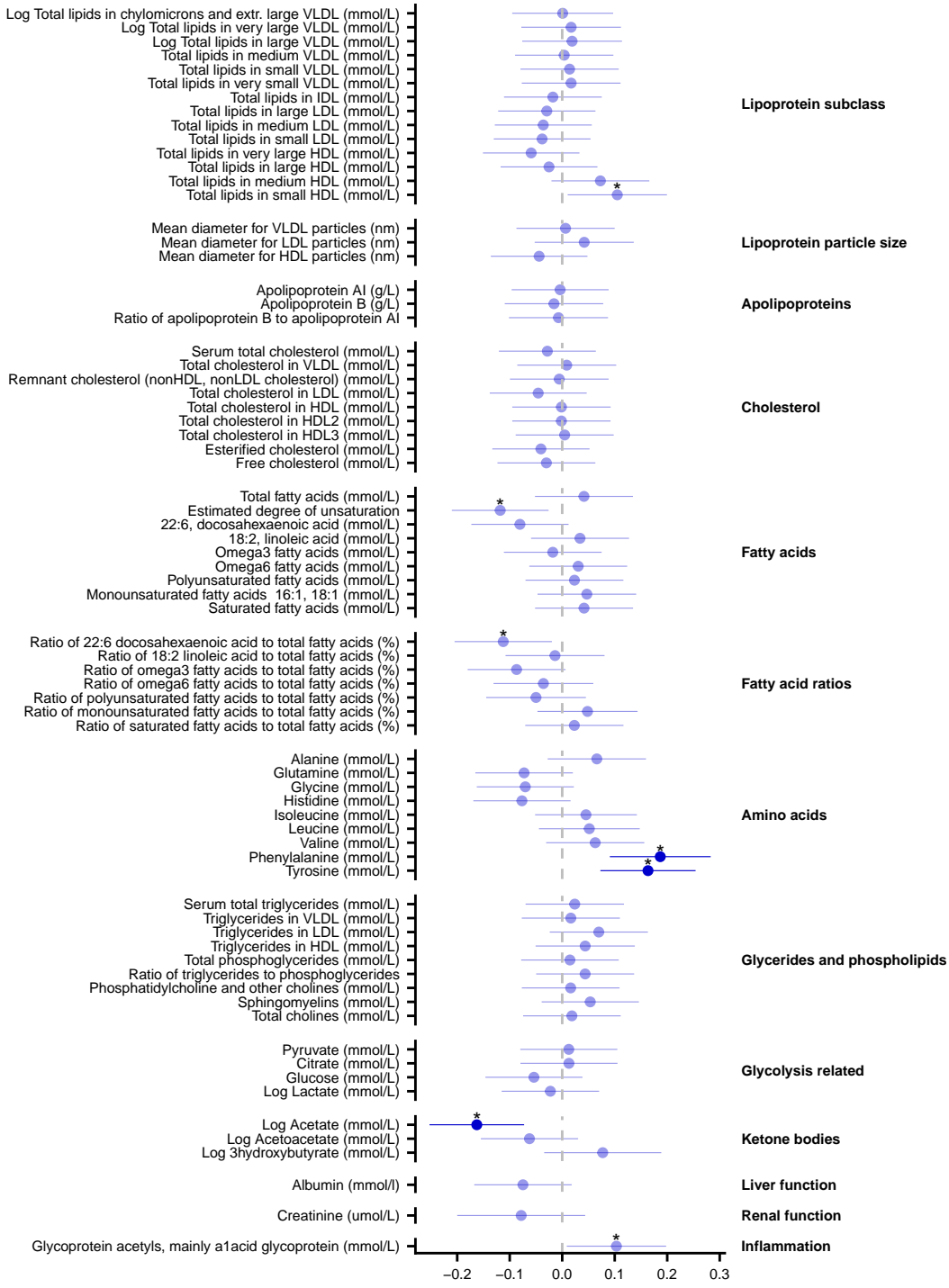
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Metabolic associations with Body Mass Index

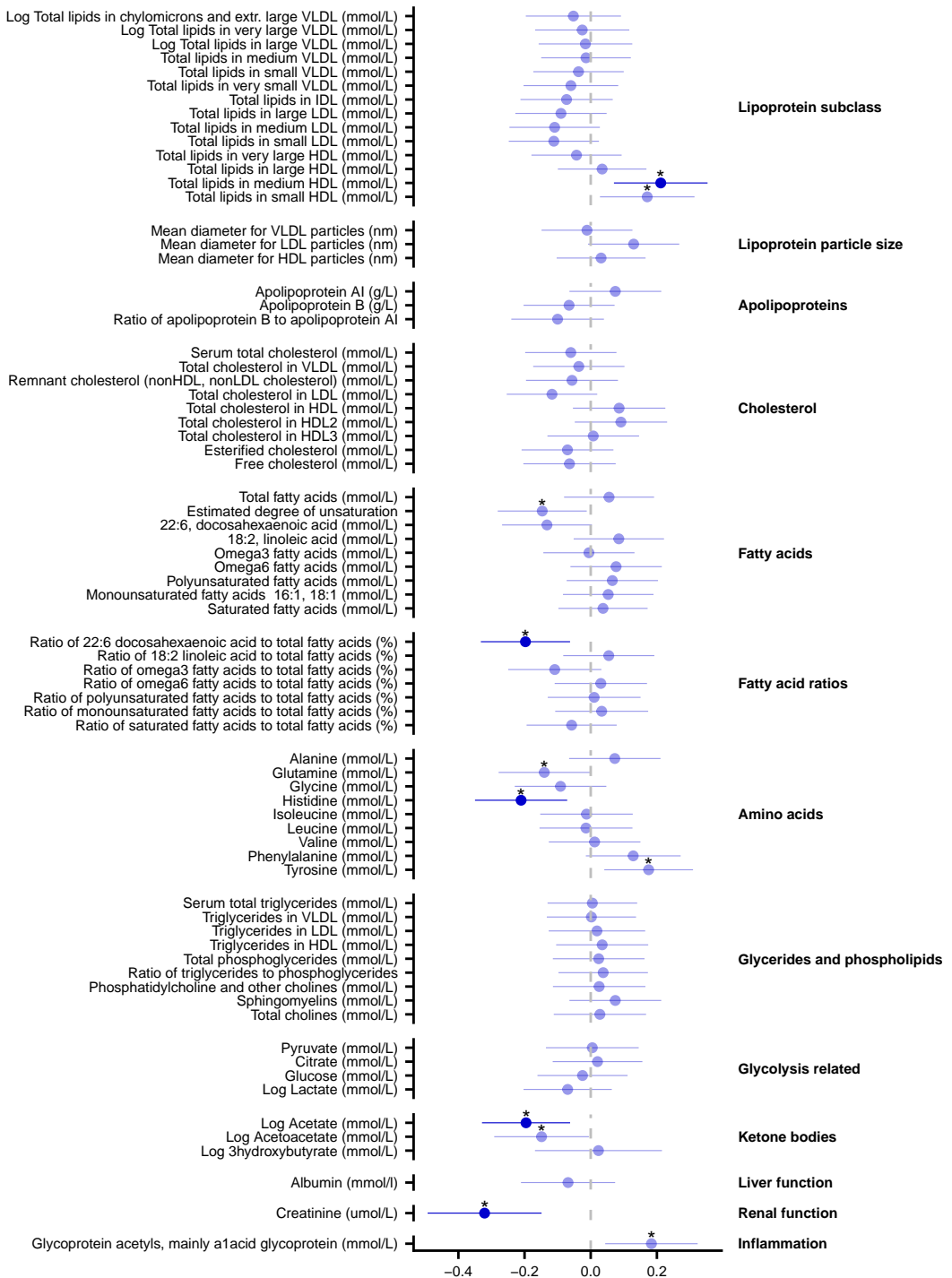SD−difference in metabolite concentration (95% CI) per 1 SD = 7kg/m2 increase in body mass index
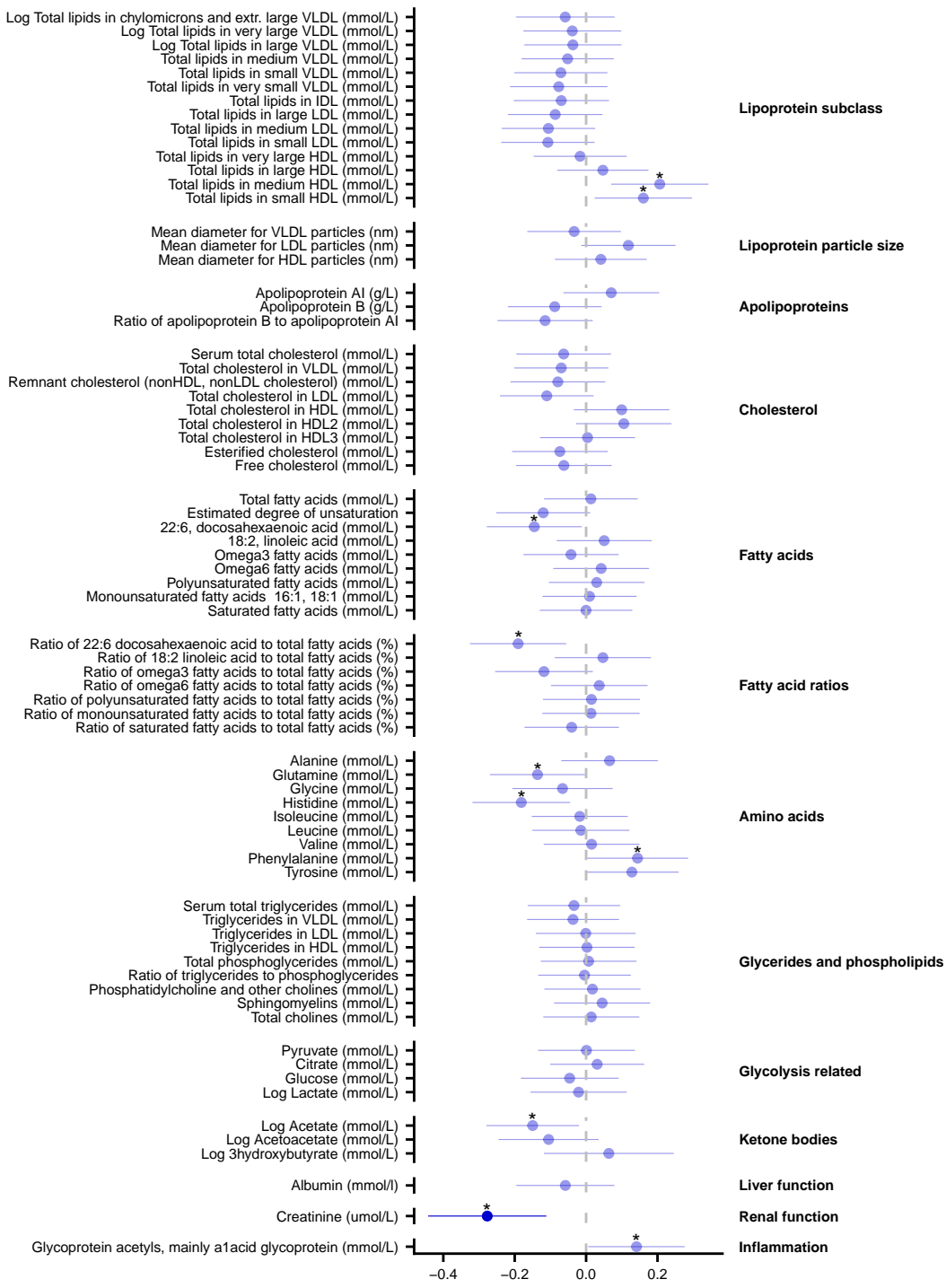
Metabolic associations with Total body fat percentage

SD−difference in metabolite concentration (95% CI) per 1 SD = 8% increase in Total body fat
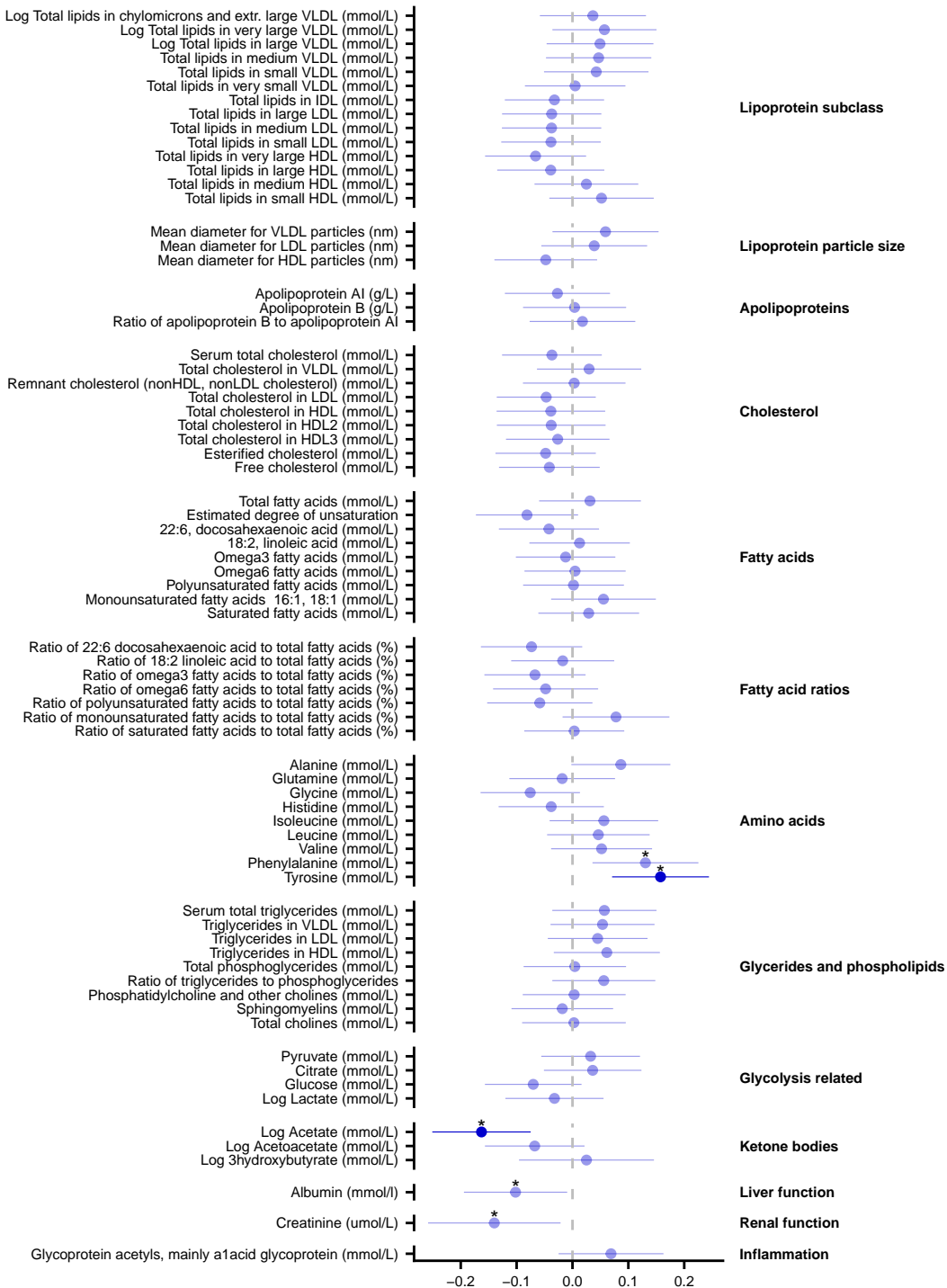
Metabolic associations with Truncal fat percentage

Metabolite (y-axis)

Lipoprotein subclass
Log Total lipids in chylomicrons and extr. large VLDL (mmol/L)
Log Total lipids in very large VLDL (mmol/L)
Log Total lipids in large VLDL (mmol/L)
Total lipids in medium VLDL (mmol/L)
Total lipids in small VLDL (mmol/L)
Total lipids in very small VLDL (mmol/L)
Total lipids in IDL (mmol/L)
Total lipids in large LDL (mmol/L)
Total lipids in medium LDL (mmol/L)
Total lipids in small LDL (mmol/L)
Total lipids in very large HDL (mmol/L)
Total lipids in large HDL (mmol/L)
Total lipids in medium HDL (mmol/L) *
Total lipids in small HDL (mmol/L) *

Lipoprotein particle size
- Mean diameter for VLDL particles (nm)
- Mean diameter for LDL particles (nm)
- Mean diameter for HDL particles (nm)

Apolipoproteins
- Apolipoprotein AI (g/L)
- Apolipoprotein B (g/L)
- Ratio of apolipoprotein B to apolipoprotein AI

Cholesterol
- Serum total cholesterol (mmol/L)
- Total cholesterol in VLDL (mmol/L)
- Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)
- Total cholesterol in LDL (mmol/L)
- Total cholesterol in HDL (mmol/L)
- Total cholesterol in HDL2 (mmol/L)
- Total cholesterol in HDL3 (mmol/L)
- Esterified cholesterol (mmol/L)
- Free cholesterol (mmol/L)

Fatty acids
- Total fatty acids (mmol/L)
- Estimated degree of unsaturation
- 22:6, docosahexaenoic acid (mmol/L) *
- 18:2, linoleic acid (mmol/L)
- Omega3 fatty acids (mmol/L)
- Omega6 fatty acids (mmol/L)
- Polyunsaturated fatty acids (mmol/L)
- Monounsaturated fatty acids 16:1, 18:1 (mmol/L)
- Saturated fatty acids (mmol/L)

Fatty acid ratios
- Ratio of 22:6 docosahexaenoic acid to total fatty acids (%) *
- Ratio of 18:2 linoleic acid to total fatty acids (%)
- Ratio of omega3 fatty acids to total fatty acids (%)
- Ratio of omega6 fatty acids to total fatty acids (%)
- Ratio of polyunsaturated fatty acids to total fatty acids (%)
- Ratio of monounsaturated fatty acids to total fatty acids (%)
- Ratio of saturated fatty acids to total fatty acids (%)

Amino acids
- Alanine (mmol/L)
- Glutamine (mmol/L) *
- Glycine (mmol/L)
- Histidine (mmol/L) *
- Isoleucine (mmol/L)
- Leucine (mmol/L)
- Valine (mmol/L)
- Phenylalanine (mmol/L) *
- Tyrosine (mmol/L)

Glycerides and phospholipids
- Serum total triglycerides (mmol/L)
- Triglycerides in VLDL (mmol/L)
- Triglycerides in LDL (mmol/L)
- Triglycerides in HDL (mmol/L)
- Total phosphoglycerides (mmol/L)
- Ratio of triglycerides to phosphoglycerides
- Phosphatidylcholine and other cholines (mmol/L)
- Sphingomyelins (mmol/L)
- Total cholines (mmol/L)

Glycolysis related
- Pyruvate (mmol/L)
- Citrate (mmol/L)
- Glucose (mmol/L)
- Log Lactate (mmol/L)

Ketone bodies
- Log Acetate (mmol/L) *
- Log Acetoacetate (mmol/L)
- Log 3hydroxybutyrate (mmol/L)

Liver function
- Albumin (mmol/l)

Renal function
- Creatinine (umol/L) *

Inflammation
- Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L) *

x-axis: −0.4, 0.0, 0.2

SD−difference in metabolite concentration (95% CI) per 1 SD = 9% increase in truncal fat
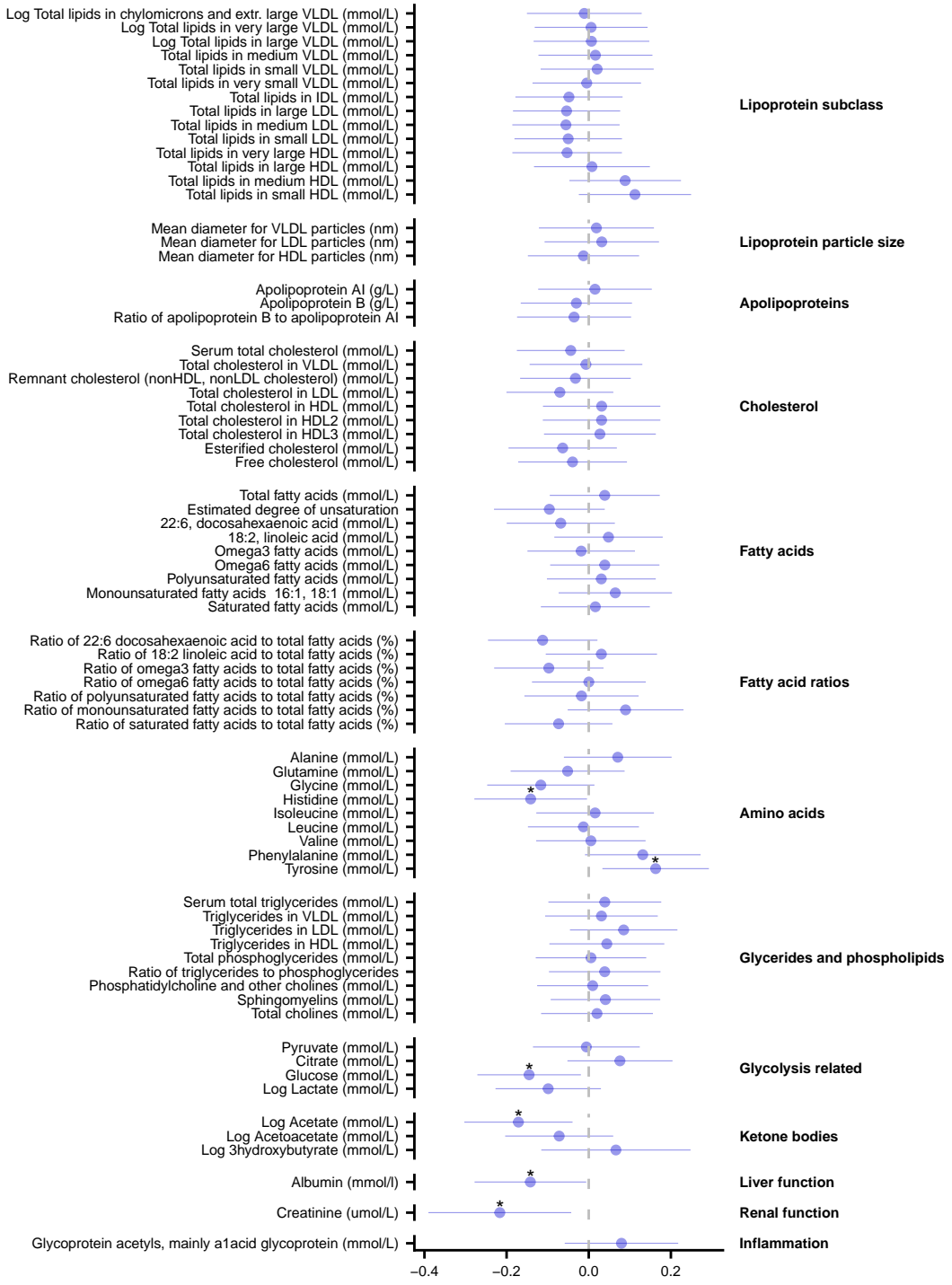
Metabolic associations with Waist circumference

SD−difference in metabolite concentration (95% CI) per 1 SD = 18.6cm increase in waist circumference

Metabolic associations with Waist to height ratio

SD−difference in metabolite concentration (95% CI) per 1 SD = 0.08 increase in waist to height ratio