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Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched chain amino acid; CDC: Centers for Disease Prevention and Control; CVD: Cardiovascular disease; CPS1: Carbamoyl-phosphate synthase 1; DHA: Docosahexaenoic acid; DOB: Date of birth; EDTA: Ethylenediaminetetraacetic acid; GlycA: Glycoprotein acetyls; HbA1c: Haemoglobin A1c; HDL: High-density lipoprotein; HOMA: Homeostatic model assessment; IDL: Intermediate density lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein; LiH: Lithium Heparin; LSAC: Longitudinal Study of Australian Children; MUFA:

Monounsaturated fatty acid; NMR: Nuclear magnetic resonance; PCOS: Polycystic Ovary Syndrome; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acids; SST: serum separating tubes; T2D: Type 2 diabetes; T2DM: Type 2 diabetes mellitus; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

ABSTRACT

Objectives: Nuclear Magnetic Resonance (NMR) metabolomics is high throughput and cost effective, with the potential to improving the understanding of disease and risk. We examine the circulating metabolic profile by quantitative NMR metabolomics of a sample of Australian 11-12 year old children and their parents, describe differences by age and sex, and explore correlation of metabolites in parent-child dyads.

Design: The population-based cross-sectional Child Health CheckPoint study nested within the Longitudinal Study of Australian Children.

Setting: Blood samples collected from CheckPoint participants at assessment centres in six Australian capital cities and eight selected regional centres between February 2015-March 2016.

Participants: 1180 children and 1325 parents provided a blood sample and had metabolomics data available. This included 1133 parent-child dyads (518 mother-daughter, 469 mother-son, 68 father-daughter, and 78 father-son).

Outcome measures: 228 metabolic measures were obtained for each participant. We focused on 70 biomarkers that captured variation in amino acid species, lipoprotein subclass measures, lipid measures, fatty acids, measures related to fatty acid saturation, and composite markers of inflammation and energy homeostasis.

Results: We identified sex-specific metabolic profiles in children and adults and differences in the level of specific metabolites between childhood and adulthood. In general, metabolite concentrations were higher in adults than children and sex differences were larger in adults than in children. Positive correlations within parent-child dyads were observed for the majority of metabolites. Correlations ranged from 0.03 (95% CI -0.05 to 0.12) for acetoacetate in mother-daughter dyads, to 0.39 (95% CI 0.31 to 0.47) for isoleucine in mother-son comparisons.

Conclusions: We report the serum metabolite profiles from mid-childhood and adulthood in a population-based sample, together with parent-child concordance. Distinct age- and sex-specific profiles were observed. These data will be informative for investigation of the

childhood origins of adult non-communicable diseases and for comparative studies in other populations.

Strengths and limitations of this study:

- In a large population-based cohort, venous blood was collected for children and their attending parent on the same day using the same methods
- Rapidly processed, high quality serum samples with standardised metabolomic data generated as a single batch
- Cross-sectional design does not enable longitudinal analysis of specific metabolite species over short term or longer periods of time
- Assessment of paternal influences on offspring metabolite measures is limited by a relatively small sample size compared to mother-child pairs, reducing the precision of estimates
- Factors known to influence metabolomic profile (such as body mass index) were not considered

INTRODUCTION

Metabolomics involves the quantitative analysis of a large number of metabolites and lipids involved in a diverse range of biochemical pathways.[1] Genetic/gene expression and environmental exposures are associated with specific metabolic changes across many tissues and body fluids.[2 3] As such, metabolomics is recognised as a powerful top-down approach to understand genetic and environmental influences on health and disease. Metabolomic profiling also has considerable potential to identify clinically relevant biomarkers for risk stratification and disease monitoring.

Recent advances in nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry have enabled the simultaneous quantitative measurement of hundreds of metabolites. These approaches are sufficiently cost effective and high throughput to be applicable to large cohort studies. For example, NMR metabolomics of serum from the Cardiovascular Risk in Young Finns Study identified many biomarkers from multiple metabolic pathways reflective of fatty liver disease.[4] These were also predictive of risk 10 years prior to diagnosis, indicating that metabolic disruptions precede overt phenotype. Similar population and disease-specific studies have identified metabolomic profiles associated with a range of exposures and health outcomes with potential to reveal clinically important biomarkers and information on disease mechanisms.[5] In addition, specific serum metabolites can also be considered 'intermediate phenotypes' linking genetic risk with disease outcomes.[6 7]

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.[8 9] However, in adults sex appears to be a major driver of variation in metabolite profile, potentially interacting with age. For example, the effects of sex appeared to be greater in younger (age 25-35) than older Japanese adults.[10] A study of 26,000 Northern European adults identified many sex-specific metabolic species at the population level.[9] In men, several lipid measures begin to rise at early middle age whereas a similar increase is only observed in females post menopause. This pattern is consistent for all cholesterol measures – very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) subclass particle concentrations - as well as for triglycerides.[9] Physiological states such as pregnancy also have consistent and measurable influence on serum metabolome.[11] However, it remains unclear how the serum metabolome responds to age, sex and hormonal-specific factors in childhood.

Moreover, factors regulating the metabolic trajectory from early life to adulthood, the role of metabolomic profile in health at the population level and the extent to which blood metabolomic profiles are concordant for parents and children have not been fully explored. One small study has reported correlations between parents (n=179) and their offspring

(n=255) for a range of cardiometabolic risk factors including standard lipid profile measured using conventional methods; this proved stronger for total cholesterol and LDL cholesterol than for high density lipoprotein (HDL) cholesterol or triglycerides.[12] Parent-child correlations of NMR metabolites have not been reported previously.

Here, we present (1) NMR-based metabolomics analysis of a population-based cohort of 11-12 year old children and their parents, (2) identify age and sex-specific metabolomic profiles and (3) report sex-specific parent-child concordance.

METHODS

Study Design: The Child Health CheckPoint comprised a detailed cross-sectional assessment of physical health and biomarkers in a population-based national sample of children (age 11-12 years) and their parents between February 2015 to March 2016. The CheckPoint was nested between waves 6 (2014) and 7 (2016) of the Longitudinal Study of Australian Children.[13] The Longitudinal Study of Australian Children commenced in 2004, when two cohorts (the 'B' and 'K' cohorts, of which the B cohort only was included in the present study) were recruited who have since been followed biennially. Further details regarding the CheckPoint study design and methods are available elsewhere.[14]

Participants: Of the 8,921 families contacted to be part of the LSAC B cohort 5,107 families (57%) agreed to take part in the first wave of data collection in 2004; 4,484 families were retained for Wave 6 in 2014. During the Wave 6 LSAC home visit, B cohort families were introduced to the upcoming Child Health CheckPoint and asked to consent to their contact details being shared with the CheckPoint team. A total of 3,513 families provided permission to receive an information pack by mail and an information and recruitment phone call regarding the CheckPoint study (78% of Wave 6 cohort, 69% of original cohort). Of the families agreeing to receive information about the CheckPoint study, 1874 families took part (53% of eligible participants, 42% of Wave 6 cohort and 37% of original cohort).

Ethics and consent: The CheckPoint data collection protocol was approved by The Royal Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26). The attending parent/caregiver provided written informed consent for themselves and their child to participate in the study, and asked to provide optional consent for the collection and use of biological samples.

Procedure: The specialised CheckPoint assessment centre sequentially visited six Australian capital cities and nine smaller regional centres between February 2015 and March 2016.[14] Each participating child attended the centre with one parent or caregiver (usually the

biological mother) at which both participated in a wide range of measures relevant to non-communicable disease. Those families who could not attend a centre were offered a home visit. Participants were included in the current analyses if metabolomic data from CheckPoint were available (figure 1). Venous blood was not available for home-visit participants, but was collected at all capital city and most regional assessment centres. Participant pairs were excluded from the concordance analyses in this study if the attending parent was not the biological parent.

An experienced phlebotomist collected approximately 28mL of blood from the brachial vein of the non-dominant arm of semi-reclining, semi-fasted participants (at the time of collection, participants reported when they last ate or drank). Blood was collected sequentially into four vacutainer tubes using a butterfly needle so only a single venepuncture was required. Order of collection was (i) 2.7mL EDTA, (ii) 9mL EDTA, (iii) 9mL serum, (iv) 7.5mL Lithium Heparin. The latter two tubes were immediately inverted 6 times to ensure mixing with anticoagulant, and all tubes were transferred to the on-site laboratory. Time of collection was scheduled earlier in the visit for parents than for children.

Collection tube barcodes were linked to the participant and samples were immediately transported to an on-site laboratory where they were processed within two hours into 0.5mL aliquots of plasma, serum, buffy coat (lymphocytes), whole blood and/or an aliquot tube containing a blood clot (1.0mL FluidX screwcap tubes, Cheshire, UK) and stored immediately at -809˚C (Thermo Fisher Scientific, Waltham, USA). Each FluidX tube contained a unique 2D barcode linked to the original collection tube and participant. As each assessment centre closed, samples were shipped on dry ice to the Melbourne Children's Bioresource Centre for long term storage at -80˚C (serum, whole blood, plasma, blood clot) or vapour phase liquid nitrogen (lymphocytes). At a later date, single 0.5ml serum aliquot was removed for every CheckPoint participant and the combined aliquots were shipped in a single batch to Nightingale Health (Helsinki, Finland) on dry ice for NMR metabolomics.

Measures

Metabolomic profiling

The Nightingale® NMR metabolomics platform (Helsinki, Finland) was used to obtain metabolomics for children and parents using the 2016-version quantification algorithm. Details of this platform and methodology have been extensively described elsewhere,[6 15] and epidemiological applications were recently reviewed.[16] Briefly, metabolites were measured from 0.35mL of serum using a single high-throughput experimental setup for the simultaneous quantification of routine lipids, lipoprotein subclass distributions, particle size and composition, fatty acids, and other low–molecular weight metabolites such as amino

acids and glycolysis-related metabolites. This generated data on 228 serum metabolite measures in absolute concentration units (eg millimoles per liter) and ratios (summarised in Table 1). Whilst widely used for epidemiological research, the NMR-based quantification has not been certified for clinical diagnostics. Further analytical validation of the quantification protocols for the biomarker subset routinely used in clinical settings (eg established cholesterol measures and creatinine) is expected to lead to recalibration of certain metabolite concentrations to better match clinical gold standards.[16]

Table 1. Summary of biomarkers and derived variables obtained via high-throughput NMR

Metabolic group	Species and derived measures
Amino acids	Alanine, Glutamine, Glycine, Histidine
	Branched chain: Isoleucine, Leucine, Valine
	Aromatic: Phenylalanine, Tyrosine
Cholesterol	VLDL, LDL, HDL, HDL2, HDL3, Total, Free, Esterified, Remnant
Triglycerides and phospholipids	Triglycerides (VLDL, LDL, HDL, total)
	Phosphoglycerides
	Ratio of triglycerides to phosphoglycerides*
	Phosphatidylcholine
	Sphingomyelins
	Total cholines
Apolipoproteins	Apolipoprotein A-1 (ApoA-1)
	Apolipoprotein B (Apo B)
	Ratio of Apolipoprotein B to Apolipoprotein A-1 (ApoB/Apo A-1)*
Fatty acids (FA)	Total, Omega-3, Omega-6, Polyunsaturated (PUFA), Saturated (SFA)
	Monounsaturated (MUFA), Docosahexaenoic acid (DHA), Linoleic (LA)
	Estimated degree of unsaturation
Fatty acid ratios	Omega-3/total FA*, Omega-6/total FA*, PUFA/total FA* (all %)
	SFA/total FA*, MUFA/total FA*, DHA/total FA*, LA/total FA* (all %)
Lipoprotein subclasses*	12 lipids in each of 14 subclasses: VLDL (XXL, XL, L, M, S, XS), IDL, LDL (L,M,S), HDL (XL, L, M,S)
Lipoprotein size*	Mean diameter of VLDL, LDL and HDL particles
Ketone bodies	Acetate, Acetoacetate, 3-hydroxybutyrate
Glycolysis related	Glucose, Lactate, Pyruvate, Citrate, Glycerol
Fluid balance	Creatinine, Albumin
Inflammation	Glycoprotein acetyls (GlycA)

Information obtained from https://nightingalehealth.com/science/biomarkers

* ratio; ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; DHA: Docosahexaenoic acid; GlycA: Glycoprotein acetyls; HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; L: Large; LDL: Low-density lipoprotein; LA: Linoleic acid; M: Medium; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; S: Small; SFA: Saturated fatty acids; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

Many of the 228 metabolomics measures correlate substantially both in adults (supplementary figure 1) and children (supplementary figure 2). For clarity, we therefore focussed on an informative subset of 70 lipid and metabolites in analyses that capture the majority of variation within the dataset. Most metabolites were reported as absolute

concentrations (ie not including ratios). We excluded glucose and lactate given the known sensitivity of these measures to variable time of fasting and specific processing variables. Some derived ratios, such as fatty acids and apolipoprotein B to apolipoprotein A-1, were included because of previous data indicating clinical utility.[17]

Other measures and sample characteristics

Age and sex: Child date of birth and sex were obtained as detailed elsewhere.[14] The attending parent reported their own date of birth and sex by questionnaire. Age at assessment was calculated to nearest week.

Body mass index: Using a portable stadiometer (Invicta IP0955, Leicester, UK), participant height was measured without shoes, in light clothing, and in duplicate, to the nearest 0.1 cm. A third measurement was taken if the difference of the first two height measurements was greater than 0.5 cm; final height was the mean of all measurements made. Weight, to the nearest 0.1 kg, was measured with an InBody230 bio-electrical impedance analysis scale (Biospace Co. Ltd. Seoul, South Korea). Body mass index (BMI; kg/m^2) was calculated, and for children was converted to age- and sex-adjusted z-score using the 1970 US Centers for Disease Control (CDC) growth reference charts.[18]

Socioeconomic Disadvantage: Neighbourhood socioeconomic position data were obtained from the 2011 Australian Census Socio-Economic Indexes for Areas scores of the postcode region where the participating family lived. This paper used the Socio-Economic Indexes for Areas Index of Relative Socio-economic Disadvantage (Disadvantage Index), a standardised score that summarises the social and economic conditions of Australian neighbourhoods (national mean of 1000 and a standard deviation (SD) of 100, where higher values represent less disadvantage).[19]

Time of blood collection, processing and fasting time: Time of blood collection and start of laboratory processing were recorded. When missing, collection time was estimated using the midpoint between the time the CheckPoint visit began and time that processing of the sample commenced. Processing lag time was calculated as the minutes between blood collection and the processing commencement. Most samples were processed within two hours.

Fasting time was calculated as the hours between last eating/drinking to time of blood collection. The last time of eating/drinking was cross-checked against when the participant was taking part in other CheckPoint stations (and known not to be eating) as well as sleep and wake times from accelerometry data (to identify usual activity, and therefore likely eating patterns) when available. Further details of cleaning processes for the time of last eat/drink can be found elsewhere.[20]

Statistical analysis

Continuous descriptive variables were summarised using weighted means and standard deviations (SD) for children and adults separately, by sex and overall. Summaries of parent and child metabolite measures we focussed on were reported using weighted means and SDs, or weighted geometric means and relative SD for skewed metabolites, for children and adults separately, by sex and overall. Population summary statistics were estimated by applying survey weights and survey procedures that corrected for sampling, participation and non-response biases, and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[21] More detail on the calculation of weights is provided elsewhere.[22]

To compare the distribution of metabolites by age and sex, density plots were examined. Skewed metabolites (skewness greater or equal to 2) were log-transformed. We used two-sided paired t-tests to compare mean metabolite concentrations between children and adults in parent-child dyads, and t-tests to compare mean metabolite concentrations between males and females for children and adults separately. The analyses were repeated using weighted multi-level survey analyses and compared to unweighted analyses. As there appeared to be no major effect of response patterns on results we reported results from unweighted analyses.

Concordance between parents and children was assessed by 1) Pearson's correlation coefficients (CC) with 95% confidence intervals, and 2) partial correlation coefficients (PCC), controlling for age, socioeconomic status, fasting time and processing lag time. In addition, the Pearson's correlation coefficients described above were repeated using weighted multi-level survey analyses and compared to unweighted analyses. As there appeared to be no major effect of response patterns on results we reported results from unweighted analyses. Scatterplots of parent versus child metabolites (log-transformed where needed as above) were examined to check for outliers and to ensure assumptions were met.

Analyses were undertaken using Stata version 14.2 and R version 3.3.2.

RESULTS

Sample characteristics

The recruitment and retention of participants in the Child Health CheckPoint are described elsewhere.[14] Of the 1874 families who participated in CheckPoint assessment centres, blood serum samples of analysable quality from 1180 children and 1325 parents (figure 1) were sent for NMR quantification of metabolites. The majority of excluded families undertook home visits or attended a regional centre, where blood samples could not be collected (n=385, 20.5%), while some participants declined a blood sample (children, n=150, 8.0%; adults, n=108, 5.8%). Few data were lost due to insufficient volume or poor quality samples at the assessment centre (figure 1). The sample characteristics of parents and children are outlined in table 2. Summary statistics for our main child and parent metabolite measures are presented in supplementary table 1.

Table 2: Sample characteristics; values are weighted mean (standard deviation)

Characteristic	All	Male	Female
Child			
n	1152-1180	558-575	594-605
Age, years	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)
BMI, (kg/m2)	19.4 (3.5)	19.2 (3.4)	19.6 (3.7)
BMI z-score	0.38 (1.0)	0.40 (1.0)	0.37 (1.0)
Disadvantage Index	1012 (63)	1011 (65)	1014 (61)
Fasting time (hours)	4.2 (1.2)	4.3 (1.3)	4.2 (1.1)
Time of day - blood collection	14.16 (2.0)	14.12 (2.0)	14.20 (2.1)
Parent			
n	1272 - 1325	174-177	1098-1148
Age, years	43.9 (5.6)	46.9 (6.9)	43.4 (5.2)
BMI, (kg/m2)	28.4 (6.4)	28.9 (4.7)	28.4 (6.6)
Fasting time (hours)	3.3 (1.6)	3.6 (2.0)	3.2 (1.5)
Time of day - blood collection	13.10 (2.0)	13.18 (2.1)	13.09 (2.0)

BMI, body mass index; Disadvantage Index: Index of Relative Socioeconomic Disadvantage; n: number of participants in cohort with this measure.

Differences in metabolite levels in children and adults

Figure 2 shows differences in mean metabolite levels for adults relative to children in standard deviation (SD) units. Most concentrations were higher in adults than children. Values that were similar in adults and children included total lipids in very large HDL lipoprotein subclass particles, acetoacetate, tyrosine and VLDL particle size. Levels in children were higher than those of adults for the glycolysis related measures (pyruvate, citrate and glycerol), the ketone body 3-hydroxybutrate, the amino acid glutamine, many fatty acid ratios and LDL and HDL particle sizes.

Supplementary table 2 lists the corresponding estimates in absolute concentration units, while supplementary figures 3-7 show density plots comparing the distributions of metabolites for boys, girls and adults.

Sex-specific differences in metabolite levels in children and adults

Figure 3 shows differences in mean metabolite levels by sex for children and adults separately in SD units, with estimates in absolute concentration units listed in supplementary table 3 and 4.

In general, sex differences appeared more pronounced in adulthood, resulting in distinct overall patterns for children and adults. Children generally showed smaller differences by sex than adults. Of note, sex differences for apolipoproteins, fatty acids and inflammation measures showed different patterns in children compared to adults.

Girls had lower levels of apolipoprotein-A-1 (ApoA-1) and higher ApoB than boys. In adults, the opposite pattern was observed with mothers having higher ApoA-1 and lower ApoB than fathers. In children, most fatty acid concentrations were similar in girls and boys. In contrast, many adult fatty acid measures were higher in fathers. There was no evidence of a difference in the level of inflammation (GlycA) by sex in children. In adults, GlycA levels were higher in fathers than mothers.

For some metabolites, sex differences in children mirrored (but were smaller than) those of adults, particularly for the ketone bodies acetate and acetoacetate and some key amino acids. At both ages, the amino acid glycine was higher in females but the branched-chain amino acids leucine and valine were higher in males.

Parent-child concordance

Figure 4 shows the correlations between metabolite measures for all children with all parents, and for boys and girls with mothers (but not with the 177 fathers, given the small numbers). The corresponding correlation coefficients and partial correlation coefficients are listed in supplementary tables 5 and 6.

A positive correlation was found for many metabolite measures irrespective of child sex, with Correlation Coefficients (CC) ranging from 0.03 (95% CI -0.05 to 0.12) for acetoacetate in mother-daughter pairs to 0.39 (95% CI 0.31 to 0.47) for isoleucine in mother-son comparisons. Additional adjustment for factors that potentially influence metabolite levels (age, socioeconomic status, fasting time and processing lag time) had little effect on the degree of correlation in any comparison (supplementary tables 5 and 6). Correlations for all parents and all children showed similar patterns to that observed for mother and child by sex.

Confidence intervals (95%) for all mother-son and mother-daughter correlations overlapped. However, some metabolites showed differences by child sex. For example, fatty acids and 3-hydroxybutyrate were more highly correlated in mother-daughter than mother-son pairs. Other metabolites were more highly correlated in mother-son than mother-daughter comparisons, including acetoacetate and the branched-chain amino acids (isoleucine, leucine and valine).

DISCUSSION

Principal findings

Here we present age and sex differences on the detailed/NMR-based metabolic profiles from 1133 Australian parent-child dyads, and demonstrate that many metabolite measures have high parent-child concordance. In accord with previous studies, we identified major differences in metabolite levels between childhood and adulthood and also sex-specific profiles in both childhood and adulthood. We also observed variability in the level of sex-specific differences for several metabolites in childhood compared to adulthood and identified a complex interplay of correlations of specific metabolites between parents and their children according to parent-child sex relationships.

Strengths and weaknesses

This is the first major cohort study to report both sex- and cross-generational differences in metabolomic concentrations in mid-childhood to adulthood utilising the NMR platform. Further strengths include the large number of parent-child dyads representing a wide range of parent ages, the national population-based sample and the state-of-the-art measurements. Replication studies exploring sex differences at earlier and later stages of childhood and adolescence would strengthen findings.

An important limitation is that paternal factors were not fully represented, as most parental samples were from mothers (a well-documented problem in longitudinal cohort studies). This also limited sex-specific parental contribution analysis; further studies including more fathers are warranted. Additional limitations are that, without samples from both parents for each child, we could not estimate heritability, and our results might not apply to mid-life adults who are not parents (although we see no good reason why these would differ greatly). The original uptake of just over 50% and subsequent attrition within LSAC and then the CheckPoint has led to a relatively advantaged sample, but nonetheless participants varied widely on key potential confounders (eg disadvantage, age) and this was at least partly offset by application or consideration of survey weights. Given the large number of metabolites and modest sample size, considerable uncertainty remains in any ranking of the various effects across metabolites.

Meaning and implications for clinicians and policymakers

Overall, we found a clear difference in metabolite profile between children and their parents. This was apparent for specific metabolite measures (such as some amino acids) as well as the

distribution of metabolites (such as lipid composition of lipoproteins of different density). Some measures were higher in adults, some similar, while a minority were lower. Previous studies, largely in adults, have identified a range of specific metabolite changes with age, particularly from mid to late adulthood.[23] This includes a general decrease in several amino acid species, which contrasts with our findings from childhood to mid adulthood.[8] Only the amino acid glutamine showed this pattern in our dataset.

Sex-specific differences in children (±0.2 SD) were generally much smaller than in adults (±0.8 SD). Large metabolomic studies using alternative platforms have previously reported reproducible, sex-specific signatures in circulating metabolite profile in adults.[24 25] This includes differences in amino acid and lipid serum concentrations, potentially influenced by sex-specific effects of genetic polymorphisms on metabolite levels. [25 26] As in our study, most amino acids have usually been reported to be higher in men than women.[25 27] For example, in a recent study of 507 metabolic markers in 1756 individuals (903 female and 853 male aged ~60 years), one third of metabolites showed significant sexual dimorphism. These were predominantly related to pathways of steroid metabolism, fatty acids, other lipids, and a large proportion of amino acids.[27] Of particular note, branched chain amino acids (BCAAs) and their related metabolic products were amongst the most differentially represented, with much higher isoleucine, leucine and valine in males. A similar finding of higher leucine and valine was also noted in the Cooperative Health Research in the Region of Augsburg (KORA) follow-ups 3 (F3) and 4 (F4) analysis of >3000 adults,[25] consistent with our observations in adulthood.

In children, we found sex-specific differences for leucine and valine were smaller but in the same direction as adults. Several lines of evidence implicate BCAA metabolism with metabolic risk in humans. For example, three candidate genes for obesity and/or type 2 diabetes mellitus (T2DM) are involved in the BCAA metabolic pathway.[28] In a recent large meta-analysis of metabolomics in diabetes, a >30% higher risk of type 2 diabetes was found per SD increase in isoleucine, leucine, valine or tyrosine, whereas glycine and glutamine were inversely associated with risk.[28] Several clinical studies have also reported that BCAAs positively correlate with insulin resistance, homeostatic model assessment (HOMA) index and levels of haemoglobin A1c (HbA1c), while longitudinal studies have reported that increased blood BCAAs are predictive of future insulin resistance and type 2 diabetes (T2D).[29] It is intriguing to speculate that the higher BCAA in males from early life could contribute to the well-described increasing prevalence of T2D in men. Levels of BCAA are

elevated in females with Polycystic Ovary Syndrome (PCOS), potentially contributing to the associated insulin resistance.[30] However, it remains unclear whether BCAA are on the causal pathway to T2D or result from adverse metabolic health. Our demonstration that the sex-specific differences in BCAA arise early in life offers potential to track their association with sex-specific measures of metabolic health from an early age to help clarify where they lie on the causal pathway.

In accord with previous adult studies[25], we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of ~10,000 adults with cardiovascular disease (CVD), with replication in >53,000 subjects, identified a genetic variant in carbamoyl-phosphate synthase 1 (*CPS1)* (linked to plasma glycine levels) to be strongly associated with a reduced risk of CVD in women ($p=6.3x10^{-5}$) but not men ($p=0.95$), suggesting a direct link between glycine levels and CVD risk, although whether this is a causal association remains unclear.[31] It will be interesting in the future to explore the link between variants in *CPS1* and circulating glycine levels from early life to adulthood in relation to markers of cardiovascular health in females.

The small sex-differences of HDL cholesterol and ApoA-1 in children compared to adults is consistent with modest differences in children, whereas substantial differences in adulthood have previously been reported.[32] ApoA-1 was more abundant in boys, while ApoB was higher in girls, leading to a higher ApoB/ApoA-1 ratio in girls. The opposite pattern was found in our limited sample of fathers relative to mothers. These data are surprising and differ from a similarly sized study of slightly older European adolescent children (mean age 15 years) that found higher ApoA-1 and ApoB in girls relative to boys.[33] Interestingly, a higher ApoB/ApoA-1 ratio has been strongly linked to increased coronary risk in adults,[34-36] suggesting that the sex-specific differences may alter with increasing age, in keeping with the increased CVD risk in adult males. ApoA-1 is the main protein component of HDL cholesterol[37] thus the differences in trajectories in lipids and HDL cholesterol for boys and girls across childhood that have been reported[38 39] could partially explain this observation.

These are the first data on the mother-child or parent-child correlations of NMR metabolites. Smaller studies have reported positive correlations between parents and children for a limited range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides measured using conventional methods. We found positive correlations between parents and children for the same lipid measures (although measured using NMR) consistent with previously reported findings. One study reported a positive

association between the serum lipid levels of 4 year old children (n=127) and their parents (122 mothers and 118 fathers) [40] while another study of children aged 6-18 (n=255) and their parents (n=179) found that the age of the child influenced the degree of correlation of several lipid measures, with older (10-18 years) children more similar to their parents in terms of triglyceride levels than younger individuals (6-9 years).[12]

Unanswered questions and future research

The temporal and sex specific dynamism of the metabolomics data we describe here offer considerable opportunities for identification of biomarkers of risk for a range of non-communicable diseases early in life, to inform targeted interventions and monitor their efficacy. Combining metabolomics with other 'omics data (such as genetics), as is increasingly reported from large adult studies, offers considerable promise in understanding the causal pathways that link early life exposures, genetics and intermediate phenotypes with later onset chronic disease, and in identifying clinically relevant biomarkers.

In conclusion, we report detailed circulating metabolite profile from mid-childhood and adulthood in a population-based sample, together with parent-child concordance and sex-specific profiles in children and adults. Distinct age- and sex-specific profiles were observed, as well as considerable evidence of parent-child concordance. These data will be informative for investigation of the childhood origins of adult non-communicable diseases and for comparative studies across populations.

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REDCap (Research Electronic Data Capture) tools[41] were used in this study. More information about this software can be found at: www.project-redcap.org.

We thank the LSAC and CheckPoint study participants, staff and students for their contributions.

COMPETING INTERESTS

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CONTRIBUTIONS

DB, RS and JC conceptualised and developed the Metabolomics Checkpoint study. SE and JC undertook all aspects of data analysis. SAC coordinated the acquisition of metabolomics data and provided critical review of this manuscript. MW, the Principal Investigator of the Child Health CheckPoint, planned the analyses and provided critical review of this manuscript. SE and RS drafted the manuscript. PW, MJ, TD, KL, JC, DB provided critical expert advice and critical review of this manuscript.

DATA SHARING STATEMENT

Dataset and technical documents available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au

FIGURE CAPTIONS AND FOOTNOTES

Figure 1: Participant flow chart.

n=number of families, c=number of children, p=number of attending adults,

MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,

LSAC=Longitudinal Study of Australian Children

*Unable to analyse due to insufficient volume or poor quality sample

^Data from 6 non-biological child-parent pairs excluded from concordance analyses

Figure 2: Differences in metabolite levels between children and adults.

Association measures are SD difference in metabolite concentration for adults compared to children. Error bars represent 95% confidence intervals. Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood.

Association measures are SD difference in metabolite concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 4: Parent:child correlation for metabolite measures.

Pearson's correlation coefficients for all children with all parents (A); and for boys (blue) with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence intervals. Correlation coefficients with associated 95% confidence intervals are listed in supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

SUPPLEMENTARY DOCUMENTS

Supplementary figure 1: Correlation of NMR metabolite measures in children.

Heatmap showing the correlation between metabolite measures in children. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 2: Correlation of NMR metabolite measures in parents.

Heatmap showing the correlation between metabolite measures in parents. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each cholesterol and apolipoprotein measure.

Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each fatty acid and fatty acid ratio measure.

Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass particles.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for total lipids within each of the 14 lipoprotein subclass particles.

Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for lipoprotein particle sizes and triglyceride measures.

Supplementary figure 7: Density plots for glycolysis related, amino acid, ketone body and inflammation measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for glycolysis related, amino acid, ketone body and inflammation measures.

Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.

Supplementary table 2: Differences in mean metabolite levels in adults compared to children in absolute concentration units.

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

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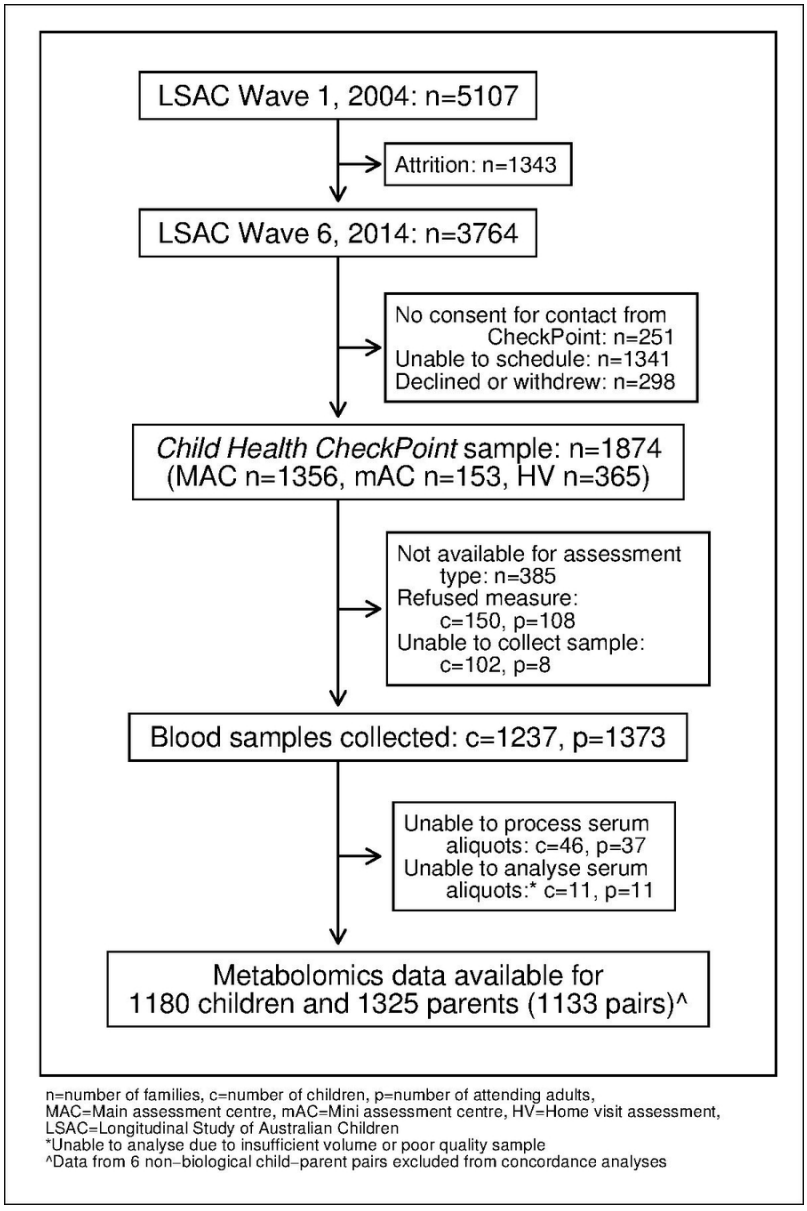

Figure 1: Participant flow chart.
n=number of families, c=number of children, p=number of attending adults,
MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,
LSAC=Longitudinal Study of Australian Children
*Unable to analyse due to insufficient volume or poor quality sample
^Data from 6 non-biological child-parent pairs excluded from concordance analyses

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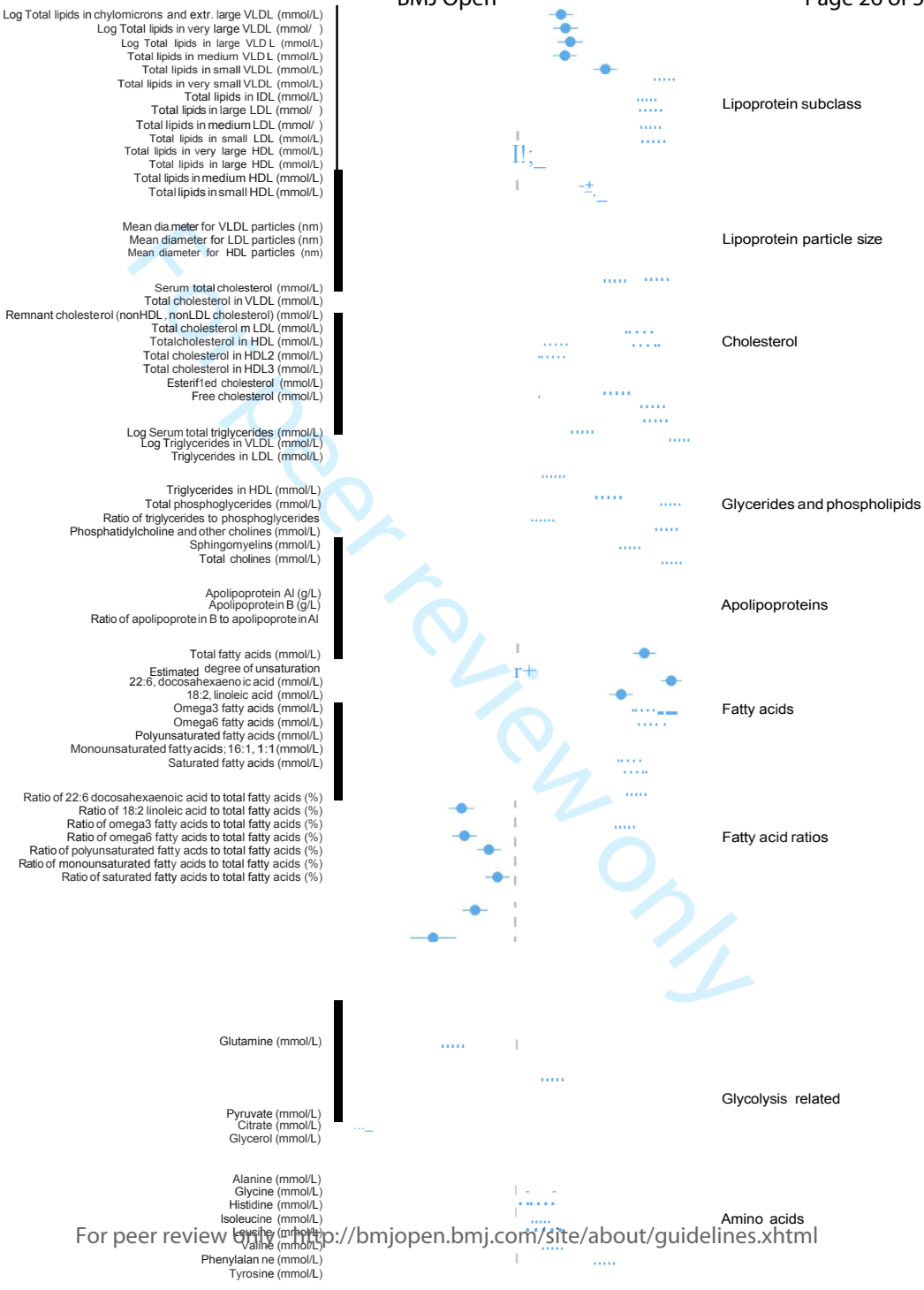
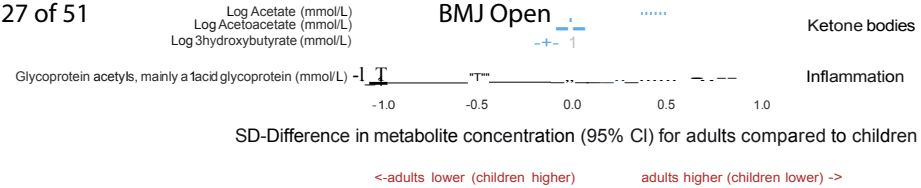
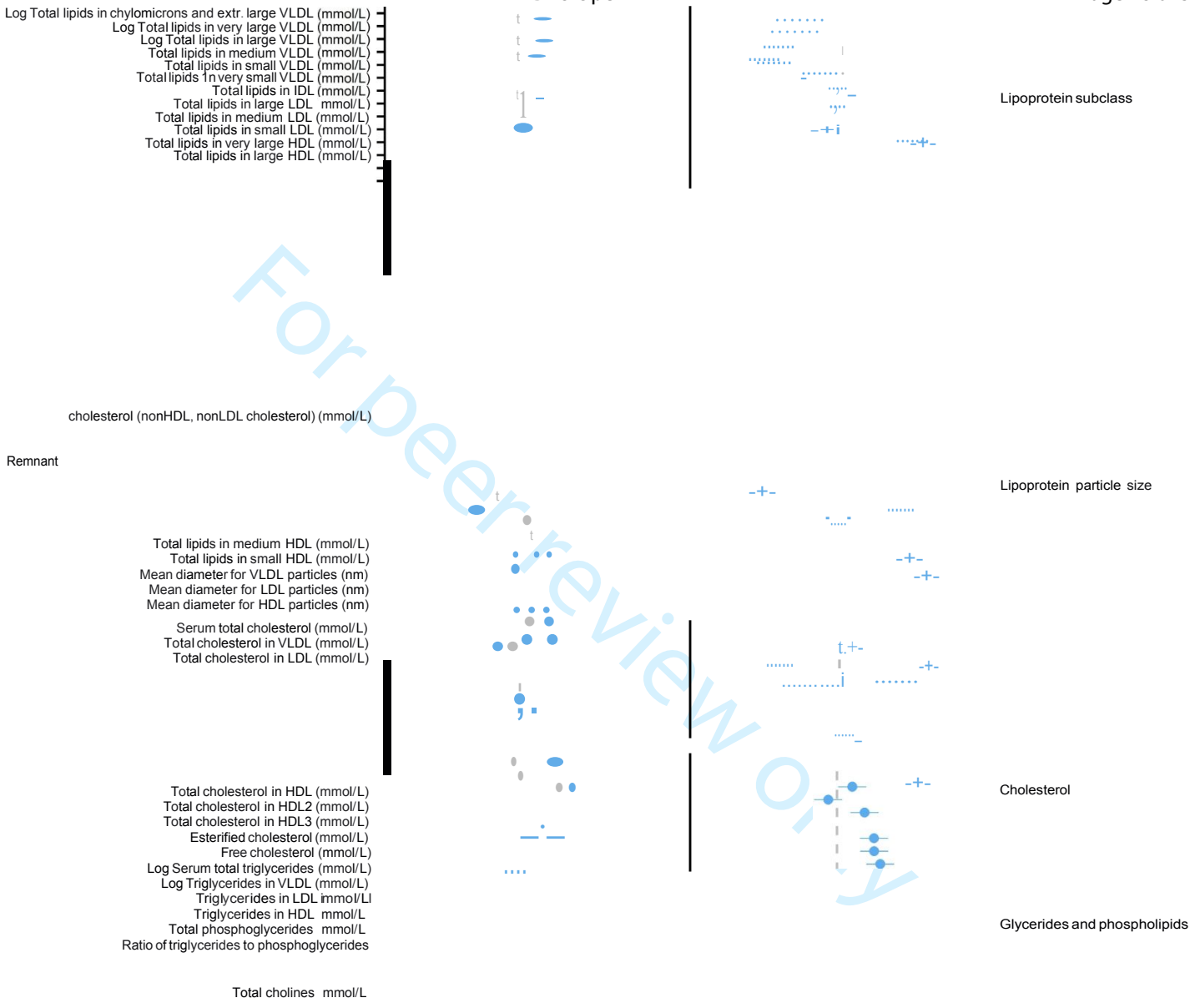
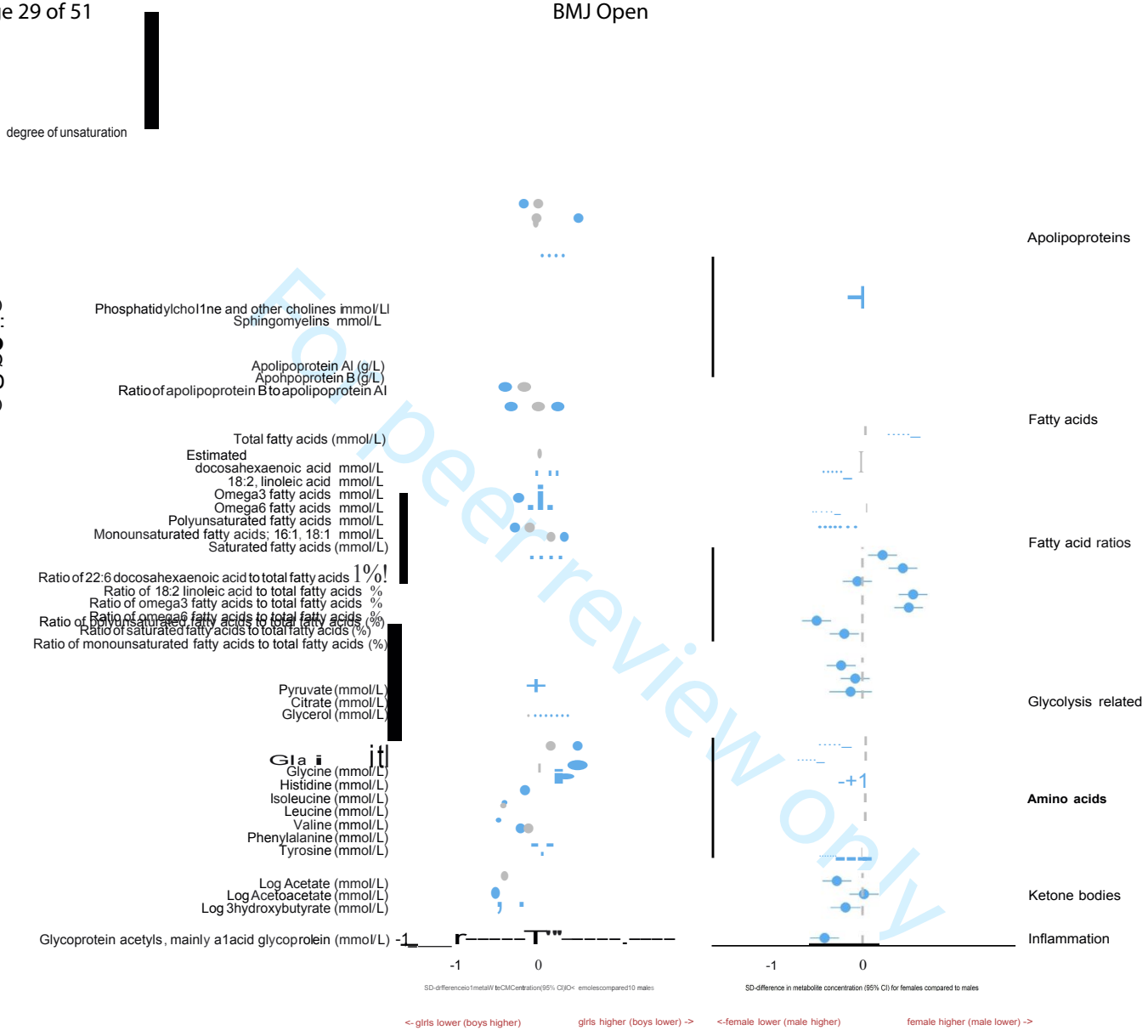
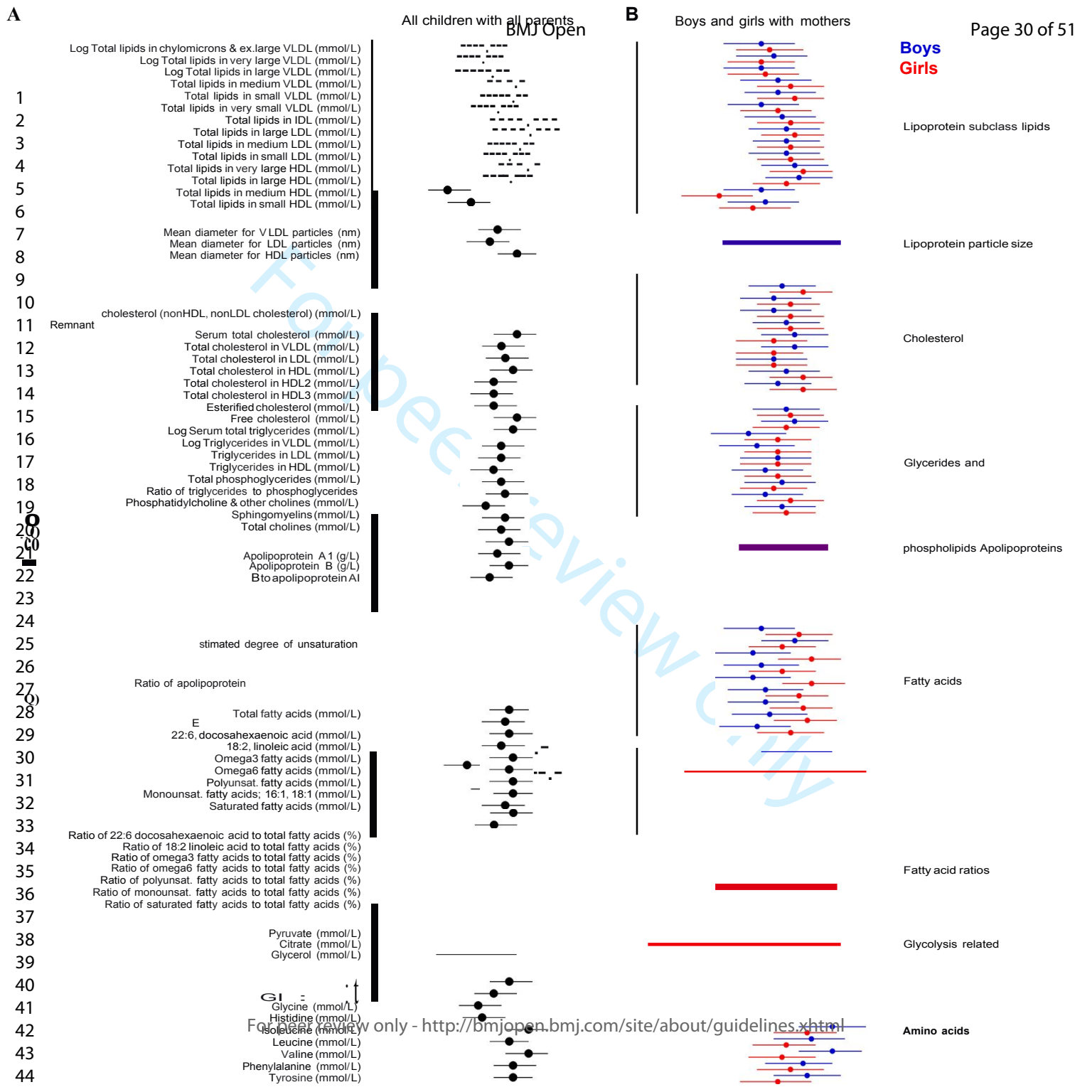
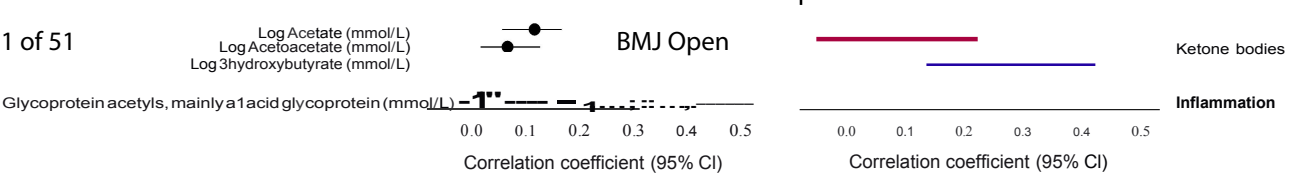




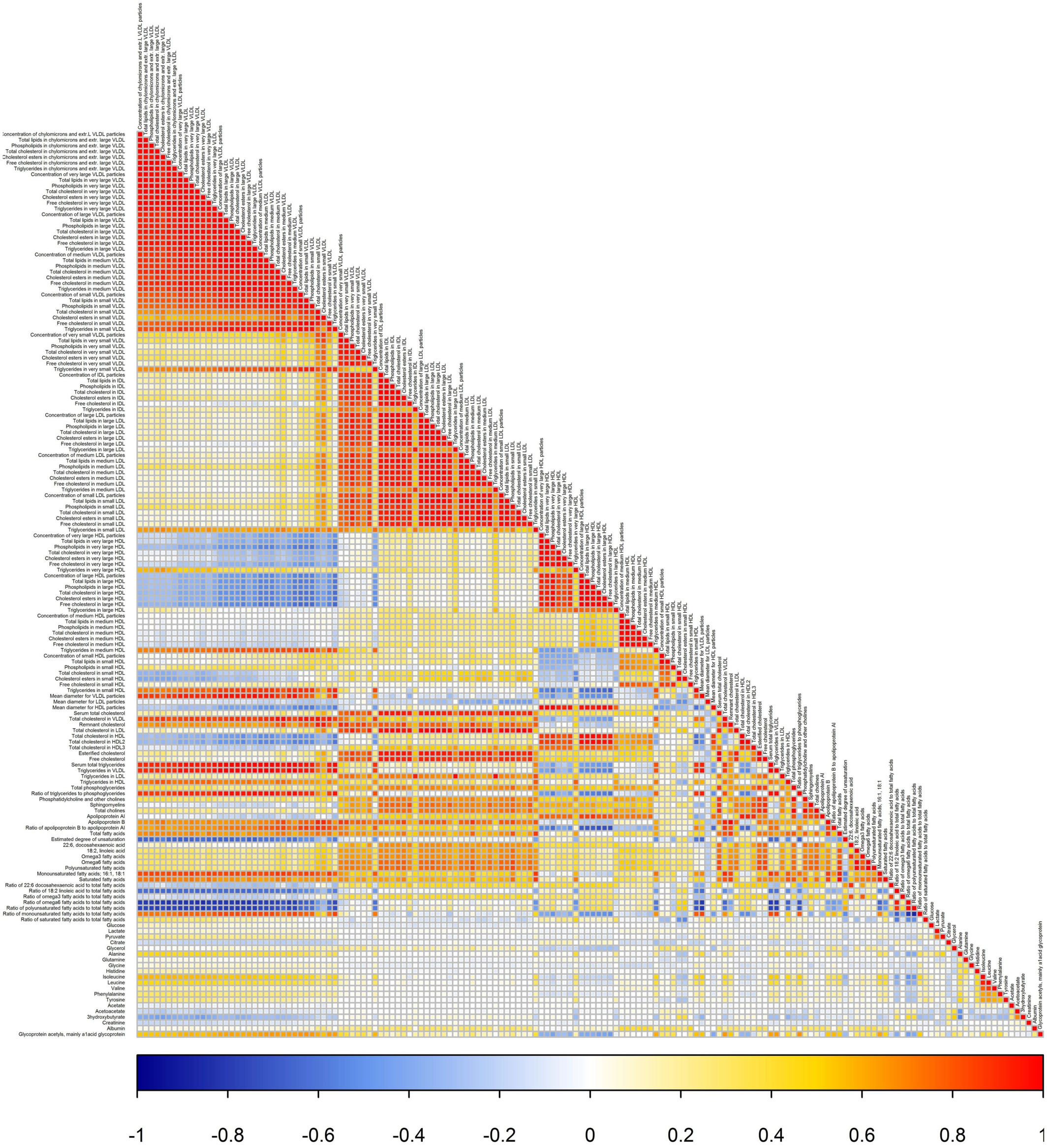






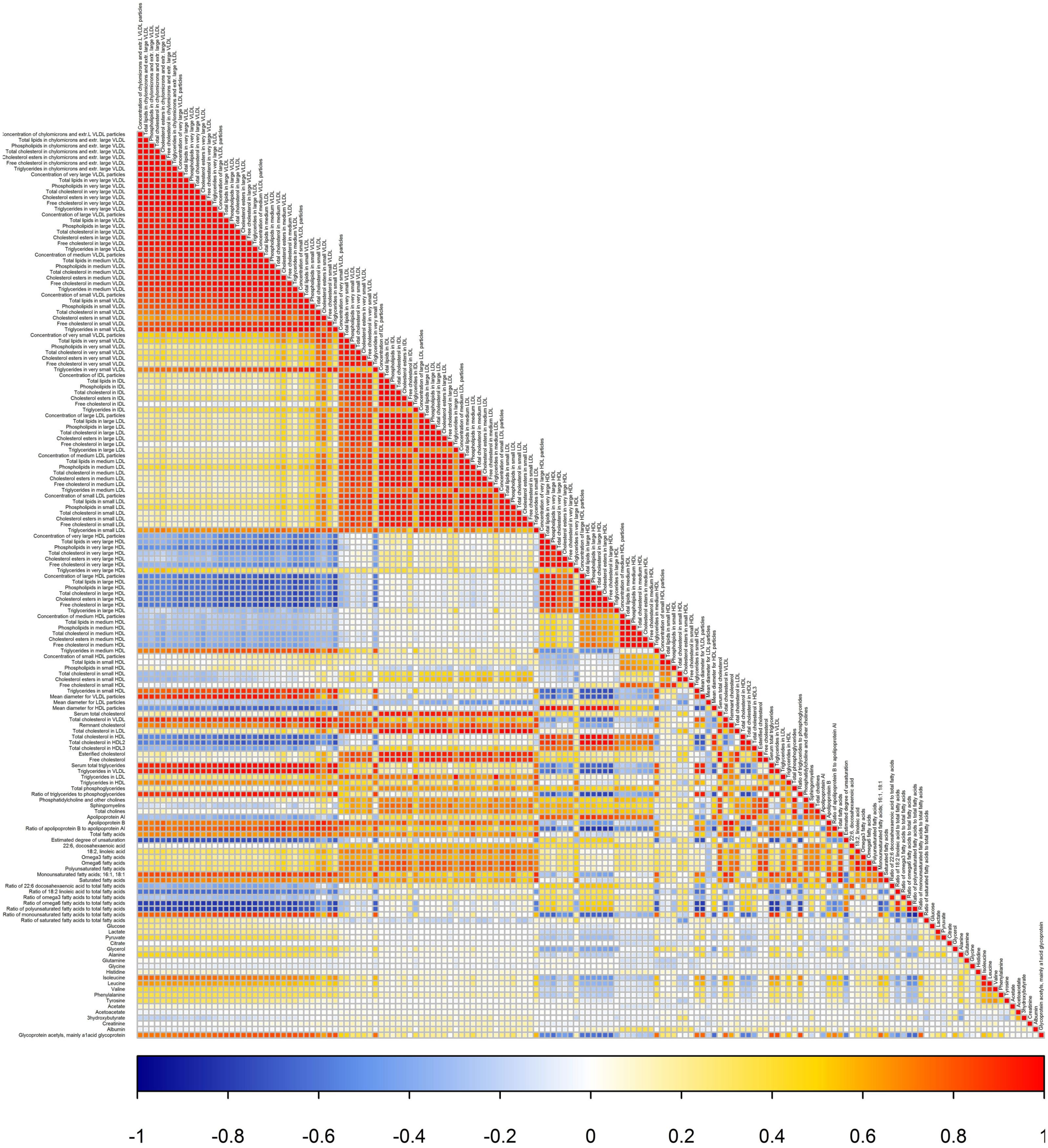


Supplementary Figure 1: Correlations between Child Health CheckPoint metabolites - children

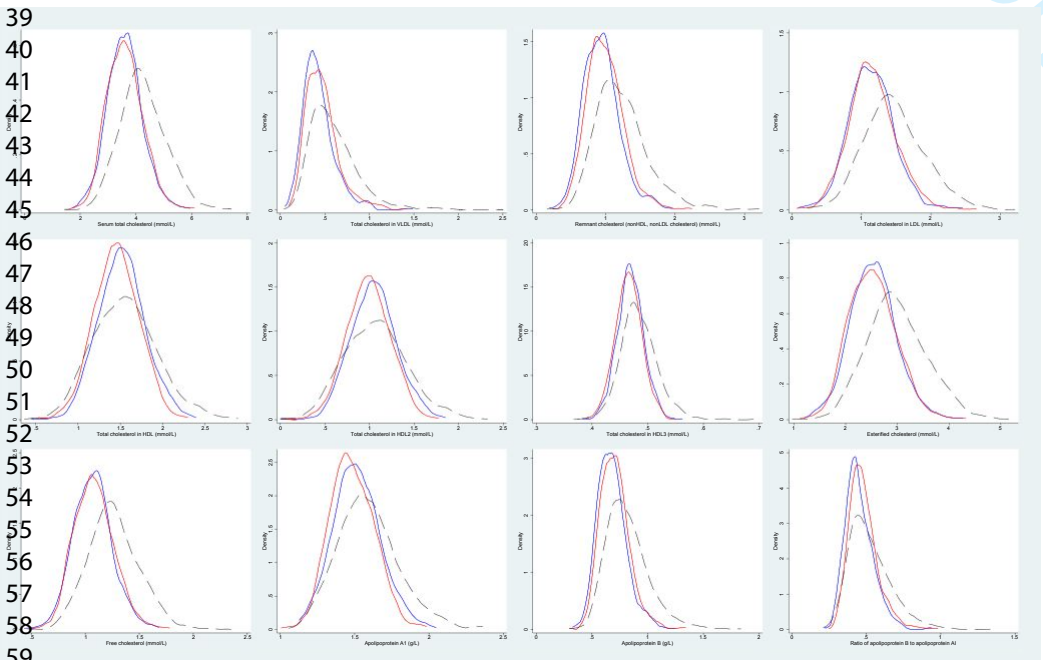


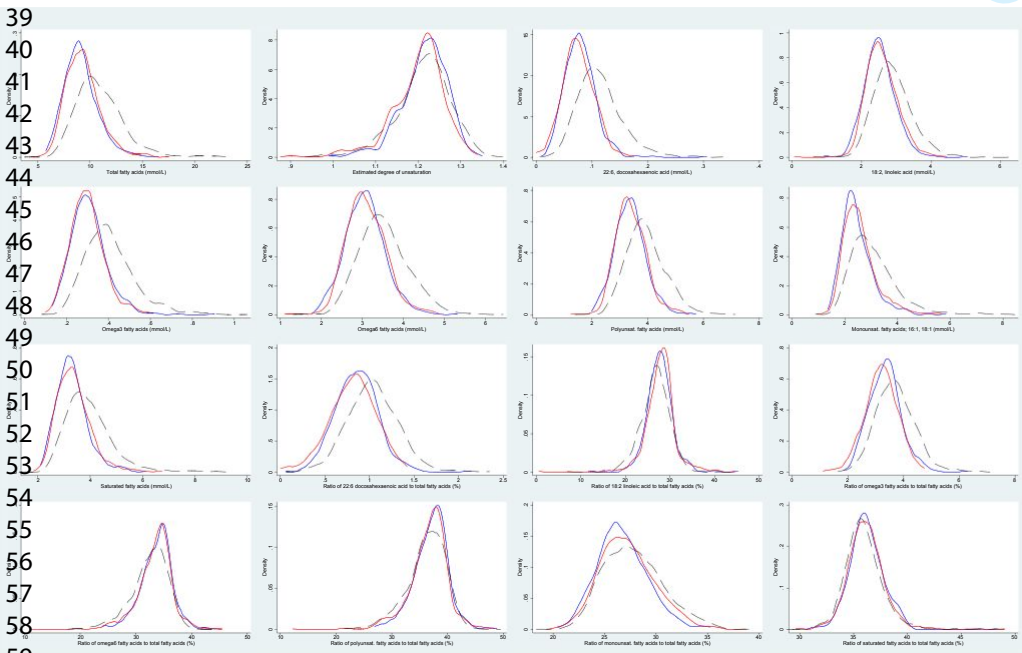
Note: Correlations (spearmans) between metabolites for the CheckPoint child metabolomics data

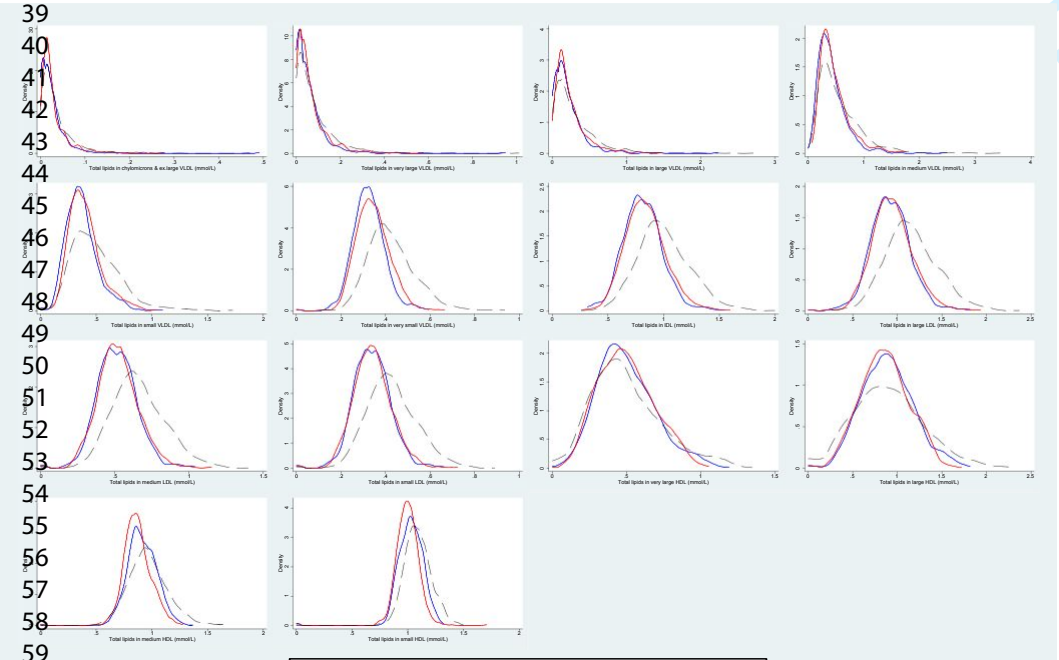
Supplementary Figure 2: Correlations between Child Health CheckPoint metabolites - parents

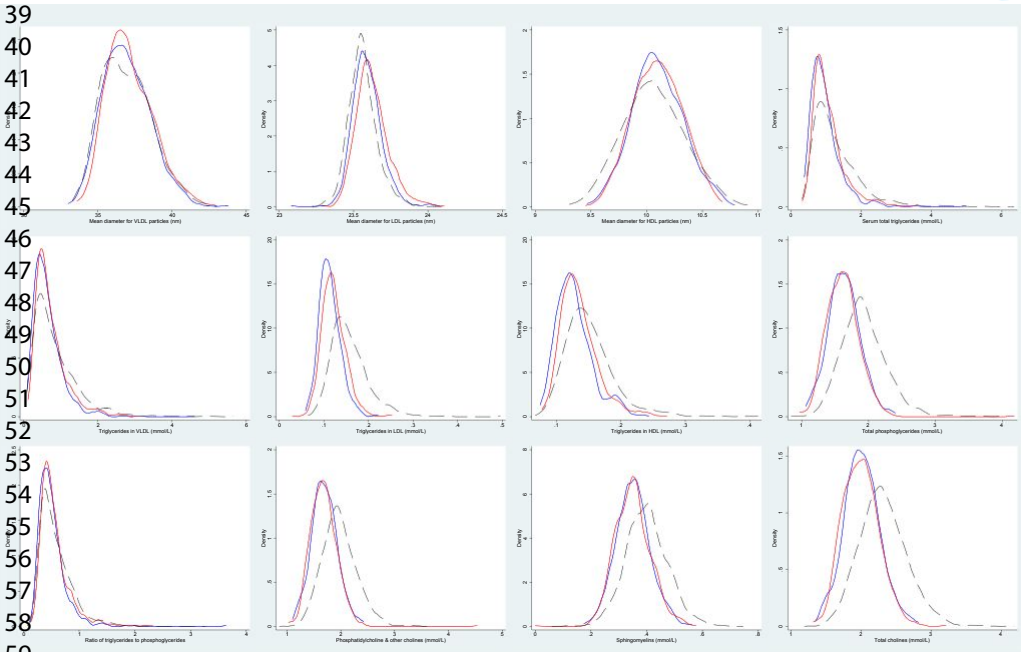


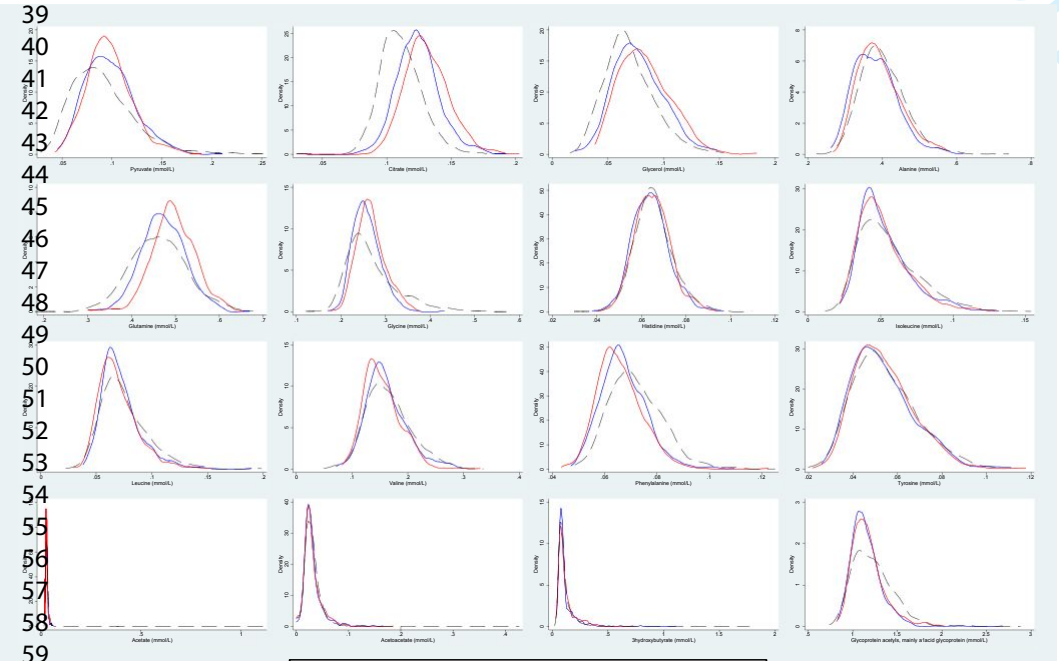
Note: Correlations (spearmans) between metabolites for the CheckPoint parent metabolomics data











Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.*

Metabolic subgroup	Children									Adults								
	Boys			Girls			All			Male			Female			All		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	575	0.005	0.60	605	0.011	0.54	1180	0.007	0.58	177	0.040	0.53	1148	0.013	0.51	1325	0.015	0.52
Total lipids in very large VLDL (mmol/L)*	575	0.006	0.73	605	0.014	0.74	1180	0.010	0.74	177	0.087	0.79	1148	0.022	0.67	1325	0.027	0.70
Total lipids in large VLDL (mmol/L)*	575	0.059	0.99	605	0.121	0.93	1180	0.085	0.99	177	0.423	1.13	1148	0.161	0.73	1325	0.182	0.78
Total lipids in medium VLDL (mmol/L)	575	0.44	0.27	605	0.48	0.27	1180	0.46	0.27	177	0.96	0.63	1148	0.55	0.37	1325	0.60	0.43
Total lipids in small VLDL (mmol/L)	575	0.38	0.15	605	0.40	0.15	1180	0.39	0.15	177	0.69	0.28	1148	0.49	0.22	1325	0.52	0.23
Total lipids in very small VLDL (mmol/L)	575	0.32	0.07	605	0.34	0.08	1180	0.33	0.07	177	0.45	0.11	1148	0.43	0.11	1325	0.43	0.11
Total lipids in IDL (mmol/L)	575	0.80	0.18	605	0.83	0.18	1180	0.82	0.18	177	0.98	0.26	1148	1.00	0.24	1325	1.00	0.24
Total lipids in large LDL (mmol/L)	575	0.92	0.22	605	0.94	0.23	1180	0.93	0.22	177	1.16	0.33	1148	1.15	0.30	1325	1.16	0.30
Total lipids in medium LDL (mmol/L)	575	0.51	0.14	605	0.52	0.14	1180	0.52	0.14	177	0.68	0.21	1148	0.66	0.18	1325	0.66	0.19
Total lipids in small LDL (mmol/L)	575	0.34	0.08	605	0.34	0.09	1180	0.34	0.08	177	0.44	0.14	1148	0.42	0.11	1325	0.43	0.12
Total lipids in very large HDL (mmol/L)	575	0.48	0.20	605	0.49	0.18	1180	0.49	0.19	177	0.32	0.19	1148	0.50	0.23	1325	0.47	0.23
Total lipids in large HDL (mmol/L)	575	0.87	0.29	605	0.86	0.27	1180	0.87	0.28	177	0.51	0.34	1148	0.90	0.38	1325	0.85	0.40
Total lipids in medium HDL (mmol/L)	575	0.92	0.13	605	0.87	0.13	1180	0.89	0.13	177	0.83	0.24	1148	0.97	0.18	1325	0.95	0.19
Total lipids in small HDL (mmol/L)	575	1.04	0.10	605	1.00	0.11	1180	1.02	0.11	177	1.06	0.25	1148	1.08	0.14	1325	1.08	0.16
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	575	37.06	1.63	605	37.24	1.56	1180	37.15	1.59	177	38.53	1.74	1148	36.94	1.60	1325	37.15	1.70
Mean diameter for LDL particles (nm)	575	23.59	0.10	605	23.63	0.11	1180	23.61	0.11	177	23.49	0.09	1148	23.57	0.10	1325	23.56	0.10
Mean diameter for HDL particles (nm)	575	10.08	0.23	605	10.10	0.22	1180	10.09	0.23	177	9.80	0.24	1148	10.07	0.26	1325	10.03	0.28
Cholesterol																		
Serum total cholesterol (mmol/L)	575	3.58	0.62	605	3.60	0.64	1180	3.59	0.63	177	4.16	0.88	1148	4.23	0.83	1325	4.22	0.83
Total cholesterol in VLDL (mmol/L)	575	0.44	0.19	605	0.47	0.19	1180	0.46	0.19	177	0.83	0.40	1148	0.59	0.26	1325	0.62	0.30
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	575	0.94	0.27	605	0.99	0.27	1180	0.97	0.27	177	1.45	0.47	1148	1.22	0.38	1325	1.25	0.40
Total cholesterol in LDL (mmol/L)	575	1.13	0.33	605	1.15	0.34	1180	1.14	0.33	177	1.49	0.50	1148	1.46	0.44	1325	1.46	0.45
Total cholesterol in HDL (mmol/L)	575	1.50	0.27	605	1.45	0.27	1180	1.48	0.27	177	1.22	0.35	1148	1.56	0.36	1325	1.51	0.38
Total cholesterol in HDL2 (mmol/L)	575	1.03	0.25	605	0.99	0.25	1180	1.01	0.25	177	0.75	0.33	1148	1.07	0.34	1325	1.03	0.35
Total cholesterol in HDL3 (mmol/L)	575	0.47	0.02	605	0.47	0.02	1180	0.47	0.02	177	0.47	0.03	1148	0.48	0.03	1325	0.48	0.03
Esterified cholesterol (mmol/L)	572	2.52	0.45	604	2.52	0.46	1176	2.52	0.45	176	2.94	0.64	1147	2.98	0.59	1323	2.97	0.60
Free cholesterol (mmol/L)	572	1.06	0.18	604	1.08	0.19	1176	1.07	0.18	176	1.21	0.27	1147	1.26	0.24	1323	1.25	0.24
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	575	0.918	4.77	605	1.005	74.68	1180	0.962	10.07	177	1.686	0.96	1148	1.129	3.73	1325	1.19	2.75
Triglycerides in VLDL (mmol/L)*	575	0.582	1.07	605	0.648	1.21	1180	0.615	1.13	177	1.249	2.80	1148	0.694	1.75	1325	0.75	2.31
Triglycerides in LDL (mmol/L)	575	0.11	0.02	605	0.12	0.03	1180	0.12	0.03	177	0.15	0.04	1148	0.16	0.04	1325	0.16	0.04
Triglycerides in HDL (mmol/L)	575	0.13	0.03	605	0.14	0.03	1180	0.13	0.03	177	0.16	0.05	1148	0.15	0.04	1325	0.15	0.04
Total phosphoglycerides (mmol/L)	572	1.63	0.24	604	1.62	0.26	1176	1.63	0.25	176	1.86	0.35	1147	1.93	0.34	1323	1.92	0.34
Ratio of triglycerides to phosphoglycerides	572	0.53	0.25	604	0.57	0.28	1176	0.55	0.27	176	0.95	0.59	1147	0.58	0.28	1323	0.63	0.36
Phosphatidylcholine & other cholines (mmol/L)	572	1.69	0.24	604	1.69	0.27	1176	1.69	0.25	175	1.88	0.32	1147	1.98	0.34	1322	1.97	0.33
Sphingomyelins (mmol/L)	572	0.35	0.06	604	0.35	0.06	1176	0.35	0.06	175	0.37	0.07	1147	0.40	0.08	1322	0.39	0.08
Total cholines (mmol/L)	572	2.01	0.26	604	2.00	0.26	1176	2.00	0.26	175	2.19	0.33	1147	2.32	0.35	1322	2.30	0.35
Apolipoproteins																		
Apolipoprotein A1 (g/L)	575	1.51	0.16	605	1.48	0.15	1180	1.50	0.15	177	1.46	0.18	1148	1.59	0.20	1325	1.57	0.21
Apolipoprotein B (g/L)	575	0.68	0.13	604	0.71	0.14	1179	0.69	0.14	177	0.96	0.25	1148	0.81	0.20	1325	0.83	0.21
Ratio of apolipoprotein B to apolipoprotein AI	575	0.46	0.10	604	0.48	0.10	1179	0.47	0.10	177	0.66	0.18	1148	0.52	0.14	1325	0.54	0.15
Fatty acids																		
Total fatty acids (mmol/L)	570	9.21	1.70	604	9.37	1.73	1174	9.29	1.71	173	11.85	2.72	1145	10.92	2.39	1318	11.03	2.45
Estimated degree of unsaturation	570	1.21	0.06	604	1.20	0.06	1174	1.20	0.06	173	1.18	0.07	1145	1.21	0.07	1318	1.21	0.07
22:6, docosahexaenoic acid (mmol/L)	570	0.08	0.03	604	0.07	0.03	1174	0.08	0.03	173	0.12	0.05	1145	0.11	0.04	1318	0.11	0.04

18:2, linoleic acid (mmol/L)	570	2.54	0.46	604	2.59	0.46	1174	2.57	0.46	173	2.92	0.57	1145	2.88	0.58	1318	2.89	0.58
Omega3 fatty acids (mmol/L)	570	0.31	0.09	604	0.30	0.08	1174	0.30	0.08	173	0.45	0.16	1145	0.40	0.12	1318	0.41	0.12
Omega6 fatty acids (mmol/L)	570	3.08	0.49	604	3.09	0.49	1174	3.09	0.49	173	3.55	0.65	1145	3.51	0.63	1318	3.51	0.63
Polyunsat. fatty acids (mmol/L)	570	3.39	0.56	604	3.39	0.56	1174	3.39	0.56	173	4.00	0.77	1145	3.91	0.72	1318	3.92	0.73
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	570	2.50	0.63	604	2.59	0.66	1174	2.54	0.65	173	3.52	1.06	1145	3.08	0.92	1318	3.14	0.94
Saturated fatty acids (mmol/L)	570	3.33	0.64	604	3.39	0.68	1174	3.36	0.66	173	4.33	1.09	1145	3.93	0.93	1318	3.98	0.96
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	570	0.84	0.24	604	0.79	0.27	1174	0.81	0.25	173	0.98	0.31	1145	1.02	0.28	1318	1.02	0.29
Ratio of 18:2 linoleic acid to total fatty acids (%)	570	27.74	3.13	604	27.87	3.27	1174	27.81	3.19	173	25.04	3.57	1145	26.66	3.42	1318	26.45	3.47
Ratio of omega3 fatty acids to total fatty acids (%)	570	3.33	0.58	604	3.15	0.60	1174	3.24	0.59	173	3.76	0.80	1145	3.66	0.70	1318	3.67	0.71
Ratio of omega6 fatty acids to total fatty acids (%)	570	33.65	3.05	604	33.31	3.23	1174	33.48	3.13	173	30.47	3.76	1145	32.50	3.31	1318	32.25	3.42
Ratio of polyunsat. fatty acids to total fatty acids (%)	570	36.98	3.24	604	36.46	3.51	1174	36.71	3.38	173	34.23	3.88	1145	36.16	3.59	1318	35.92	3.67
Ratio of monounsat. fatty acids to total fatty acids (%)	570	26.91	2.57	604	27.38	2.68	1174	27.15	2.63	173	29.37	2.97	1145	27.89	2.84	1318	28.07	2.89
Ratio of saturated fatty acids to total fatty acids (%)	570	36.11	1.68	604	36.17	1.80	1174	36.14	1.73	173	36.40	2.03	1145	35.95	2.03	1318	36.01	2.03
Amino acids																		
Pyruvate (mmol/L)	574	0.10	0.02	605	0.10	0.02	1179	0.10	0.02	177	0.10	0.03	1147	0.09	0.03	1324	0.09	0.03
Citrate (mmol/L)	575	0.12	0.02	604	0.13	0.02	1179	0.13	0.02	177	0.11	0.02	1148	0.11	0.02	1325	0.11	0.02
Glycerol (mmol/L)	240	0.08	0.02	283	0.08	0.02	523	0.08	0.02	84	0.07	0.02	470	0.07	0.02	554	0.07	0.02
Ketone bodies																		
Alanine (mmol/L)	575	0.39	0.06	605	0.40	0.06	1180	0.39	0.06	176	0.42	0.06	1147	0.40	0.06	1323	0.40	0.06
Glutamine (mmol/L)	575	0.47	0.05	605	0.50	0.05	1180	0.49	0.05	177	0.49	0.06	1148	0.46	0.07	1325	0.46	0.07
Glycine (mmol/L)	574	0.26	0.03	604	0.27	0.03	1178	0.27	0.03	176	0.24	0.03	1148	0.27	0.06	1324	0.27	0.06
Histidine (mmol/L)	574	0.06	0.01	605	0.07	0.01	1179	0.07	0.01	176	0.07	0.01	1148	0.07	0.01	1324	0.07	0.01
Isoleucine (mmol/L)	574	0.05	0.02	605	0.05	0.02	1179	0.05	0.02	174	0.07	0.02	1146	0.05	0.02	1320	0.06	0.02
Leucine (mmol/L)	575	0.07	0.02	605	0.07	0.02	1180	0.07	0.02	177	0.10	0.03	1148	0.07	0.02	1325	0.08	0.02
Valine (mmol/L)	575	0.16	0.04	604	0.16	0.04	1179	0.16	0.04	177	0.19	0.04	1147	0.16	0.04	1324	0.17	0.04
Phenylalanine (mmol/L)	575	0.07	0.01	605	0.07	0.01	1180	0.07	0.01	177	0.07	0.01	1148	0.07	0.01	1325	0.07	0.01
Tyrosine (mmol/L)	574	0.05	0.01	605	0.05	0.01	1179	0.05	0.01	176	0.06	0.01	1148	0.05	0.01	1324	0.05	0.01
Glycolysis related																		
Acetate (mmol/L)*	575	0.031	0.05	605	0.03	0.04	1180	0.03	0.05	177	0.037	0.11	1146	0.033	0.09	1323	0.034	0.09
Acetoacetate (mmol/L)*	575	0.025	0.27	605	0.023	0.30	1180	0.024	0.28	177	0.023	0.45	1147	0.024	0.26	1324	0.024	0.29
3hydroxybutyrate (mmol/L)*	555	0.10	0.21	580	0.103	0.23	1135	0.101	0.22	170	0.104	0.16	1098	0.096	0.20	1268	0.097	0.20
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	575	1.17	0.19	605	1.17	0.19	1180	1.17	0.19	177	1.38	0.37	1148	1.24	0.23	1325	1.26	0.26

* geometric mean [relative SD] when skewed variable

Supplementary table 2: Differences in mean metabolite levels in adults compared to children in absolute concentration units.

Metabolic subgroup	Differences by age (Parents - Child) Unadjusted			
	Estimate	95% CI	p-value	Conversion factor (SD) #
Lipoprotein subclass lipids				
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.704	(0.519, 0.890)	<0.001	2.53
Total lipids in very large VLDL (mmol/L)*	0.922	(0.700, 1.145)	<0.001	3.03
Total lipids in large VLDL (mmol/L)*	0.648	(0.502, 0.795)	<0.001	1.95
Total lipids in medium VLDL (mmol/L)	0.105	(0.080, 0.129)	<0.001	0.35
Total lipids in small VLDL (mmol/L)	0.107	(0.094, 0.121)	<0.001	0.20
Total lipids in very small VLDL (mmol/L)	0.093	(0.086, 0.099)	<0.001	0.10
Total lipids in IDL (mmol/L)	0.181	(0.166, 0.196)	<0.001	0.23
Total lipids in large LDL (mmol/L)	0.229	(0.211, 0.247)	<0.001	0.29
Total lipids in medium LDL (mmol/L)	0.144	(0.132, 0.155)	<0.001	0.18
Total lipids in small LDL (mmol/L)	0.089	(0.082, 0.096)	<0.001	0.11
Total lipids in very large HDL (mmol/L)	0.012	(-0.003, 0.027)	0.128	0.22
Total lipids in large HDL (mmol/L)	0.032	(0.007, 0.057)	0.011	0.35
Total lipids in medium HDL (mmol/L)	0.076	(0.064, 0.089)	<0.001	0.17
Total lipids in small HDL (mmol/L)	0.068	(0.058, 0.078)	<0.001	0.14
Lipoprotein particle size				
Mean diameter for VLDL particles (nm)	-0.147	(-0.263, -0.031)	0.013	1.63
Mean diameter for LDL particles (nm)	-0.044	(-0.052, -0.037)	<0.001	0.11
Mean diameter for HDL particles (nm)	-0.027	(-0.045, -0.010)	0.002	0.26
Cholesterol				
Serum total cholesterol (mmol/L)	0.670	(0.619, 0.721)	<0.001	0.80
Total cholesterol in VLDL (mmol/L)	0.146	(0.129, 0.163)	<0.001	0.25
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.261	(0.237, 0.284)	<0.001	0.36
Total cholesterol in LDL (mmol/L)	0.327	(0.300, 0.354)	<0.001	0.42
Total cholesterol in HDL (mmol/L)	0.082	(0.058, 0.106)	<0.001	0.34
Total cholesterol in HDL2 (mmol/L)	0.064	(0.042, 0.086)	<0.001	0.31
Total cholesterol in HDL3 (mmol/L)	0.018	(0.016, 0.020)	<0.001	0.03
Esterified cholesterol (mmol/L)	0.474	(0.437, 0.511)	<0.001	0.58
Free cholesterol (mmol/L)	0.195	(0.180, 0.210)	<0.001	0.23
Glycerides and phospholipids				
Serum total triglycerides (mmol/L)*	0.176	(0.145, 0.206)	<0.001	0.44
Triglycerides in VLDL (mmol/L)*	0.140	(0.096, 0.183)	<0.001	0.62
Triglycerides in LDL (mmol/L)	0.040	(0.038, 0.043)	<0.001	0.04
Triglycerides in HDL (mmol/L)	0.020	(0.017, 0.022)	<0.001	0.04
Total phosphoglycerides (mmol/L)	0.311	(0.290, 0.332)	<0.001	0.34
Ratio of triglycerides to phosphoglycerides	0.049	(0.027, 0.071)	<0.001	0.30
Phosphatidylcholine & other cholines (mmol/L)	0.295	(0.274, 0.316)	<0.001	0.33
Sphingomyelins (mmol/L)	0.052	(0.047, 0.057)	<0.001	0.08
Total cholines (mmol/L)	0.323	(0.302, 0.345)	<0.001	0.35
Apolipoproteins				
Apolipoprotein A1 (g/L)	0.099	(0.086, 0.112)	<0.001	0.19
Apolipoprotein B (g/L)	0.125	(0.113, 0.137)	<0.001	0.18
Ratio of apolipoprotein B to apolipoprotein AI	0.055	(0.046, 0.064)	<0.001	0.13
Fatty acids				
Total fatty acids (mmol/L)	1.738	(1.592, 1.885)	<0.001	2.24
Estimated degree of unsaturation	0.005	(0.000, 0.009)	0.030	0.06
22:6, docosahexaenoic acid (mmol/L)	0.037	(0.035, 0.040)	<0.001	0.04
18:2, linoleic acid (mmol/L)	0.347	(0.310, 0.384)	<0.001	0.54
Omega3 fatty acids (mmol/L)	0.105	(0.098, 0.113)	<0.001	0.12
Omega6 fatty acids (mmol/L)	0.453	(0.414, 0.492)	<0.001	0.60
Polyunsat. fatty acids (mmol/L)	0.558	(0.513, 0.603)	<0.001	0.70
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.568	(0.512, 0.625)	<0.001	0.85
Saturated fatty acids (mmol/L)	0.612	(0.554, 0.669)	<0.001	0.86
Fatty acid ratios				
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.209	(0.190, 0.228)	<0.001	0.29
Ratio of 18:2 linoleic acid to total fatty acids (%)	-1.079	(-1.331, -0.827)	<0.001	3.39
Ratio of omega3 fatty acids to total fatty acids (%)	0.446	(0.403, 0.490)	<0.001	0.69
Ratio of omega6 fatty acids to total fatty acids (%)	-0.992	(-1.227, -0.757)	<0.001	3.30
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.546	(-0.794, -0.298)	<0.001	3.51
Ratio of monounsat. fatty acids to total fatty acids (%)	0.741	(0.547, 0.934)	<0.001	2.80

Ratio of saturated fatty acids to total fatty acids (%)	-0.195	(-0.328, -0.062)	0.004	1.86
Glycolysis related				
Pyruvate (mmol/L)	-0.007	(-0.009, -0.005)	<0.001	0.03
Citrate (mmol/L)	-0.017	(-0.018, -0.016)	<0.001	0.02
Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	<0.001	0.02
Amino acids				
Alanine (mmol/L)	0.013	(0.009, 0.017)	<0.001	0.06
Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	<0.001	0.06
Glycine (mmol/L)	0.007	(0.003, 0.010)	<0.001	0.05
Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	0.01
Isoleucine (mmol/L)	0.003	(0.002, 0.004)	<0.001	0.02
Leucine (mmol/L)	0.004	(0.003, 0.006)	<0.001	0.02
Valine (mmol/L)	0.009	(0.006, 0.011)	<0.001	0.04
Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	<0.001	0.01
Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	0.01
Ketone bodies				
Acetate (mmol/L)*	0.101	(0.084, 0.117)	<0.001	0.23
Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	1.02
3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	0.49
Inflammation				
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.062	(0.047, 0.078)	<0.001	0.22

*metabolite has been log transformed

Associations in Figure 2 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.
Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Metabolic subgroup	Differences for children (Girls - Boys) Unadjusted			
	Estimate	95% CI	p-value	Conversion factor (SD) #
Lipoprotein subclass lipids				
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.737	(0.414, 1.059)	<0.001	2.84
Total lipids in very large VLDL (mmol/L)*	0.744	(0.355, 1.134)	<0.001	3.43
Total lipids in large VLDL (mmol/L)*	0.663	(0.390, 0.936)	<0.001	2.41
Total lipids in medium VLDL (mmol/L)	0.049	(0.018, 0.080)	0.002	0.27
Total lipids in small VLDL (mmol/L)	0.032	(0.015, 0.048)	<0.001	0.15
Total lipids in very small VLDL (mmol/L)	0.018	(0.010, 0.027)	<0.001	0.07
Total lipids in IDL (mmol/L)	0.025	(0.004, 0.046)	0.017	0.18
Total lipids in large LDL (mmol/L)	0.020	(-0.006, 0.046)	0.132	0.23
Total lipids in medium LDL (mmol/L)	0.008	(-0.008, 0.024)	0.338	0.14
Total lipids in small LDL (mmol/L)	0.001	(-0.008, 0.011)	0.788	0.09
Total lipids in very large HDL (mmol/L)	-0.002	(-0.023, 0.020)	0.882	0.19
Total lipids in large HDL (mmol/L)	-0.033	(-0.066, -0.001)	0.044	0.28
Total lipids in medium HDL (mmol/L)	-0.045	(-0.059, -0.030)	<0.001	0.13
Total lipids in small HDL (mmol/L)	-0.035	(-0.048, -0.022)	<0.001	0.12
Lipoprotein particle size				
Mean diameter for VLDL particles (nm)	0.215	(0.035, 0.395)	0.019	1.58
Mean diameter for LDL particles (nm)	0.035	(0.023, 0.047)	<0.001	0.11
Mean diameter for HDL particles (nm)	0.003	(-0.023, 0.028)	0.847	0.23
Cholesterol				
Serum total cholesterol (mmol/L)	0.007	(-0.066, 0.079)	0.857	0.63
Total cholesterol in VLDL (mmol/L)	0.040	(0.019, 0.061)	<0.001	0.18
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.053	(0.023, 0.083)	0.001	0.27
Total cholesterol in LDL (mmol/L)	0.016	(-0.023, 0.054)	0.427	0.34
Total cholesterol in HDL (mmol/L)	-0.062	(-0.093, -0.031)	<0.001	0.27
Total cholesterol in HDL2 (mmol/L)	-0.059	(-0.087, -0.030)	<0.001	0.25
Total cholesterol in HDL3 (mmol/L)	-0.003	(-0.006, -0.001)	0.013	0.02
Esterified cholesterol (mmol/L)	-0.008	(-0.060, 0.044)	0.755	0.45
Free cholesterol (mmol/L)	0.013	(-0.008, 0.034)	0.211	0.18
Glycerides and phospholipids				
Serum total triglycerides (mmol/L)*	0.101	(0.056, 0.145)	<0.001	0.39
Triglycerides in VLDL (mmol/L)*	0.125	(0.062, 0.187)	<0.001	0.55
Triglycerides in LDL (mmol/L)	0.009	(0.006, 0.012)	<0.001	0.03
Triglycerides in HDL (mmol/L)	0.007	(0.004, 0.011)	<0.001	0.03
Total phosphoglycerides (mmol/L)	-0.018	(-0.047, 0.010)	0.206	0.25
Ratio of triglycerides to phosphoglycerides	0.051	(0.020, 0.083)	0.001	0.27
Phosphatidylcholine & other cholines (mmol/L)	-0.011	(-0.040, 0.018)	0.447	0.25
Sphingomyelins (mmol/L)	-0.001	(-0.009, 0.006)	0.706	0.06
Total cholines (mmol/L)	-0.017	(-0.046, 0.013)	0.268	0.26
Apolipoproteins				
Apolipoprotein A1 (g/L)	-0.030	(-0.048, -0.013)	0.001	0.16
Apolipoprotein B (g/L)	0.027	(0.012, 0.042)	0.001	0.13
Ratio of apolipoprotein B to apolipoprotein AI	0.027	(0.016, 0.038)	<0.001	0.10
Fatty acids				
Total fatty acids (mmol/L)	0.200	(0.011, 0.389)	0.038	1.65
Estimated degree of unsaturation	-0.016	(-0.022, -0.009)	<0.001	0.06
22:6, docosahexaenoic acid (mmol/L)	-0.004	(-0.007, -0.000)	0.033	0.03
18:2, linoleic acid (mmol/L)	0.068	(0.015, 0.120)	0.011	0.46
Omega3 fatty acids (mmol/L)	-0.008	(-0.018, 0.001)	0.083	0.08
Omega6 fatty acids (mmol/L)	0.031	(-0.024, 0.087)	0.271	0.48
Polyunsat. fatty acids (mmol/L)	0.023	(-0.041, 0.086)	0.483	0.55
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.105	(0.034, 0.176)	0.004	0.62
Saturated fatty acids (mmol/L)	0.073	(-0.000, 0.146)	0.052	0.64
Fatty acid ratios				
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	-0.049	(-0.078, -0.019)	0.001	0.25
Ratio of 18:2 linoleic acid to total fatty acids (%)	0.197	(-0.173, 0.567)	0.297	3.23
Ratio of omega3 fatty acids to total fatty acids (%)	-0.145	(-0.213, -0.078)	<0.001	0.59
Ratio of omega6 fatty acids to total fatty acids (%)	-0.302	(-0.658, 0.054)	0.096	3.11
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.447	(-0.830, -0.065)	0.022	3.35
Ratio of monounsat. fatty acids to total fatty acids (%)	0.499	(0.202, 0.796)	0.001	2.61
Ratio of saturated fatty acids to total fatty acids (%)	-0.051	(-0.251, 0.148)	0.614	1.74
Glycolysis related				
Pyruvate (mmol/L)	-0.001	(-0.004, 0.002)	0.524	0.02
Citrate (mmol/L)	0.007	(0.005, 0.009)	<0.001	0.02
Glycerol (mmol/L)	0.006	(0.002, 0.010)	0.004	0.02
Amino acids				
Alanine (mmol/L)	0.011	(0.004, 0.017)	0.002	0.06
Glutamine (mmol/L)	0.023	(0.018, 0.029)	<0.001	0.05
Glycine (mmol/L)	0.010	(0.006, 0.014)	<0.001	0.03
Histidine (mmol/L)	0.001	(-0.000, 0.002)	0.075	0.01
Isoleucine (mmol/L)	-0.000	(-0.003, 0.002)	0.637	0.02
Leucine (mmol/L)	-0.002	(-0.005, -0.000)	0.022	0.02
Valine (mmol/L)	-0.007	(-0.011, -0.003)	0.001	0.04
Phenylalanine (mmol/L)	-0.002	(-0.003, -0.001)	0.003	0.01
Tyrosine (mmol/L)	0.000	(-0.001, 0.002)	0.582	0.01
Ketone bodies				
Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.17
Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.99

3hydroxybutyrate (mmol/L)*	0.041	(-0.019, 0.101)	0.178	0.51
Inflammation				
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.017	(-0.004, 0.038)	0.104	0.18

*metabolite has been log transformed

Associations for children in Figure 3 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure in children can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.

Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Metabolic subgroup	Differences for adults (Female - Male)			
	Unadjusted			
	Estimate	95% CI	p-value	Conversion factor (SD) #
Lipoprotein subclass lipids				
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	-0.930	(-1.271, -0.589)	<0.001	2.17
Total lipids in very large VLDL (mmol/L)*	-1.217	(-1.617, -0.818)	<0.001	2.55
Total lipids in large VLDL (mmol/L)*	-0.900	(-1.107, -0.693)	<0.001	1.34
Total lipids in medium VLDL (mmol/L)	-0.325	(-0.385, -0.264)	<0.001	0.40
Total lipids in small VLDL (mmol/L)	-0.167	(-0.201, -0.133)	<0.001	0.22
Total lipids in very small VLDL (mmol/L)	-0.022	(-0.039, -0.005)	0.013	0.11
Total lipids in IDL (mmol/L)	0.014	(-0.023, 0.051)	0.465	0.24
Total lipids in large LDL (mmol/L)	-0.011	(-0.058, 0.035)	0.634	0.29
Total lipids in medium LDL (mmol/L)	-0.028	(-0.057, 0.001)	0.058	0.18
Total lipids in small LDL (mmol/L)	-0.017	(-0.035, 0.001)	0.061	0.11
Total lipids in very large HDL (mmol/L)	0.195	(0.158, 0.231)	<0.001	0.24
Total lipids in large HDL (mmol/L)	0.395	(0.334, 0.455)	<0.001	0.41
Total lipids in medium HDL (mmol/L)	0.129	(0.101, 0.158)	<0.001	0.18
Total lipids in small HDL (mmol/L)	0.002	(-0.021, 0.025)	0.850	0.14
Lipoprotein particle size				
Mean diameter for VLDL particles (nm)	-1.414	(-1.669, -1.159)	<0.001	1.68
Mean diameter for LDL particles (nm)	0.081	(0.066, 0.096)	<0.001	0.10
Mean diameter for HDL particles (nm)	0.278	(0.236, 0.320)	<0.001	0.28
Cholesterol				
Serum total cholesterol (mmol/L)	0.112	(-0.018, 0.241)	0.091	0.82
Total cholesterol in VLDL (mmol/L)	-0.187	(-0.230, -0.145)	<0.001	0.28
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	-0.184	(-0.244, -0.124)	<0.001	0.38
Total cholesterol in LDL (mmol/L)	-0.048	(-0.117, 0.021)	0.175	0.44
Total cholesterol in HDL (mmol/L)	0.344	(0.286, 0.401)	<0.001	0.38
Total cholesterol in HDL2 (mmol/L)	0.324	(0.271, 0.378)	<0.001	0.35
Total cholesterol in HDL3 (mmol/L)	0.019	(0.014, 0.025)	<0.001	0.03
Esterified cholesterol (mmol/L)	0.070	(-0.023, 0.163)	0.139	0.59
Free cholesterol (mmol/L)	0.046	(0.009, 0.084)	0.016	0.24
Glycerides and phospholipids				
Serum total triglycerides (mmol/L)*	-0.344	(-0.416, -0.273)	<0.001	0.47
Triglycerides in VLDL (mmol/L)*	-0.530	(-0.630, -0.429)	<0.001	0.66
Triglycerides in LDL (mmol/L)	0.008	(0.001, 0.014)	0.033	0.04
Triglycerides in HDL (mmol/L)	-0.004	(-0.010, 0.002)	0.228	0.04
Total phosphoglycerides (mmol/L)	0.106	(0.052, 0.159)	<0.001	0.34
Ratio of triglycerides to phosphoglycerides	-0.289	(-0.337, -0.241)	<0.001	0.32
Phosphatidylcholine & other cholines (mmol/L)	0.138	(0.086, 0.190)	<0.001	0.33
Sphingomyelins (mmol/L)	0.032	(0.020, 0.045)	<0.001	0.08
Total cholines (mmol/L)	0.170	(0.115, 0.224)	<0.001	0.35
Apolipoproteins				
Apolipoprotein A1 (g/L)	0.146	(0.114, 0.178)	<0.001	0.21
Apolipoprotein B (g/L)	-0.115	(-0.146, -0.084)	<0.001	0.20
Ratio of apolipoprotein B to apolipoprotein AI	-0.126	(-0.148, -0.105)	<0.001	0.14
Fatty acids				
Total fatty acids (mmol/L)	-0.711	(-1.091, -0.330)	<0.001	2.39
Estimated degree of unsaturation	0.031	(0.021, 0.042)	<0.001	0.07
22:6, docosahexaenoic acid (mmol/L)	-0.002	(-0.008, 0.005)	0.622	0.04
18:2, linoleic acid (mmol/L)	0.004	(-0.087, 0.094)	0.934	0.57
Omega3 fatty acids (mmol/L)	-0.035	(-0.054, -0.016)	<0.001	0.12
Omega6 fatty acids (mmol/L)	0.004	(-0.094, 0.102)	0.936	0.61
Polyunsat. fatty acids (mmol/L)	-0.031	(-0.144, 0.082)	0.592	0.71
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	-0.372	(-0.520, -0.225)	<0.001	0.93
Saturated fatty acids (mmol/L)	-0.307	(-0.455, -0.159)	<0.001	0.93
Fatty acid ratios				
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.064	(0.018, 0.110)	0.006	0.29
Ratio of 18:2 linoleic acid to total fatty acids (%)	1.527	(0.984, 2.070)	<0.001	3.43
Ratio of omega3 fatty acids to total fatty acids (%)	-0.038	(-0.152, 0.075)	0.508	0.71
Ratio of omega6 fatty acids to total fatty acids (%)	1.882	(1.351, 2.412)	<0.001	3.38
Ratio of polyunsat. fatty acids to total fatty acids (%)	1.843	(1.272, 2.414)	<0.001	3.62
Ratio of monounsat. fatty acids to total fatty acids (%)	-1.456	(-1.914, -0.998)	<0.001	2.90
Ratio of saturated fatty acids to total fatty acids (%)	-0.387	(-0.700, -0.074)	0.015	1.96

Glycolysis related				
Pyruvate (mmol/L)	-0.007	(-0.013, -0.002)	0.004	0.03
Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.02
Glycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.02
Amino acids				
Alanine (mmol/L)	-0.020	(-0.030, -0.011)	<0.001	0.06
Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	<0.001	0.07
Glycine (mmol/L)	0.029	(0.020, 0.038)	<0.001	0.06
Histidine (mmol/L)	-0.001	(-0.003, 0.000)	0.116	0.01
Isoleucine (mmol/L)	-0.016	(-0.019, -0.013)	<0.001	0.02
Leucine (mmol/L)	-0.019	(-0.022, -0.016)	<0.001	0.02
Valine (mmol/L)	-0.029	(-0.035, -0.022)	<0.001	0.04
Phenylalanine (mmol/L)	-0.000	(-0.002, 0.001)	0.576	0.01
Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	<0.001	0.01
Ketone bodies				
Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.27
Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	1.05
3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.47
Inflammation				
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	0.24

*metabolite has been log transformed

Associations for parents in Figure 3 are presented in SD-units.

The conversion factor provided (unweighted standard deviation of each metabolite measure in adults/parents) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor. Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Metabolic subgroup	Mother																	
	Boys						Girls						All Children					
	n	CC	95% CI	n	PCC#	95% CI	n	CC	95% CI	n	PCC#	95% CI	n	CC	95% CI	n	PCC#	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	468	0.22	0.13 - 0.31	518	0.24	0.16 - 0.32	517	0.23	0.14 - 0.31	987	0.23	0.17 - 0.29	985	0.23	0.17 - 0.29
Total lipids in very large VLDL (mmol/L)*	469	0.25	0.16 - 0.33	468	0.25	0.16 - 0.33	518	0.22	0.14 - 0.30	517	0.21	0.13 - 0.29	987	0.24	0.18 - 0.29	985	0.23	0.17 - 0.29
Total lipids in large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	468	0.22	0.13 - 0.30	518	0.23	0.14 - 0.31	517	0.21	0.13 - 0.29	987	0.22	0.16 - 0.28	985	0.21	0.15 - 0.27
Total lipids in medium VLDL (mmol/L)	469	0.26	0.17 - 0.34	468	0.25	0.17 - 0.34	518	0.29	0.21 - 0.37	517	0.28	0.20 - 0.36	987	0.28	0.22 - 0.34	985	0.27	0.22 - 0.33
Total lipids in small VLDL (mmol/L)	469	0.26	0.18 - 0.35	468	0.26	0.17 - 0.34	518	0.30	0.21 - 0.37	517	0.29	0.21 - 0.36	987	0.29	0.23 - 0.34	985	0.28	0.22 - 0.33
Total lipids in very small VLDL (mmol/L)	469	0.22	0.14 - 0.31	468	0.22	0.13 - 0.30	518	0.26	0.17 - 0.34	517	0.26	0.18 - 0.34	987	0.25	0.19 - 0.30	985	0.24	0.18 - 0.30
Total lipids in IDL (mmol/L)	469	0.27	0.18 - 0.35	468	0.26	0.17 - 0.34	518	0.29	0.21 - 0.37	517	0.30	0.22 - 0.37	987	0.29	0.23 - 0.34	985	0.28	0.22 - 0.34
Total lipids in large LDL (mmol/L)	469	0.28	0.19 - 0.36	468	0.27	0.18 - 0.35	518	0.30	0.22 - 0.37	517	0.30	0.22 - 0.38	987	0.29	0.23 - 0.35	985	0.29	0.23 - 0.34
Total lipids in medium LDL (mmol/L)	469	0.28	0.20 - 0.36	468	0.27	0.19 - 0.35	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	987	0.29	0.23 - 0.35	985	0.29	0.23 - 0.34
Total lipids in small LDL (mmol/L)	469	0.28	0.19 - 0.36	468	0.27	0.18 - 0.35	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	987	0.28	0.23 - 0.34	985	0.28	0.22 - 0.34
Total lipids in very large HDL (mmol/L)	469	0.30	0.22 - 0.38	468	0.29	0.21 - 0.38	518	0.32	0.24 - 0.39	517	0.32	0.24 - 0.39	987	0.31	0.25 - 0.36	985	0.30	0.25 - 0.36
Total lipids in large HDL (mmol/L)	469	0.31	0.23 - 0.39	468	0.31	0.22 - 0.39	518	0.28	0.20 - 0.36	517	0.28	0.20 - 0.36	987	0.30	0.24 - 0.35	985	0.29	0.23 - 0.35
Total lipids in medium HDL (mmol/L)	469	0.22	0.13 - 0.30	468	0.21	0.12 - 0.30	518	0.12	0.03 - 0.20	517	0.13	0.04 - 0.21	987	0.17	0.11 - 0.23	985	0.17	0.11 - 0.23
Total lipids in small HDL (mmol/L)	469	0.23	0.14 - 0.31	468	0.22	0.13 - 0.31	518	0.20	0.12 - 0.29	517	0.20	0.12 - 0.29	987	0.21	0.15 - 0.27	985	0.21	0.15 - 0.27
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	469	0.30	0.22 - 0.38	468	0.30	0.22 - 0.38	518	0.27	0.19 - 0.35	517	0.26	0.18 - 0.34	987	0.29	0.23 - 0.35	985	0.28	0.22 - 0.34
Mean diameter for LDL particles (nm)	469	0.22	0.13 - 0.31	468	0.22	0.13 - 0.31	518	0.30	0.22 - 0.38	517	0.31	0.23 - 0.38	987	0.26	0.20 - 0.31	985	0.26	0.20 - 0.32
Mean diameter for HDL particles (nm)	469	0.32	0.23 - 0.40	468	0.31	0.23 - 0.39	518	0.33	0.26 - 0.41	517	0.33	0.25 - 0.41	987	0.33	0.27 - 0.38	985	0.32	0.26 - 0.38
Cholesterol																		
Serum total cholesterol (mmol/L)	469	0.27	0.19 - 0.35	468	0.27	0.18 - 0.35	518	0.32	0.24 - 0.39	517	0.32	0.24 - 0.40	987	0.30	0.24 - 0.35	985	0.29	0.24 - 0.35
Total cholesterol in VLDL (mmol/L)	469	0.25	0.17 - 0.34	468	0.25	0.16 - 0.33	518	0.29	0.21 - 0.36	517	0.28	0.20 - 0.36	987	0.28	0.22 - 0.33	985	0.27	0.21 - 0.33
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	469	0.25	0.17 - 0.34	468	0.25	0.16 - 0.33	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	987	0.28	0.22 - 0.34	985	0.28	0.22 - 0.33
Total cholesterol in LDL (mmol/L)	469	0.28	0.20 - 0.36	468	0.27	0.19 - 0.35	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	987	0.29	0.23 - 0.34	985	0.29	0.23 - 0.34
Total cholesterol in HDL (mmol/L)	469	0.30	0.22 - 0.38	468	0.30	0.21 - 0.38	518	0.25	0.16 - 0.33	517	0.24	0.16 - 0.32	987	0.28	0.22 - 0.33	985	0.27	0.21 - 0.32
Total cholesterol in HDL2 (mmol/L)	469	0.30	0.22 - 0.38	468	0.30	0.21 - 0.38	518	0.25	0.16 - 0.32	517	0.24	0.16 - 0.32	987	0.28	0.22 - 0.33	985	0.27	0.21 - 0.33
Total cholesterol in HDL3 (mmol/L)	469	0.25	0.16 - 0.33	468	0.24	0.16 - 0.33	518	0.25	0.16 - 0.33	517	0.24	0.16 - 0.32	987	0.25	0.19 - 0.31	985	0.24	0.18 - 0.30
Esterified cholesterol (mmol/L)	465	0.28	0.19 - 0.36	464	0.27	0.19 - 0.35	518	0.32	0.24 - 0.39	517	0.32	0.24 - 0.39	983	0.30	0.24 - 0.35	981	0.30	0.24 - 0.35
Free cholesterol (mmol/L)	465	0.26	0.18 - 0.34	464	0.26	0.17 - 0.34	518	0.32	0.24 - 0.40	517	0.33	0.25 - 0.40	983	0.30	0.24 - 0.35	981	0.29	0.24 - 0.35
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	469	0.28	0.20 - 0.36	468	0.28	0.19 - 0.36	518	0.29	0.21 - 0.37	517	0.28	0.20 - 0.36	987	0.29	0.23 - 0.35	985	0.28	0.23 - 0.34
Triglycerides in VLDL (mmol/L)*	469	0.30	0.22 - 0.38	468	0.29	0.21 - 0.38	518	0.28	0.20 - 0.36	517	0.27	0.19 - 0.35	987	0.29	0.23 - 0.35	985	0.28	0.22 - 0.34
Triglycerides in LDL (mmol/L)	469	0.19	0.10 - 0.28	468	0.19	0.10 - 0.28	518	0.26	0.18 - 0.34	517	0.26	0.18 - 0.34	987	0.23	0.17 - 0.29	985	0.23	0.17 - 0.29
Triglycerides in HDL (mmol/L)	469	0.21	0.12 - 0.30	468	0.21	0.13 - 0.30	518	0.26	0.18 - 0.34	517	0.25	0.17 - 0.33	987	0.24	0.18 - 0.30	985	0.24	0.18 - 0.29
Total phosphoglycerides (mmol/L)	465	0.26	0.17 - 0.34	464	0.26	0.17 - 0.34	518	0.26	0.17 - 0.34	517	0.26	0.18 - 0.34	983	0.26	0.20 - 0.32	981	0.26	0.20 - 0.32
Ratio of triglycerides to phosphoglycerides	465	0.23	0.15 - 0.32	464	0.23	0.14 - 0.32	518	0.26	0.18 - 0.34	517	0.25	0.17 - 0.33	983	0.25	0.20 - 0.31	981	0.25	0.19 - 0.30
Phosphatidylcholine & other cholines (mmol/L)	465	0.27	0.18 - 0.35	464	0.27	0.18 - 0.35	518	0.25	0.17 - 0.33	517	0.25	0.17 - 0.33	983	0.26	0.20 - 0.32	981	0.26	0.20 - 0.32
Sphingomyelins (mmol/L)	465	0.23	0.15 - 0.32	464	0.23	0.15 - 0.32	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	983	0.27	0.21 - 0.32	981	0.26	0.20 - 0.32
Total cholines (mmol/L)	465	0.27	0.18 - 0.35	464	0.27	0.18 - 0.35	518	0.28	0.20 - 0.35	517	0.28	0.20 - 0.36	983	0.27	0.21 - 0.33	981	0.27	0.21 - 0.33
Apolipoproteins																		
Apolipoprotein A1 (g/L)	469	0.28	0.20 - 0.36	468	0.28	0.19 - 0.36	518	0.26	0.18 - 0.34	517	0.26	0.18 - 0.34	987	0.27	0.21 - 0.33	985	0.27	0.21 - 0.32
Apolipoprotein B (g/L)	469	0.26	0.18 - 0.35	468	0.26	0.17 - 0.34	517	0.30	0.22 - 0.38	516	0.30	0.22 - 0.38	986	0.29	0.23 - 0.35	984	0.29	0.23 - 0.35
Ratio of apolipoprotein B to apolipoprotein AI	469	0.28	0.20 - 0.36	468	0.27	0.19 - 0.35	517	0.25	0.17 - 0.33	516	0.24	0.16 - 0.32	986	0.27	0.21 - 0.33	984	0.26	0.20 - 0.32
Fatty acids																		
Total fatty acids (mmol/L)	462	0.22	0.13 - 0.30	461	0.23	0.14 - 0.31	517	0.31	0.23 - 0.39	516	0.31	0.23 - 0.39	979	0.27	0.22 - 0.33	977	0.28	0.22 - 0.33
Estimated degree of unsaturation	462	0.30	0.22 - 0.38	461	0.30	0.21 - 0.38	517	0.27	0.19 - 0.35	516	0.25	0.17 - 0.33	979	0.29	0.23 - 0.34	977	0.28	0.22 - 0.33
22:6, docosahexaenoic acid (mmol/L)	462	0.20	0.11 - 0.29	461	0.19	0.10 - 0.28	517	0.34	0.26 - 0.41	516	0.33	0.25 - 0.40	979	0.27	0.21 - 0.32	977	0.26	0.20 - 0.31
18:2, linoleic acid (mmol/L)	462	0.22	0.13 - 0.31	461	0.23	0.15 - 0.32	517	0.27	0.19 - 0.35	516	0.27	0.19 - 0.35	979	0.25	0.19 - 0.31	977	0.26	0.20 - 0.32
Omega3 fatty acids (mmol/L)	462	0.20	0.11 - 0.29	461	0.21	0.12 - 0.30	517	0.34	0.27 - 0.42	516	0.34	0.26 - 0.41	979	0.27	0.21 - 0.33	977	0.27	0.21 - 0.33
Omega6 fatty acids (mmol/L)	462	0.23	0.14 - 0.32	461	0.24	0.16 - 0.33	517	0.31	0.23 - 0.38	516	0.31	0.23 - 0.38	979	0.27	0.21 - 0.33	977	0.28	0.22 - 0.33
Polyunsat. fatty acids (mmol/L)	462	0.23	0.14 - 0.31	461	0.24	0.15 - 0.33	517	0.32	0.24 - 0.39	516	0.32	0.24 - 0.39	979	0.28	0.22 - 0.33	977	0.28	0.22 - 0.34
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	462	0.24	0.15 - 0.33	461	0.24	0.16 - 0.33	517	0.33	0.25 - 0.40	516	0.32	0.24 - 0.39	979	0.29	0.24 - 0.35	977	0.29	0.23 - 0.35
Saturated fatty acids (mmol/L)	462	0.21	0.12 - 0.29	461	0.21	0.12 - 0.30	517	0.29	0.21 - 0.37	516	0.29	0.21 - 0.37	979	0.26	0.20 - 0.32	977	0.26	0.20 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	462	0.31	0.22 - 0.39	461	0.29	0.21 - 0.38	517	0.35	0.27 - 0.42	516	0.33	0.25 - 0.41	979	0.33	0.27 - 0.39	977	0.31	0.25 - 0.37
Ratio of 18:2 linoleic acid to total fatty acids (%)	462	0.13	0.04 - 0.22	461	0.13	0.03 - 0.21	517	0.20	0.11 - 0.28	516	0.19	0.10 - 0.27	979	0.17	0.11 - 0.23	977	0.16	0.10 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	462	0.32	0.24 - 0.40	461	0.33	0.24 - 0.40	517	0.40	0.33 - 0.47	516	0.38	0.30 - 0.45	979	0.36	0.31 - 0.41	977	0.35	0.29 - 0.40
Ratio of omega6 fatty acids to total fatty acids (%)	462	0.23	0.15 - 0.32	461	0.23	0.14 - 0.32	517	0.25	0.17 - 0.33	516	0.24	0.15 - 0.32	979	0.24	0.18 - 0.30	977	0.24	0.18 - 0.30
Ratio of polyunsat. fatty acids to total fatty acids (%)	462	0.27	0.19 - 0.36	461	0.27	0.18 - 0.35	517	0.28	0.20 - 0.36	516	0.26	0.18 - 0.34	979	0.28	0.22 - 0.33	977	0.27	0.21 - 0.32
Ratio of monounsat. fatty acids to total fatty acids (%)	462	0.29	0.21 - 0.38	461	0.29	0.20 - 0.37	517	0.33	0.25 - 0.40	516	0.31	0.23 - 0.39	979	0.32	0.26 - 0.37	977	0.30	0.25 - 0.36
Ratio of saturated fatty acids to total fatty acids (%)	462	0.20	0.11 - 0.29	461	0.20	0.11 - 0.28	517	0.26	0.18 - 0.34	516	0.26	0.18 - 0.34	979	0.23	0.17 - 0.29	977	0.23	0.17 - 0.29
Glycolysis related																		
Pyruvate (mmol/L)	467	0.12	0.03 - 0.21	466	0.12	0.02 - 0.20	518	0.23	0.14 - 0.31	517	0.23	0.14 - 0.31	985	0.17	0.11 - 0.23	983	0.17	0.11 - 0.23
Citrate (mmol/L)	469	0.24	0.15 - 0.32	468	0.24	0.15 - 0.32	517	0.16	0.07 - 0.24	516	0.16	0.08 - 0.25	986	0.18	0.12 - 0.24	984	0.19	0.13 - 0.25
Glycerol (mmol/L)	124	0.25	0.07 - 0.41	123	0.24	0.06 - 0.40	155	0.10	-0.05 - 0.26	155	0.11	-0.04 - 0.27	279	0.16	0.05 - 0.28	278	0.17	0.06 - 0.29
Amino acids																		
Alanine (mmol/L)	469	0.22	0.13 - 0.31	468	0.23	0.14 - 0.31	517	0.32	0.24 - 0.40	516	0.32	0.24 - 0.40	986	0.28	0.22 - 0.33	984	0.28	0.22 - 0.34
Glutamine (mmol/L)	469	0.28	0.20 - 0.36	468	0.29	0.20 - 0.37	518	0.19	0.10 - 0.27	517	0.19	0.10 - 0.27	987	0.23	0.17 - 0.29	985	0.23	0.17 - 0.29
Glycine (mmol/L)	468	0.22	0.13 - 0.30	467	0.23	0.15 - 0.32	517	0.19	0.10 - 0.27	516	0.18	0.10 - 0.26	985	0.20	0.14 - 0.26	983	0.21	0.15 - 0.27
Histidine (mmol/L)	468	0.20	0.11 - 0.29	467	0.20	0.11 - 0.28	518	0.21	0.13 - 0.29	517	0.21	0.13 - 0.29	986	0.21	0.15 - 0.27	984	0.21	0.15 - 0.27
Isoleucine (mmol/L)	466	0.39	0.31 - 0.47	465	0.39	0.31 - 0.47	518	0.33	0.25 - 0.40	517	0.32	0.24 - 0.39	984	0.36	0.30 - 0.41	982	0.35	0.29 - 0.40
Leucine (mmol/L)	469	0.34	0.25 - 0.42	468	0.34	0.25 - 0.42	518	0.28	0.20 - 0.35	517	0.27	0.19 - 0.35	987	0.30	0.25 - 0.36	985	0.30	0.24 - 0.36
Valine (mmol/L)	469	0.39	0.31 - 0.46	468	0.39	0.31 - 0.46	516	0.27	0.19 - 0.35	515	0.27	0.19 - 0.35	985	0.33	0.27 - 0.38	983	0.33	0.27 - 0.38
Phenylalanine (mmol/L)	469	0.32	0.23 - 0.39	468	0.31	0.23 - 0.39	518	0.29	0.21 - 0.37	517	0.29	0.21 - 0.37	987	0.30	0.24 - 0.36	985	0.30	0.24 - 0.36
Tyrosine (mmol/L)	468	0.33	0.25 - 0.41	467	0.33	0.25 - 0.41	518	0.26	0.17 - 0.34	517	0.25	0.17 - 0.33	986	0.29	0.23 - 0.35	984	0.29	0.23 - 0.35
Ketone bodies																		
Acetate (mmol/L)*	468	0.11	0.02 - 0.20	467	0.09	-0.00 - 0.18	517	0.14	0.06 - 0.22	516	0.14	0.05 - 0.22	985	0.13	0.07 - 0.19	983	0.12	0.05 - 0.18
Acetoacetate (mmol/L)*	469	0.13	0.04 - 0.22	468	0.13	0.04 - 0.22	517	0.03	-0.05 - 0.12	516	0.02	-0.06 - 0.11	986	0.08	0.01 - 0.14	984	0.08	0.01 - 0.14
3hydroxybutyrate (mmol/L)*	437	0.23	0.14 - 0.32	436	0.23	0.14 - 0.32	486	0.34	0.26 - 0.42	485	0.33	0.25 - 0.41	923	0.28	0.22 - 0.34	921	0.28	0.22 - 0.34
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	469	0.23	0.15 - 0.32	468	0.22	0.14 - 0.31	518	0.28	0.19 - 0.35	517	0.27	0.18 - 0.34	987	0.26	0.20 - 0.32	985	0.25	0.19 - 0.31

*log transformation has been applied to metabolite
#adjusted for age, socioeconomic status, fasting time and processing lag time

Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

Metabolic subgroup	All Parents																	
	Boys						Girls						All Children					
	n	CC	95% CI	n	PCC#	95% CI	n	CC	95% CI	n	PCC#	95% CI	n	CC	95% CI	n	PCC#	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	547	0.22	0.14 - 0.30	544	0.22	0.14 - 0.30	586	0.20	0.12 - 0.28	585	0.19	0.11 - 0.27	1133	0.22	0.16 - 0.27	1129	0.21	0.16 - 0.27
Total lipids in very large VLDL (mmol/L)*	547	0.23	0.15 - 0.31	544	0.23	0.15 - 0.31	586	0.17	0.10 - 0.25	585	0.17	0.09 - 0.24	1133	0.21	0.15 - 0.26	1129	0.20	0.15 - 0.26
Total lipids in large VLDL (mmol/L)*	547	0.20	0.12 - 0.28	544	0.20	0.12 - 0.28	586	0.20	0.12 - 0.28	585	0.18	0.10 - 0.26	1133	0.20	0.15 - 0.26	1129	0.19	0.13 - 0.25
Total lipids in medium VLDL (mmol/L)	547	0.27	0.19 - 0.35	544	0.26	0.18 - 0.34	586	0.25	0.17 - 0.32	585	0.23	0.16 - 0.31	1133	0.26	0.21 - 0.31	1129	0.25	0.19 - 0.30
Total lipids in small VLDL (mmol/L)	547	0.27	0.19 - 0.34	544	0.26	0.18 - 0.33	586	0.26	0.18 - 0.33	585	0.25	0.17 - 0.32	1133	0.26	0.21 - 0.32	1129	0.25	0.20 - 0.31
Total lipids in very small VLDL (mmol/L)	547	0.23	0.15 - 0.31	544	0.23	0.14 - 0.30	586	0.25	0.17 - 0.33	585	0.26	0.18 - 0.33	1133	0.25	0.19 - 0.30	1129	0.24	0.19 - 0.30
Total lipids in IDL (mmol/L)	547	0.28	0.20 - 0.35	544	0.26	0.19 - 0.34	586	0.29	0.21 - 0.36	585	0.29	0.22 - 0.36	1133	0.29	0.23 - 0.34	1129	0.28	0.23 - 0.33
Total lipids in large LDL (mmol/L)	547	0.28	0.20 - 0.36	544	0.27	0.19 - 0.35	586	0.29	0.21 - 0.36	585	0.29	0.21 - 0.36	1133	0.29	0.23 - 0.34	1129	0.28	0.23 - 0.34
Total lipids in medium LDL (mmol/L)	547	0.29	0.21 - 0.36	544	0.28	0.20 - 0.35	586	0.28	0.21 - 0.36	585	0.28	0.21 - 0.36	1133	0.28	0.23 - 0.34	1129	0.28	0.23 - 0.33
Total lipids in small LDL (mmol/L)	547	0.28	0.20 - 0.35	544	0.27	0.19 - 0.35	586	0.28	0.20 - 0.35	585	0.28	0.20 - 0.35	1133	0.28	0.22 - 0.33	1129	0.27	0.22 - 0.33
Total lipids in very large HDL (mmol/L)	547	0.29	0.21 - 0.37	544	0.28	0.20 - 0.36	586	0.29	0.21 - 0.36	585	0.28	0.21 - 0.36	1133	0.29	0.24 - 0.34	1129	0.28	0.23 - 0.33
Total lipids in large HDL (mmol/L)	547	0.29	0.21 - 0.36	544	0.27	0.20 - 0.35	586	0.24	0.17 - 0.32	585	0.24	0.16 - 0.31	1133	0.27	0.21 - 0.32	1129	0.25	0.20 - 0.31
Total lipids in medium HDL (mmol/L)	547	0.15	0.07 - 0.24	544	0.14	0.06 - 0.23	586	0.10	0.02 - 0.18	585	0.10	0.02 - 0.18	1133	0.12	0.07 - 0.18	1129	0.12	0.06 - 0.18
Total lipids in small HDL (mmol/L)	547	0.19	0.10 - 0.27	544	0.18	0.10 - 0.26	586	0.18	0.10 - 0.26	585	0.18	0.10 - 0.26	1133	0.18	0.12 - 0.23	1129	0.18	0.12 - 0.23
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	547	0.29	0.21 - 0.36	544	0.28	0.20 - 0.36	586	0.22	0.14 - 0.29	585	0.21	0.13 - 0.28	1133	0.25	0.20 - 0.31	1129	0.24	0.19 - 0.30
Mean diameter for LDL particles (nm)	547	0.19	0.11 - 0.27	544	0.19	0.11 - 0.27	586	0.27	0.19 - 0.34	585	0.27	0.20 - 0.35	1133	0.23	0.17 - 0.28	1129	0.23	0.17 - 0.28
Mean diameter for HDL particles (nm)	547	0.31	0.23 - 0.38	544	0.30	0.22 - 0.37	586	0.30	0.22 - 0.37	585	0.29	0.22 - 0.36	1133	0.30	0.25 - 0.35	1129	0.29	0.24 - 0.35
Cholesterol																		
Serum total cholesterol (mmol/L)	547	0.28	0.20 - 0.36	544	0.27	0.19 - 0.35	586	0.31	0.24 - 0.38	585	0.31	0.24 - 0.39	1133	0.30	0.24 - 0.35	1129	0.29	0.24 - 0.34
Total cholesterol in VLDL (mmol/L)	547	0.26	0.18 - 0.34	544	0.26	0.18 - 0.33	586	0.26	0.18 - 0.33	585	0.25	0.17 - 0.32	1133	0.26	0.21 - 0.32	1129	0.25	0.20 - 0.31
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	547	0.27	0.19 - 0.34	544	0.26	0.18 - 0.34	586	0.28	0.20 - 0.35	585	0.28	0.20 - 0.35	1133	0.27	0.22 - 0.33	1129	0.27	0.21 - 0.32
Total cholesterol in LDL (mmol/L)	547	0.29	0.21 - 0.36	544	0.28	0.20 - 0.36	586	0.28	0.21 - 0.35	585	0.28	0.21 - 0.36	1133	0.29	0.23 - 0.34	1129	0.28	0.23 - 0.34
Total cholesterol in HDL (mmol/L)	547	0.27	0.19 - 0.35	544	0.25	0.17 - 0.33	586	0.22	0.14 - 0.29	585	0.21	0.13 - 0.28	1133	0.24	0.19 - 0.30	1129	0.23	0.17 - 0.28
Total cholesterol in HDL2 (mmol/L)	547	0.27	0.19 - 0.34	544	0.25	0.17 - 0.33	586	0.21	0.14 - 0.29	585	0.20	0.13 - 0.28	1133	0.24	0.18 - 0.29	1129	0.23	0.17 - 0.28
Total cholesterol in HDL3 (mmol/L)	547	0.25	0.17 - 0.33	544	0.23	0.15 - 0.31	586	0.23	0.15 - 0.31	585	0.22	0.15 - 0.30	1133	0.24	0.19 - 0.30	1129	0.23	0.17 - 0.28
Esterified cholesterol (mmol/L)	543	0.28	0.20 - 0.36	540	0.27	0.19 - 0.35	584	0.31	0.24 - 0.38	583	0.31	0.24 - 0.38	1127	0.30	0.24 - 0.35	1123	0.29	0.24 - 0.34
Free cholesterol (mmol/L)	543	0.27	0.19 - 0.34	540	0.26	0.18 - 0.33	584	0.32	0.24 - 0.39	583	0.32	0.24 - 0.39	1127	0.29	0.24 - 0.35	1123	0.29	0.23 - 0.34
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	547	0.28	0.20 - 0.35	544	0.27	0.19 - 0.34	586	0.25	0.17 - 0.32	585	0.24	0.16 - 0.31	1133	0.26	0.21 - 0.32	1129	0.25	0.20 - 0.31
Triglycerides in VLDL (mmol/L)*	547	0.28	0.20 - 0.36	544	0.28	0.20 - 0.35	586	0.23	0.15 - 0.30	585	0.21	0.14 - 0.29	1133	0.26	0.20 - 0.31	1129	0.25	0.19 - 0.30
Triglycerides in LDL (mmol/L)	547	0.20	0.12 - 0.28	544	0.19	0.11 - 0.27	586	0.27	0.19 - 0.34	585	0.27	0.19 - 0.34	1133	0.24	0.18 - 0.29	1129	0.23	0.18 - 0.29
Triglycerides in HDL (mmol/L)	547	0.25	0.17 - 0.33	544	0.25	0.17 - 0.33	586	0.27	0.20 - 0.35	585	0.27	0.19 - 0.34	1133	0.26	0.21 - 0.32	1129	0.26	0.20 - 0.31
Total phosphoglycerides (mmol/L)	543	0.28	0.20 - 0.36	540	0.27	0.19 - 0.35	584	0.27	0.19 - 0.34	583	0.27	0.20 - 0.35	1127	0.27	0.22 - 0.33	1123	0.27	0.22 - 0.32
Ratio of triglycerides to phosphoglycerides	543	0.23	0.15 - 0.31	540	0.22	0.14 - 0.30	584	0.21	0.13 - 0.28	583	0.19	0.11 - 0.27	1127	0.22	0.16 - 0.27	1123	0.21	0.15 - 0.26
Phosphatidylcholine & other cholines (mmol/L)	542	0.28	0.20 - 0.36	539	0.27	0.19 - 0.35	584	0.26	0.19 - 0.34	583	0.26	0.19 - 0.34	1126	0.27	0.21 - 0.32	1122	0.27	0.21 - 0.32
Sphingomyelins (mmol/L)	542	0.23	0.15 - 0.31	539	0.22	0.13 - 0.30	584	0.28	0.21 - 0.36	583	0.28	0.21 - 0.36	1126	0.26	0.20 - 0.31	1122	0.25	0.19 - 0.30
Total cholines (mmol/L)	542	0.27	0.19 - 0.35	539	0.27	0.19 - 0.34	584	0.29	0.21 - 0.36	583	0.29	0.21 - 0.36	1126	0.28	0.22 - 0.33	1122	0.27	0.22 - 0.33
Apolipoproteins																		
Apolipoprotein A1 (g/L)	547	0.26	0.18 - 0.34	544	0.25	0.17 - 0.33	586	0.25	0.17 - 0.33	585	0.25	0.17 - 0.32	1133	0.25	0.20 - 0.31	1129	0.24	0.19 - 0.30
Apolipoprotein B (g/L)	547	0.27	0.19 - 0.35	544	0.27	0.19 - 0.34	585	0.28	0.20 - 0.35	584	0.28	0.20 - 0.35	1132	0.28	0.23 - 0.33	1128	0.28	0.22 - 0.33
Ratio of apolipoprotein B to apolipoprotein AI	547	0.26	0.18 - 0.33	544	0.25	0.17 - 0.33	585	0.21	0.13 - 0.29	584	0.20	0.12 - 0.28	1132	0.23	0.18 - 0.29	1128	0.23	0.17 - 0.28
Fatty acids																		
Total fatty acids (mmol/L)	537	0.26	0.18 - 0.33	534	0.26	0.18 - 0.34	583	0.30	0.22 - 0.37	582	0.29	0.22 - 0.37	1120	0.28	0.23 - 0.33	1116	0.28	0.22 - 0.33
Estimated degree of unsaturation	537	0.30	0.22 - 0.37	534	0.30	0.22 - 0.37	583	0.24	0.17 - 0.32	582	0.23	0.15 - 0.30	1120	0.27	0.21 - 0.32	1116	0.26	0.20 - 0.31
22:6, docosahexaenoic acid (mmol/L)	537	0.23	0.15 - 0.31	534	0.22	0.14 - 0.30	583	0.33	0.26 - 0.40	582	0.33	0.25 - 0.40	1120	0.28	0.23 - 0.34	1116	0.27	0.22 - 0.33
18:2, linoleic acid (mmol/L)	537	0.24	0.16 - 0.32	534	0.26	0.17 - 0.33	583	0.27	0.19 - 0.34	582	0.27	0.19 - 0.34	1120	0.26	0.21 - 0.32	1116	0.26	0.21 - 0.32
Omega3 fatty acids (mmol/L)	537	0.24	0.16 - 0.32	534	0.24	0.16 - 0.32	583	0.34	0.27 - 0.41	582	0.34	0.27 - 0.41	1120	0.29	0.23 - 0.34	1116	0.29	0.23 - 0.34
Omega6 fatty acids (mmol/L)	537	0.26	0.18 - 0.33	534	0.26	0.18 - 0.34	583	0.30	0.23 - 0.38	582	0.30	0.23 - 0.38	1120	0.28	0.23 - 0.34	1116	0.28	0.23 - 0.34
Polyunsat. fatty acids (mmol/L)	537	0.26	0.17 - 0.33	534	0.26	0.18 - 0.34	583	0.31	0.24 - 0.39	582	0.31	0.24 - 0.39	1120	0.29	0.23 - 0.34	1116	0.29	0.23 - 0.34
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	537	0.27	0.19 - 0.35	534	0.27	0.19 - 0.35	583	0.30	0.22 - 0.37	582	0.29	0.22 - 0.37	1120	0.29	0.24 - 0.34	1116	0.28	0.23 - 0.34
Saturated fatty acids (mmol/L)	537	0.25	0.17 - 0.33	534	0.25	0.17 - 0.33	583	0.28	0.20 - 0.35	582	0.28	0.20 - 0.35	1120	0.27	0.21 - 0.32	1116	0.27	0.21 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	537	0.31	0.23 - 0.38	534	0.30	0.22 - 0.37	583	0.33	0.26 - 0.40	582	0.32	0.24 - 0.39	1120	0.32	0.27 - 0.37	1116	0.31	0.25 - 0.36
Ratio of 18:2 linoleic acid to total fatty acids (%)	537	0.15	0.07 - 0.23	534	0.15	0.06 - 0.23	583	0.18	0.10 - 0.26	582	0.17	0.09 - 0.25	1120	0.17	0.11 - 0.23	1116	0.16	0.11 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	537	0.32	0.24 - 0.39	534	0.32	0.24 - 0.39	583	0.40	0.33 - 0.47	582	0.39	0.32 - 0.46	1120	0.36	0.31 - 0.41	1116	0.35	0.30 - 0.40
Ratio of omega6 fatty acids to total fatty acids (%)	537	0.24	0.16 - 0.32	534	0.24	0.16 - 0.32	583	0.23	0.15 - 0.30	582	0.22	0.14 - 0.29	1120	0.24	0.18 - 0.29	1116	0.23	0.17 - 0.28
Ratio of polyunsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.35	534	0.27	0.19 - 0.34	583	0.26	0.18 - 0.33	582	0.24	0.16 - 0.32	1120	0.26	0.21 - 0.32	1116	0.25	0.20 - 0.31
Ratio of monounsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.34	534	0.26	0.17 - 0.33	583	0.30	0.23 - 0.37	582	0.29	0.21 - 0.36	1120	0.29	0.23 - 0.34	1116	0.27	0.22 - 0.33
Ratio of saturated fatty acids to total fatty acids (%)	537	0.22	0.14 - 0.30	534	0.22	0.13 - 0.30	583	0.26	0.19 - 0.34	582	0.26	0.19 - 0.34	1120	0.24	0.19 - 0.30	1116	0.24	0.19 - 0.30
Glycolysis related																		
Pyruvate (mmol/L)	545	0.11	0.03 - 0.20	542	0.11	0.03 - 0.20	586	0.22	0.15 - 0.30	585	0.22	0.15 - 0.30	1131	0.17	0.11 - 0.22	1127	0.17	0.11 - 0.22
Citrate (mmol/L)	547	0.23	0.15 - 0.31	544	0.23	0.15 - 0.31	585	0.14	0.06 - 0.22	584	0.14	0.06 - 0.22	1132	0.17	0.12 - 0.23	1128	0.18	0.12 - 0.23
Glycerol (mmol/L)	151	0.30	0.15 - 0.44	149	0.28	0.13 - 0.42	175	0.12	-0.03 - 0.26	175	0.13	-0.02 - 0.27	326	0.20	0.09 - 0.30	324	0.20	0.10 - 0.31
Amino acids																		
Alanine (mmol/L)	546	0.24	0.16 - 0.31	543	0.24	0.16 - 0.32	585	0.32	0.25 - 0.39	584	0.32	0.25 - 0.39	1131	0.28	0.23 - 0.34	1127	0.29	0.23 - 0.34
Glutamine (mmol/L)	547	0.30	0.22 - 0.37	544	0.30	0.22 - 0.37	586	0.20	0.12 - 0.27	585	0.20	0.12 - 0.27	1133	0.24	0.19 - 0.30	1129	0.24	0.18 - 0.29
Glycine (mmol/L)	545	0.21	0.13 - 0.29	542	0.22	0.14 - 0.30	585	0.19	0.11 - 0.27	584	0.19	0.11 - 0.27	1130	0.20	0.15 - 0.26	1126	0.21	0.15 - 0.26
Histidine (mmol/L)	545	0.21	0.13 - 0.29	542	0.21	0.13 - 0.29	586	0.22	0.14 - 0.29	585	0.22	0.14 - 0.29	1131	0.21	0.16 - 0.27	1127	0.21	0.16 - 0.27
Isoleucine (mmol/L)	541	0.36	0.29 - 0.44	538	0.36	0.29 - 0.43	586	0.29	0.22 - 0.36	585	0.28	0.20 - 0.35	1127	0.33	0.27 - 0.38	1123	0.32	0.26 - 0.37
Leucine (mmol/L)	547	0.32	0.24 - 0.39	544	0.32	0.24 - 0.40	586	0.24	0.16 - 0.32	585	0.23	0.15 - 0.31	1133	0.28	0.23 - 0.33	1129	0.28	0.22 - 0.33
Valine (mmol/L)	547	0.38	0.31 - 0.45	544	0.39	0.31 - 0.45	584	0.27	0.19 - 0.34	583	0.26	0.18 - 0.33	1131	0.33	0.27 - 0.38	1127	0.32	0.27 - 0.38
Phenylalanine (mmol/L)	547	0.29	0.22 - 0.37	544	0.29	0.22 - 0.37	586	0.30	0.22 - 0.37	585	0.30	0.22 - 0.37	1133	0.29	0.24 - 0.35	1129	0.29	0.24 - 0.35
Tyrosine (mmol/L)	545	0.31	0.24 - 0.39	542	0.32	0.24 - 0.39	586	0.27	0.19 - 0.34	585	0.27	0.19 - 0.34	1131	0.29	0.24 - 0.34	1127	0.29	0.24 - 0.34
Ketone bodies																		
Acetate (mmol/L)*	546	0.09	0.01 - 0.17	543	0.07	-0.02 - 0.15	585	0.15	0.06 - 0.22	584	0.14	0.06 - 0.22	1131	0.12	0.06 - 0.17	1127	0.11	0.05 - 0.16
Acetoacetate (mmol/L)*	547	0.11	0.03 - 0.20	544	0.12	0.03 - 0.20	585	0.04	-0.04 - 0.12	584	0.04	-0.04 - 0.12	1132	0.07	0.02 - 0.13	1128	0.08	0.02 - 0.13
3hydroxybutyrate (mmol/L)*	511	0.22	0.13 - 0.30	508	0.22	0.13 - 0.30	551	0.34	0.26 - 0.41	550	0.33	0.25 - 0.40	1062	0.27	0.21 - 0.33	1058	0.27	0.21 - 0.32
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	547	0.24	0.16 - 0.32	544	0.23	0.15 - 0.31	586	0.24	0.17 - 0.32	585	0.23	0.16 - 0.31	1133	0.24	0.19 - 0.30	1129	0.23	0.18 - 0.29

*log transformation has been applied to metabolite
#adjusted for age, socioeconomic status, fasting time and processing lag time

STROBE Statement—checklist of items that should be included in reports of observational studies

Paper title: Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

Person completing checklist: Susan Ellul

	Item No	Recommendation	Page number	Line number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2	14-15
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	9-13 26-32
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5	Pg 4 (3-9 ,21-22, 31-32, 33-35) Pg 5 (3-4)
Objectives	3	State specific objectives, including any prespecified hypotheses	5	5-7
Methods				
Study design	4	Present key elements of study design early in the paper	5	10-17
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6	Pg 5 (33-35) Pg 6 (1-27)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ~~*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale~~ for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	18-26
		~~(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed~~ ~~*Case-control study*—For matched studies, give matching criteria and the number of controls per case~~		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8	Pg 6 (29-36) Pg 7 (1-6, table 1, 15-19) Pg 8 (1-33)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8	Pg 6 (29-36) Pg 7 (1-6, table 1, 15-19) Pg 8 (1-33)
Bias	9	Describe any efforts to address potential sources of bias	9	6-10
Study size	10	Explain how the study size was arrived at	10	5-14, figure 1

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9	2-26
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9	2-26
		(*b*) Describe any methods used to examine subgroups and interactions	9	2-26
		(*c*) Explain how missing data were addressed	9	6-10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed ~~*Case-control study*—If applicable, explain how matching of cases and controls was addressed~~	9	6-10, 15-17, 20-23
		(*e*) Describe any sensitivity analyses	9	15-17, 20-23

Results			**Page number**	**Line number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10 and figure 1	5-12
		(b) Give reasons for non-participation at each stage	10 6 figure 1	8-12 5-6
		(c) Consider use of a flow diagram	figure 1	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11 (table 2)	2
		(b) Indicate number of participants with missing data for each variable of interest	figure 1 table 2 sup Table 1	2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	sup table 1	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12 supp tables 2-6	20-25
		(*b*) Report category boundaries when continuous variables were categorized	NA	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA	

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	supp tables 2-6	
Discussion				
Key results	18	Summarise key results with reference to study objectives	13	3-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13	18-28
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-16	Pg 13 (30-32) Pg 14 (all) Pg 15 (all) Pg 16 (1-2)
Generalisability	21	Discuss the generalisability (external validity) of the study results	13	19-27
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17-18	Pg 17 (28-33) Pg 18 (1-3)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords:	Metabolomics, Lipids, Reference values, Children, Inheritance patterns, Epidemiologic studies

Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords: Metabolomics; lipids; inflammation; reference values; parents; children; inheritance patterns; correlation studies; epidemiologic studies; cross-sectional studies.

Word count: 4270

Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched chain amino acid; BD: Becton Dickinson; CDC: Centers for Disease Prevention and Control; CVD: Cardiovascular disease; CPS1: Carbamoyl-phosphate synthase 1; DHA: Docosahexaenoic acid; DOB: Date of birth; EDTA: Ethylenediaminetetraacetic acid; FDR: False discovery rate; GlycA: Glycoprotein acetyls; HbA1c: Haemoglobin A1c; HDL: High-density lipoprotein; HOMA: Homeostatic model assessment; IDL: Intermediate density lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein; LiH: Lithium Heparin; LSAC:

Longitudinal Study of Australian Children; MUFA: Monounsaturated fatty acid; NMR: Nuclear magnetic resonance; PCOS: Polycystic Ovary Syndrome; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acids; SST: serum separating tubes; T2D: Type 2 diabetes; T2DM: Type 2 diabetes mellitus; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

ABSTRACT

Objectives: Nuclear Magnetic Resonance (NMR) metabolomics is high throughput and cost effective, with the potential to improve the understanding of disease and risk. We examine the circulating metabolic profile by quantitative NMR metabolomics of a sample of Australian 11-12 year old children and their parents, describe differences by age and sex, and explore correlation of metabolites in parent-child dyads.

Design: The population-based cross-sectional Child Health CheckPoint study nested within the Longitudinal Study of Australian Children.

Setting: Blood samples collected from CheckPoint participants at assessment centres in seven Australian cities and eight regional towns; February 2015-March 2016.

Participants: 1180 children and 1325 parents provided a blood sample and had metabolomics data available. This included 1133 parent-child dyads (518 mother-daughter, 469 mother-son, 68 father-daughter, and 78 father-son).

Outcome measures: 228 metabolic measures were obtained for each participant. We focused on 74 biomarkers including amino acid species, lipoprotein subclass measures, lipid measures, fatty acids, measures related to fatty acid saturation, and composite markers of inflammation and energy homeostasis.

Results: We identified sex-specific metabolic profiles in children and adults and differences in the concentration of specific metabolites between childhood and adulthood. In general, metabolite concentrations were higher in adults than children and sex differences were larger in adults than in children. Positive correlations were observed for the majority of metabolites including for isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total cholesterol (CC 0.30, 95% CI 0.24 to 0.35) and omega 6 fatty acids (CC 0.28, 95% CI 0.23 to 0.34) in parent-child comparisons.

Conclusions: We describe the serum metabolite profiles from mid-childhood and adulthood in a population-based sample, together with parent-child concordance. Distinct age- and sex-specific profiles were observed. These data will be informative for investigation of the childhood origins of adult non-communicable diseases and for comparative studies in other populations.

Strengths and limitations of this study:

- In a large population-based cohort, venous blood was collected for children and their attending parent on the same day using the same methods
- Rapidly processed, high quality serum samples with standardised metabolomic data generated as a single batch
- Cross-sectional design does not enable longitudinal analysis of specific metabolite species over short term or longer periods of time
- Assessment of paternal associations with offspring metabolite measures is limited by a relatively small sample size compared to mother-child pairs, reducing the precision of estimates
- Factors known to influence metabolomic profile (such as body mass index) were not considered as the aim was to describe the distribution of metabolites in children and their parents.

INTRODUCTION

Metabolomics involves the quantitative analysis of a large number of metabolites and lipids involved in a diverse range of biochemical pathways.[1] Genetic/gene expression and environmental exposures are associated with specific metabolic changes across many tissues and body fluids.[2 3] As such, metabolomics is recognised as a powerful top-down approach to understanding genetic and environmental influences on health and disease. Metabolomic profiling also has considerable potential to identify clinically relevant biomarkers for risk stratification and disease monitoring.

Recent advances in nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry have enabled the simultaneous quantitative measurement of hundreds of metabolites. These approaches are sufficiently cost effective and high throughput to be applicable to large cohort studies. For example, NMR metabolomics of serum from the Cardiovascular Risk in Young Finns Study identified many biomarkers from multiple metabolic pathways reflective of fatty liver disease.[4] These were also predictive of risk 10 years prior to diagnosis, indicating that metabolic disruptions precede overt phenotype. Similar population and disease-specific studies have identified metabolomic profiles associated with a range of exposures and health outcomes with potential to reveal clinically important biomarkers and information on disease mechanisms.[5] In addition, specific serum metabolites can also be considered 'intermediate phenotypes' linking genetic risk with disease outcomes.[6 7]

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.[8 9] However, in adults sex appears to be a major driver of variation in metabolite profile, potentially interacting with age. For example, the effects of sex appeared to be greater in younger (age 25-35) than older Japanese adults.[10] A study of 26,000 Northern European adults identified many sex-specific metabolic species at the population level.[9] In men, several lipid measures begin to rise at early middle age whereas a similar increase is only observed in females post menopause. This pattern is consistent for all non-HDL cholesterol measures – very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) subclass particle concentrations - as well as for triglycerides.[9] Physiological states such as pregnancy also have consistent and measurable influence on serum metabolome.[11] However, it remains unclear how the serum metabolome differs in adults compared to children and by sex particularly in childhood.

Moreover, factors regulating the metabolic trajectory from early life to adulthood, the role of metabolomic profile in health at the population level and the extent to which blood metabolomic profiles are concordant for parents and children have not been fully explored. One small study has reported correlations between parents (n=179) and their offspring

(n=255) for a range of cardiometabolic risk factors including standard lipid profile measured using conventional methods; this proved stronger for total cholesterol and LDL cholesterol than for high density lipoprotein (HDL) cholesterol or triglycerides.[12] Considerable evidence exists that the metabolomic profile is regulated, at least in part, by genetic factors[13 14] and is also influenced by dietary and lifestyle factors. Each of these influences is likely to be shared between parents and their offspring to varying degrees, however, parent-child correlations of metabolites from NMR-based platforms have not been reported previously.

Here, we describe (1) the distribution of NMR-based metabolite measures in a population-based cohort of 11-12 year old children and their parents, differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults; and (4) report sex-specific parent-child concordance.

METHODS

Study Design: Details of the initial Longitudinal Study of Australian Children (LSAC) study design and recruitment are outlined elsewhere.[15 16] The LSAC commenced in 2004, when two cohorts (the 'B' and 'K' cohorts, of which the B cohort only was included in the present study) were recruited who have since been followed biennially. The Child Health CheckPoint comprised a detailed cross-sectional assessment of physical health and biomarkers in a population-based national sample of children (age 11-12 years) and their parents between February 2015 to March 2016. The CheckPoint was nested between waves 6 (2014) and 7 (2016) of the LSAC. Further details regarding the CheckPoint study design and methods are available elsewhere. [17 18]

Participants: Of the 8,921 families contacted to be part of the LSAC B cohort 5,107 families (57%) agreed to take part in the first wave of data collection in 2004; 4,484 families were retained for Wave 6 in 2014. During the Wave 6 LSAC home visit, B cohort families were introduced to the upcoming Child Health CheckPoint and asked to consent to their contact details being shared with the CheckPoint team. A total of 3,513 families provided permission to receive an information pack by mail and an information and recruitment phone call regarding the CheckPoint study (78% of Wave 6 cohort, 69% of original cohort). Of the families agreeing to receive information about the CheckPoint study, 1874 families took part (53% of eligible participants, 42% of Wave 6 cohort and 37% of original cohort).

Ethics and consent: The CheckPoint data collection protocol was approved by The Royal Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26). The attending parent/caregiver provided written informed consent for themselves and their child to

participate in the study, and asked to provide optional consent for the collection and use of biological samples.

Procedure: The specialised CheckPoint assessment centre sequentially visited seven Australian cities and eight regional towns between February 2015 and March 2016.[18] Each participating child attended the centre with one parent or caregiver (usually the biological mother) at which both participated in a wide range of measures relevant to non-communicable disease. Those families who could not attend a centre were offered a home visit. Participants were included in the current analyses if metabolomic data from CheckPoint were available (figure 1). Venous blood was not available for home-visit participants, but was collected at all city and most regional assessment centres. Participant pairs were excluded from the concordance analyses in this study if the attending parent was not the biological parent.

An experienced phlebotomist collected approximately 28mL of blood from the brachial vein of the non-dominant arm of semi-reclining, semi-fasted participants (at the time of collection, participants reported when they last ate or drank). Blood was collected sequentially into four Becton Dickinson (BD) Vacutainer® tubes using a butterfly needle so only a single venepuncture was required. Order of collection was (i) 2.7mL EDTA, (ii) 9mL EDTA, (iii) 9mL serum, (iv) 7.5mL Lithium Heparin. The latter two tubes were immediately inverted 6 times to ensure mixing with anticoagulant, and all tubes were transferred to the on-site laboratory. Time of collection was scheduled earlier in the visit for parents than for children.

Collection tube barcodes were linked to the participant and samples were immediately transported to an on-site laboratory where they were processed within two hours. Blood clotting was allowed at room temperature for at least 30 minutes after collection. The sample tubes were spun at 550g relative centrifugal force for 10 minutes at room temperature and distributed into 0.5mL aliquots of plasma, serum, buffy coat (lymphocytes), whole blood and/or an aliquot tube containing a blood clot (1.0mL FluidX screwcap tubes, Cheshire, UK) and stored immediately at -80˚C (Thermo Fisher Scientific, Waltham, USA). Each FluidX tube contained a unique 2D barcode linked to the original collection tube and participant. As each assessment centre closed, samples were shipped on dry ice to the Melbourne Children's Bioresource Centre for long term storage at -80˚C (serum, whole blood, plasma, blood clot) or vapour phase liquid nitrogen (lymphocytes). At a later date, single 0.5ml serum aliquot was removed for every CheckPoint participant and the combined aliquots were shipped in a single batch to Nightingale Health (Helsinki, Finland) on dry ice for NMR metabolomics.

Measures

Metabolomic profiling

The Nightingale® NMR metabolomics platform (Helsinki, Finland) was used to obtain metabolomics for children and parents using the 2016-version quantification algorithm. Details of this platform and methodology have been extensively described elsewhere,[6 19] and epidemiological applications were recently reviewed.[20] Briefly, metabolites were measured from 0.35mL of serum using a single high-throughput experimental setup for the simultaneous quantification of routine lipids, lipoprotein subclass distributions, particle size and composition, fatty acids, and other low–molecular weight metabolites such as amino acids and glycolysis-related metabolites. This generated data on 228 serum metabolite measures in absolute concentration units (eg millimoles per liter) and ratios (summarised in Table 1). Whilst widely used for epidemiological research, the NMR-based quantification has not been certified for clinical diagnostics. Further analytical validation of the quantification protocols for the biomarker subset routinely used in clinical settings (eg established cholesterol measures and creatinine) is expected to lead to recalibration of certain metabolite concentrations to better match clinical gold standards.[20]

Table 1. Summary of biomarkers and derived variables obtained via high-throughput NMR

Metabolic group	Species and derived measures
Amino acids	Alanine, Glutamine, Glycine, Histidine
	Branched chain: Isoleucine, Leucine, Valine
	Aromatic: Phenylalanine, Tyrosine
Cholesterol	VLDL, LDL, HDL, HDL2, HDL3, Total, Free, Esterified, Remnant
Triglycerides and phospholipids	Triglycerides (VLDL, LDL, HDL, total)
	Phosphoglycerides
	Ratio of triglycerides to phosphoglycerides*
	Phosphatidylcholine
	Sphingomyelins
	Total cholines
Apolipoproteins	Apolipoprotein A-1 (ApoA-1)
	Apolipoprotein B (Apo B)
	Ratio of Apolipoprotein B to Apolipoprotein A-1 (ApoB/Apo A-1)*
Fatty acids (FA)	Total, Omega-3, Omega-6, Polyunsaturated (PUFA), Saturated (SFA)
	Monounsaturated (MUFA), Docosahexaenoic acid (DHA), Linoleic (LA)
	Estimated degree of unsaturation
Fatty acid ratios	Omega-3/total FA*, Omega-6/total FA*, PUFA/total FA* (all %)
	SFA/total FA*, MUFA/total FA*, DHA/total FA*, LA/total FA* (all %)
Lipoprotein subclasses*	12 lipid measures in each of 14 subclasses VLDL (XXL, XL, L, M, S, XS), IDL, LDL (L,M,S), HDL (XL, L, M,S): Particle concentration, Total lipids, Esterified cholesterol, Total cholesterol, Phospholipids, Free cholesterol, Triglycerides and Esterified cholesterol/Total lipids (%), Free cholesterol/Total lipids (%), Total cholesterol/Total lipids (%), Triglycerides/Total lipids (%) and Phospholipids/Total lipids (%).
Lipoprotein size*	Mean diameter of VLDL, LDL and HDL particles
Ketone bodies	Acetate, Acetoacetate, 3-hydroxybutyrate
Glycolysis related	Glucose, Lactate, Pyruvate, Citrate, Glycerol
Fluid balance	Creatinine, Albumin
Inflammation	Glycoprotein acetyls (GlycA)

Information obtained from https://nightingalehealth.com/science/biomarkers

* ratio; ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; DHA: Docosahexaenoic acid; GlycA: Glycoprotein acetyls; HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; L: Large; LDL: Low-density lipoprotein; LA: Linoleic acid; M: Medium; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; S: Small; SFA: Saturated fatty acids; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

Many of the 228 metabolomics measures correlate substantially both in adults (supplementary figure 1) and children (supplementary figure 2) and the pattern of correlations were similar for adults and children. For clarity, we therefore focused on a subset of 74 metabolites in analyses. We eliminated the 5 ratio measures for each of the 14 lipoprotein subclass particles. In addition, the 7 other measures within each of the lipoproteins (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, total lipids and particle concentration) are all highly correlated and therefore we only reported total lipids for each of the lipoprotein subclass particles.

Other measures and sample characteristics

Age and sex: For children, LSAC provided date of birth (DOB) and sex, which was originally exported from the Medicare Australia database. In parents, DOB and sex was self-reported in the CheckPoint questionnaire. Age in years was calculated as the difference between date of the CheckPoint assessment and DOB divided by 365.

Disadvantage index: LSAC provided contact details of families consenting to be contacted by CheckPoint. The family's residential postcode was confirmed during the CheckPoint recruitment phone call and updated, if required. The disadvantage index score of postcode was used to summarise neighbourhood socioeconomic position. Generated by the ABS from the 2011 national Census, the index numerically summarises the social and economic conditions of Australian neighbourhoods; national mean 1000, standard deviation 100; higher scores indicate less disadvantage.[21]

Time of blood collection, processing and fasting time: Time of blood collection and start of laboratory processing were recorded. When missing, collection time was estimated using the midpoint between the time the CheckPoint visit began and time that processing of the sample commenced. Processing lag time was calculated as the minutes between blood collection and the processing commencement. Most samples were processed within two hours.

Fasting time was calculated as the hours between last eating/drinking to time of blood collection. The last time of eating/drinking was cross-checked against when the participant was taking part in other CheckPoint stations (and known not to be eating) as well as sleep and wake times from accelerometry data (to identify usual activity, and therefore likely eating patterns) when available. Further details of cleaning processes for the time of last eat/drink can be found elsewhere.[22]

Statistical analysis

Sample Characteristics

Continuous descriptive variables and metabolite measures were summarised using means and standard deviations (SD) for children and adults separately, by sex and overall. For skewed metabolites, geometric means and relative SD were reported. To provide visual comparisons of distributions of metabolites by age and sex, density plots were used. Population summary statistics were estimated by applying survey weights and survey procedures that corrected for sampling, participation and non-response biases, and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[23] More detail on the calculation of weights is provided elsewhere.[24]

Differences in metabolite concentration by age (adults compared to children) and by sex (adults, children)

Skewed metabolites (skewness greater or equal to 2) were log-transformed. We used two-sided paired and unpaired t-tests (as appropriate) to assess differences in mean metabolite concentrations between adults and children in parent-child dyads, and between males and females for adults and children separately. P-values were adjusted using Benjamini-Hochberg (B-H) with a false discovery rate (FDR) of 10% to account for multiple comparisons.

Parent-Child concordance

Concordance between parents and children was assessed by 1) Pearson's correlation coefficients (CC) with 95% confidence intervals, and 2) partial correlation coefficients (PCC), adjusting for child and parent age, disadvantage index, fasting time, processing lag time (and for child and parent sex where appropriate). Scatterplots of parent versus child metabolites (log-transformed where needed as above) were examined to check for outliers and to ensure assumptions were met.

The analyses were repeated using weighted multi-level survey analyses and compared to unweighted analyses. As there appeared to be no major effect of response patterns on results we reported results from unweighted analyses. Analyses were undertaken using Stata version 14.2 (StataCorp, College Station, TX) and R version 3.3.2. [25]

Patient and Public Involvement: Because LSAC is a population-based longitudinal study, no patient groups were involved in its design or conduct. To our knowledge, the public was not involved in the study design, recruitment or conduct of LSAC study or its CheckPoint module. Parents received a summary health report for their child and themselves at or soon after the assessment visit. They consented to take part knowing that they would not otherwise receive individual results about themselves or their child.

RESULTS

Sample characteristics

The recruitment and retention of participants in the Child Health CheckPoint are described elsewhere.[18] Of the 1874 families who participated in CheckPoint assessment centres, blood serum samples of analysable quality from 1180 children and 1325 parents (figure 1) were sent for NMR quantification of metabolites. The majority of excluded families undertook home visits or attended a regional centre, where blood samples could not be collected (n=385, 20.5%), while some participants declined a blood sample (children, n=150, 8.0%; adults, n=108, 5.8%). Few data were lost due to insufficient volume or poor quality samples at the assessment centre (figure 1). The sample characteristics of parents and children are outlined in table 2. Summary statistics for our main child and parent metabolite measures are presented in supplementary table 1. Supplementary figures 3-7 show density plots comparing the distributions of metabolites for boys, girls and adults.

Table 2: Sample characteristics; values are weighted mean (standard deviation)

Characteristic	All	Male	Female
Child			
n	1152-1180	558-575	594-605
Age, years	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)
Disadvantage Index	1012 (63)	1011 (65)	1014 (61)
Fasting time (hours)	4.2 (1.2)	4.3 (1.3)	4.2 (1.1)
Time of day - blood collection	14.16 (2.0)	14.12 (2.0)	14.20 (2.1)
Processing lag time (hours)	1.16 (0.5)	1.18 (0.5)	1.14 (0.5)
Parent			
n	1272-1325	174-177	1098-1148
Age, years	43.9 (5.6)	46.9 (6.9)	43.4 (5.2)
Fasting time (hours)	3.3 (1.6)	3.6 (2.0)	3.2 (1.5)
Time of day - blood collection	13.10 (2.0)	13.18 (2.1)	13.09 (2.0)
Processing lag time (hours)	1.26 (0.5)	1.31 (0.5)	1.26 (0.5)

Disadvantage Index: Index of Relative Socioeconomic Disadvantage; n: number of participants in cohort with this measure.

Differences in metabolite levels by age (adults compared to children)

Figure 2 shows mean differences in metabolite levels for adults relative to children in standard deviation (SD) units. Most concentrations were higher in adults than children. Values that were similar in adults and children included total lipids in very large HDL lipoprotein subclass particles, acetoacetate, tyrosine and glucose. Levels in children were higher than those of adults for the majority of glycolysis related measures (lactate, pyruvate, citrate and glycerol), the ketone body 3-hydroxybutrate, the amino acid glutamine, many fatty acid ratios and all lipoprotein particle sizes. Supplementary table 2 lists the corresponding estimates in absolute concentration units.

Sex-specific differences in metabolite levels in children and adults

Figure 3 shows differences in mean metabolite levels by sex for children and adults separately in SD units, with estimates in absolute concentration units listed in supplementary table 3 and 4.

In general, sex differences were more pronounced in adulthood, resulting in distinct overall patterns for children and adults. Children generally showed smaller differences by sex than

adults. Of note, sex differences for apolipoproteins and fatty acid measures showed different patterns in children compared to adults.

Girls had lower levels of apolipoprotein-A-1 (ApoA-1) and higher ApoB than boys. In adults, the opposite pattern was observed with females having higher ApoA-1 and lower ApoB than males. In children, some fatty acid concentrations were higher in girls than boys. In contrast, many adult fatty acid measures were higher in males. There was no evidence of a difference in the level of inflammation (GlycA) by sex in children, while in adults, GlycA levels tended to be higher in males than females.

For some metabolites, sex differences in children mirrored (but were smaller in magnitude than) those of adults, particularly for the ketone body acetate and some key amino acids. At both ages, the amino acid glycine was higher in females but the branched-chain amino acids leucine and valine tended to be higher in males.

Parent-child concordance

Figure 4 shows the correlations between metabolite measures for all children with all parents, and for boys and girls with mothers (but not with the 177 fathers, given the small numbers). The corresponding correlation coefficients and partial correlation coefficients are listed in supplementary tables 5 and 6.

Correlations for all parents and all children showed similar patterns to that observed for mother and child by sex. While there was little suggestion of substantial correlation within parent-child dyads for some metabolites (eg glucose, acetate) a positive correlation was found for many metabolite measures irrespective of child sex. For example, positive correlations were observed for isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total serum cholesterol (CC 0.30, 95% CI 0.24 to 0.35) and omega 6 fatty acids (CC 0.28, 95% CI 0.23 to 0.34) in parent-child comparisons. Additional adjustment for factors that potentially influence metabolite levels (age, socioeconomic status, fasting time and processing lag time) had little effect on the degree of correlation in any comparison (supplementary tables 5 and 6).

DISCUSSION

Principal findings

Here we present age and sex differences, describing the distribution of detailed/NMR-based metabolite measures in Australian 11-12 year old children and their parents, and demonstrate that many metabolite measures have high parent-child concordance. In accord with previous studies, we observed major differences in metabolite levels between childhood and adulthood and also differences by sex in both childhood and adulthood. We also observed variability in the magnitude of differences by sex for several metabolites in childhood compared to adulthood and identified a complex interplay of correlations of specific metabolites between parents and their children according to parent-child sex relationships.

Strengths and weaknesses

This is the first major cohort study to report both sex- and cross-generational differences in metabolomic concentrations in mid-childhood to adulthood utilising the NMR platform. Further strengths include the large number of parent-child dyads representing a wide range of parent ages, the national population-based sample and the state-of-the-art measurements. Replication studies exploring sex differences at earlier and later stages of childhood and adolescence would strengthen findings.

An important limitation is that paternal factors were not fully represented, as most parental samples were from mothers (a well-documented problem in longitudinal cohort studies). This also limited sex-specific parental contribution analysis; further studies including more fathers are warranted. Additional limitations are that, without samples from both parents for each child, we could not estimate heritability, and our results might not apply to mid-life adults who are not parents (although we see no good reason why these would differ greatly). The original uptake of just over 50% and subsequent attrition within LSAC and then the CheckPoint has led to a relatively advantaged sample, but nonetheless participants varied widely on key potential confounders (eg disadvantage, age) and this was at least partly offset by application or consideration of survey weights. Given the large number of metabolites and modest sample size, considerable uncertainty remains in any ranking of the various effects across metabolites.

Meaning and implications for clinicians and policymakers

Overall, we found a clear difference in metabolite profile between children and their parents. This was apparent for specific metabolite measures (such as some amino acids) as well as the

distribution of metabolites (such as lipid composition of lipoproteins of different density). Some measures were higher in adults, some similar, while a minority were lower. Previous studies, largely in adults, have identified a range of specific metabolite changes with age, particularly from mid to late adulthood.[26] This includes a general decrease in several amino acid species, which contrasts with our findings from childhood to mid adulthood.[8] Only the amino acid glutamine showed this pattern in our dataset.

Sex-specific differences in children (±0.2 SD) were generally much smaller than in adults (±0.8 SD). Large metabolomic studies using alternative platforms have previously reported reproducible, sex-specific signatures in circulating metabolite profile in adults.[27] [28] This includes differences in amino acid and lipid serum concentrations, potentially influenced by sex-specific effects of genetic polymorphisms on metabolite levels. [28] [29] As in our study, most amino acids have usually been reported to be higher in men than women.[28] [30] For example, in a recent study of 507 metabolic markers in 1756 individuals (903 female and 853 male aged ~60 years), one third of metabolites showed significant sexual dimorphism. These were predominantly related to pathways of steroid metabolism, fatty acids, other lipids, and a large proportion of amino acids.[30] Of particular note, branched chain amino acids (BCAAs) and their related metabolic products were amongst the most differentially represented, with much higher isoleucine, leucine and valine in males. A similar finding of higher leucine and valine was also noted in the Cooperative Health Research in the Region of Augsburg (KORA) follow-ups 3 (F3) and 4 (F4) analysis of >3000 adults,[28] consistent with our observations in adulthood.

In children, we found sex-specific differences for leucine and valine were smaller but in the same direction as adults. Several lines of evidence implicate BCAA metabolism with metabolic risk in humans. For example, three candidate genes for obesity and/or type 2 diabetes mellitus (T2DM) are involved in the BCAA metabolic pathway.[31] In a recent large meta-analysis of metabolomics in diabetes, a >30% higher risk of type 2 diabetes was found per SD increase in isoleucine, leucine, valine or tyrosine, whereas glycine and glutamine were inversely associated with risk.[31] Several clinical studies have also reported that BCAAs positively correlate with insulin resistance, homeostatic model assessment (HOMA) index and levels of haemoglobin A1c (HbA1c), while longitudinal studies have reported that increased blood BCAAs are predictive of future insulin resistance and type 2 diabetes (T2D).[32] It is intriguing to speculate that the higher BCAA in males from early life could contribute to the well-described increasing prevalence of T2D in men. Levels of BCAA are

elevated in females with Polycystic Ovary Syndrome (PCOS), potentially contributing to the associated insulin resistance.[33] However, it remains unclear whether BCAA are on the causal pathway to T2D or result from adverse metabolic health. Our demonstration that the sex-specific differences in BCAA arise early in life offers potential to track their association with sex-specific measures of metabolic health from an early age to help clarify where they lie on the causal pathway.

In accord with previous adult studies[28], we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of ~10,000 adults with cardiovascular disease (CVD), with replication in >53,000 subjects, identified a genetic variant in carbamoyl-phosphate synthase 1 (*CPS1)* (linked to plasma glycine levels) to be strongly associated with a reduced risk of CVD in women ($p=6.3x10^{-5}$) but not men (p=0.95), suggesting a direct link between glycine levels and CVD risk, although whether this is a causal association remains unclear.[34] It will be interesting in the future to explore the link between variants in *CPS1* and circulating glycine levels from early life to adulthood in relation to markers of cardiovascular health in females.

The small sex-differences of HDL cholesterol and ApoA-1 in children compared to adults is consistent with modest differences in children, whereas substantial differences in adulthood have previously been reported.[35] ApoA-1 was more abundant in boys, while ApoB was higher in girls, leading to a higher ApoB/ApoA-1 ratio in girls. The opposite pattern was found in our limited sample of fathers relative to mothers. These data are surprising and differ from a similarly sized study of slightly older European adolescent children (mean age 15 years) that found higher ApoA-1 and ApoB in girls relative to boys.[36] Interestingly, a higher ApoB/ApoA-1 ratio has been strongly linked to increased coronary risk in adults,[37-39] suggesting that the sex-specific differences may alter with increasing age, in keeping with the increased CVD risk in adult males. ApoA-1 is the main protein component of HDL cholesterol[40] thus the differences in trajectories in lipids and HDL cholesterol for boys and girls across childhood that have been reported[41 42] could partially explain this observation.

These are the first data on the mother-child or parent-child correlations of NMR metabolites. Smaller studies have reported positive correlations between parents and children for a limited range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides measured using conventional methods. We found positive correlations between parents and children for the same lipid measures (although measured using NMR) consistent with previously reported findings. One study reported a positive

association between the serum lipid levels of 4 year old children (n=127) and their parents (122 mothers and 118 fathers) [43] while another study of children aged 6-18 (n=255) and their parents (n=179) found that the age of the child influenced the degree of correlation of several lipid measures, with older (10-18 years) children more similar to their parents in terms of triglyceride levels than younger individuals (6-9 years).[12]

Unanswered questions and future research

The temporal and sex specific dynamism of the metabolomics data we describe here offer considerable opportunities for identification of biomarkers of risk for a range of non-communicable diseases early in life, to inform targeted interventions and monitor their efficacy. Combining metabolomics with other 'omics data (such as genetics), as is increasingly reported from large adult studies, offers considerable promise in understanding the causal pathways that link early life exposures, genetics and intermediate phenotypes with later onset chronic disease, and in identifying clinically relevant biomarkers.

In conclusion, we describe the metabolite profile from mid-childhood and adulthood in a population-based sample, together with parent-child concordance and sex-specific differences in children and adults. In this descriptive paper, distinct age- and sex-specific profiles were observed, as well as considerable evidence of correlation between parent and child measures. These data will be informative for investigation of the childhood origins of adult non-communicable diseases and for comparative studies across populations.

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REDCap (Research Electronic Data Capture) tools ENREF 43[44] were used in this study. More information about this software can be found at: www.project-redcap.org.

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COMPETING INTERESTS

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and decision to submit the manuscript for publication. Research at the MCRI is supported by the Victorian Government's Operational Infrastructure Support Program.

CONTRIBUTIONS

DB, RS and JC conceptualised and developed the Metabolomics Checkpoint study. SE and JC undertook all aspects of data analysis. SAC coordinated the acquisition of metabolomics data and provided critical review of this manuscript. MW, the Principal Investigator of the Child Health CheckPoint, planned the analyses and provided critical review of this manuscript. SE and RS drafted the manuscript. PW, MJ, TD, KL, JC, DB provided critical expert advice and critical review of this manuscript.

DATA SHARING STATEMENT

Dataset and technical documents available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au

FIGURE CAPTIONS AND FOOTNOTES

Figure 1: Participant flow chart.

n=number of families, c=number of children, p=number of attending adults,

MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,

LSAC=Longitudinal Study of Australian Children

*Unable to analyse due to insufficient volume or poor quality sample

^Data from 6 non-biological child-parent pairs excluded from concordance analyses

Figure 2: Differences in metabolite levels between children and adults.

Association measures are SD difference in metabolite concentration for adults compared to children. Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood.

Association measures are SD difference in metabolite concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 4: Parent:child correlation for metabolite measures.

Pearson's correlation coefficients for all children with all parents (A); and for boys (blue) with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence intervals. Correlation coefficients with associated 95% confidence intervals are listed in

supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

SUPPLEMENTARY DOCUMENTS

Supplementary figure 1: Correlation of NMR measures in children.

Heatmap showing the correlation between metabolite measures in children. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 2: Correlation of NMR metabolite measures in parents.

Heatmap showing the correlation between metabolite measures in parents. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each cholesterol and apolipoprotein measure.

Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each fatty acid and fatty acid ratio measure.

Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass particles.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for total lipids within each of the 14 lipoprotein subclass particles.

Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for lipoprotein particle sizes and triglyceride measures.

Supplementary figure 7: Density plots for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.

Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.

Supplementary table 2: Mean difference in metabolite levels in adults compared to children in absolute concentration units.

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

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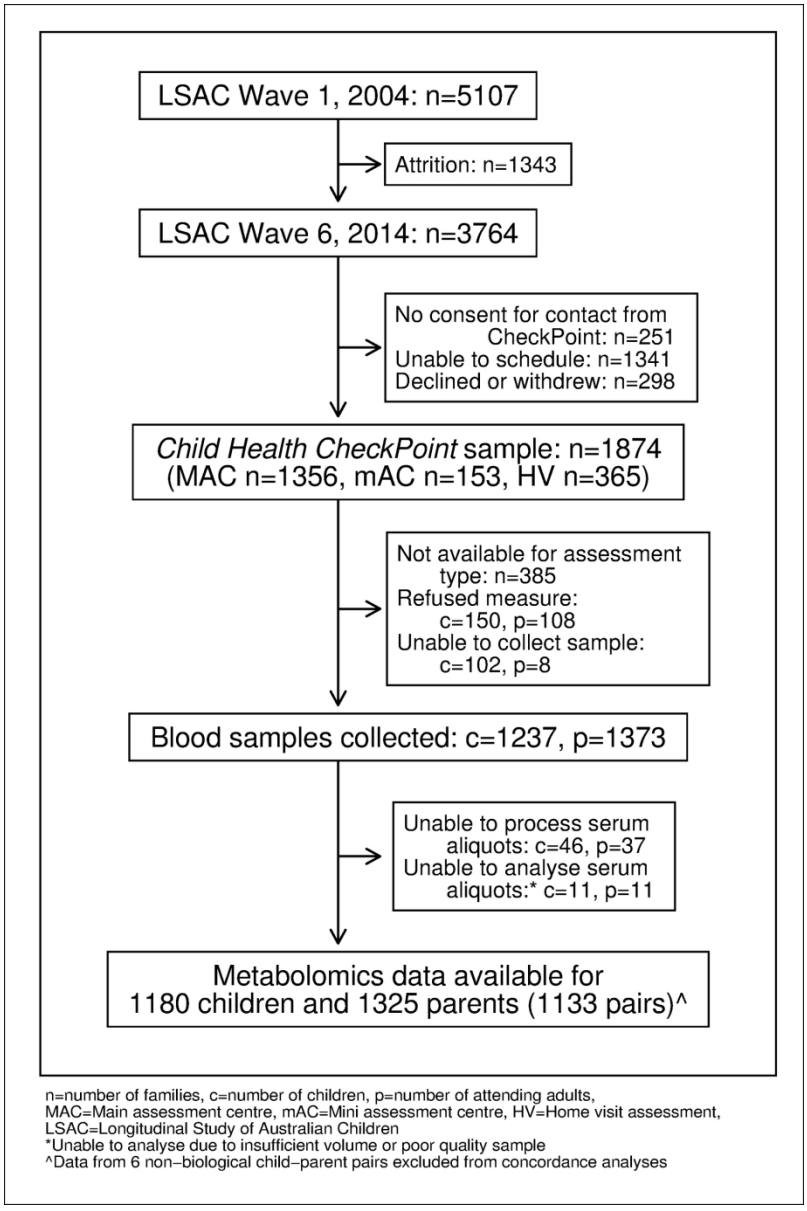

Figure 1: Participant flow chart.
n=number of families, c=number of children, p=number of attending adults,
MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,
LSAC=Longitudinal Study of Australian Children
*Unable to analyse due to insufficient volume or poor quality sample
^Data from 6 non-biological child-parent pairs excluded from concordance analyses

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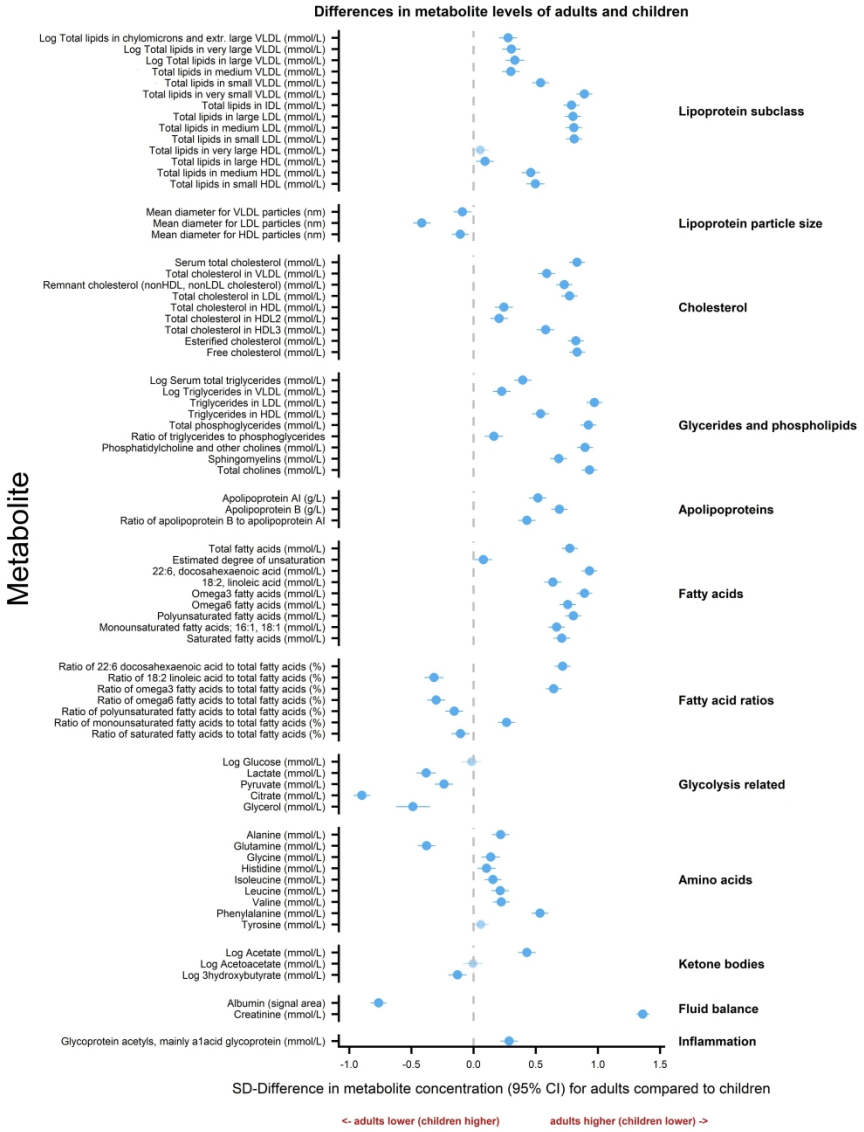

Figure 2: Differences in metabolite levels between children and adults.
Association measures are SD difference in metabolite concentration for adults compared to children. Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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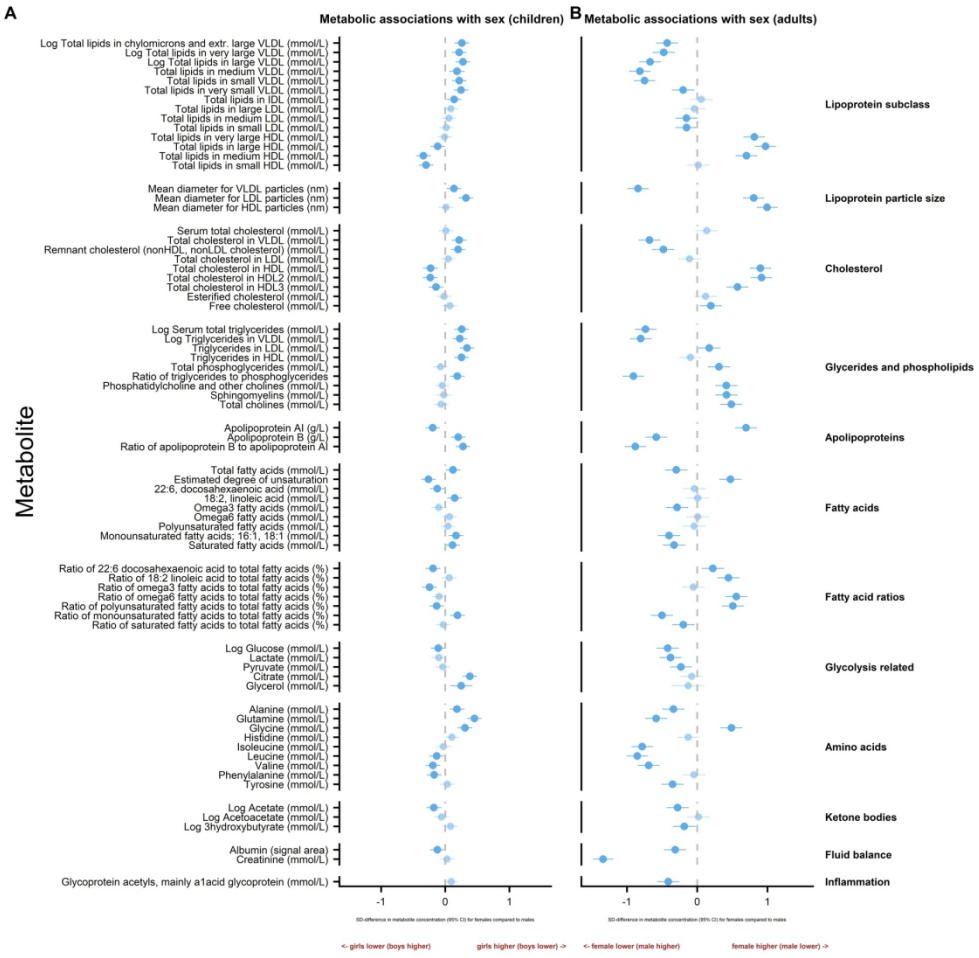

Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood.
Association measures are SD difference in metabolite concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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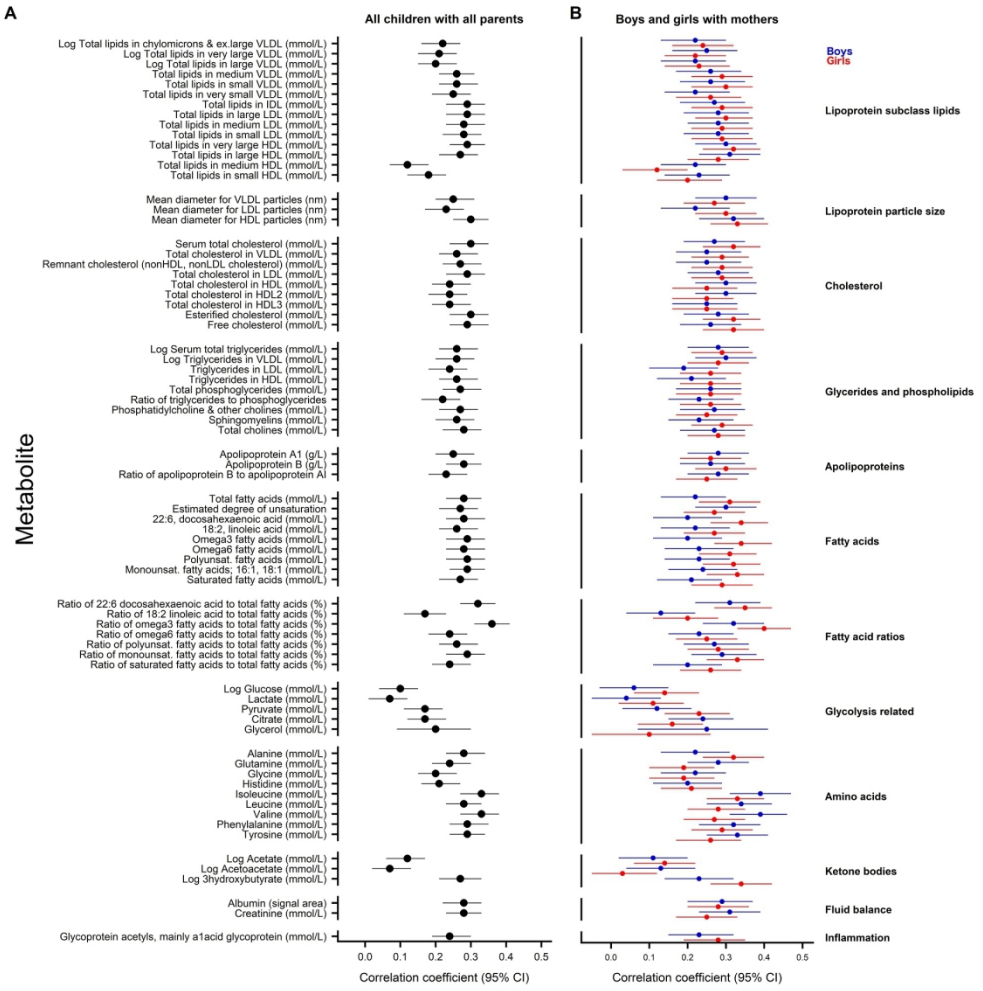

Figure 4: Parent:child correlation for metabolite measures. Pearson's correlation coefficients for all children with all parents (A); and for boys (blue) with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence intervals. Correlation coefficients with associated 95% confidence intervals are listed in supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.*

Metabolic subgroup	Children									Adults								
	Male			Female			All			Male			Female			All		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	575	0.005	4.826	605	0.011	3.260	1180	0.007	4.054	177	0.040	2.101	1148	0.013	2.822	1325	0.015	2.777
Total lipids in very large VLDL (mmol/L)*	575	0.006	6.246	605	0.014	4.800	1180	0.010	5.521	177	0.087	2.419	1148	0.022	3.458	1325	0.027	3.398
Total lipids in large VLDL (mmol/L)*	575	0.059	3.945	605	0.121	2.488	1180	0.085	3.234	177	0.423	1.287	1148	0.161	1.666	1325	0.182	1.665
Total lipids in medium VLDL (mmol/L)	575	0.441	0.270	605	0.478	0.272	1180	0.460	0.271	177	0.959	0.630	1148	0.548	0.366	1325	0.602	0.432
Total lipids in small VLDL (mmol/L)	575	0.381	0.153	605	0.405	0.146	1180	0.393	0.149	177	0.690	0.279	1148	0.494	0.216	1325	0.520	0.234
Total lipids in very small VLDL (mmol/L)	575	0.325	0.070	605	0.342	0.076	1180	0.334	0.073	177	0.451	0.111	1148	0.426	0.110	1325	0.429	0.110
Total lipids in IDL (mmol/L)	575	0.804	0.176	605	0.834	0.183	1180	0.819	0.180	177	0.985	0.261	1148	0.999	0.240	1325	0.997	0.242
Total lipids in large LDL (mmol/L)	575	0.917	0.220	605	0.941	0.228	1180	0.929	0.224	177	1.162	0.327	1148	1.155	0.298	1325	1.156	0.301
Total lipids in medium LDL (mmol/L)	575	0.511	0.136	605	0.519	0.140	1180	0.515	0.138	177	0.676	0.214	1148	0.655	0.185	1325	0.658	0.189
Total lipids in small LDL (mmol/L)	575	0.338	0.083	605	0.340	0.087	1180	0.339	0.085	177	0.439	0.135	1148	0.425	0.114	1325	0.427	0.117
Total lipids in very large HDL (mmol/L)	575	0.482	0.196	605	0.495	0.184	1180	0.488	0.189	177	0.320	0.189	1148	0.497	0.229	1325	0.474	0.232
Total lipids in large HDL (mmol/L)	575	0.874	0.291	605	0.859	0.275	1180	0.866	0.282	177	0.509	0.335	1148	0.900	0.382	1325	0.849	0.399
Total lipids in medium HDL (mmol/L)	575	0.917	0.127	605	0.871	0.126	1180	0.894	0.128	177	0.828	0.241	1148	0.971	0.175	1325	0.952	0.191
Total lipids in small HDL (mmol/L)	575	1.039	0.103	605	0.997	0.115	1180	1.018	0.111	177	1.055	0.254	1148	1.085	0.138	1325	1.081	0.157
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	575	37.063	1.633	605	37.238	1.557	1180	37.152	1.591	177	38.527	1.737	1148	36.943	1.599	1325	37.152	1.701
Mean diameter for LDL particles (nm)	575	23.587	0.103	605	23.628	0.109	1180	23.608	0.107	177	23.487	0.093	1148	23.573	0.100	1325	23.562	0.104
Mean diameter for HDL particles (nm)	575	10.081	0.233	605	10.102	0.221	1180	10.092	0.226	177	9.798	0.244	1148	10.068	0.262	1325	10.032	0.275
Cholesterol																		
Serum total cholesterol (mmol/L)	575	3.576	0.620	605	3.596	0.643	1180	3.586	0.629	177	4.161	0.885	1148	4.234	0.828	1325	4.225	0.835
Total cholesterol in VLDL (mmol/L)	575	0.438	0.188	605	0.472	0.189	1180	0.455	0.189	177	0.826	0.395	1148	0.592	0.265	1325	0.623	0.295
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	575	0.944	0.269	605	0.994	0.271	1180	0.970	0.270	177	1.452	0.472	1148	1.219	0.383	1325	1.250	0.402
Total cholesterol in LDL (mmol/L)	575	1.130	0.330	605	1.149	0.341	1180	1.139	0.334	177	1.492	0.498	1148	1.460	0.442	1325	1.464	0.449
Total cholesterol in HDL (mmol/L)	575	1.503	0.274	605	1.453	0.266	1180	1.477	0.270	177	1.217	0.350	1148	1.556	0.363	1325	1.511	0.379
Total cholesterol in HDL2 (mmol/L)	575	1.035	0.254	605	0.988	0.246	1180	1.011	0.250	177	0.751	0.327	1148	1.072	0.335	1325	1.030	0.351
Total cholesterol in HDL3 (mmol/L)	575	0.468	0.024	605	0.466	0.024	1180	0.467	0.024	177	0.466	0.035	1148	0.483	0.033	1325	0.481	0.034
Esterified cholesterol (mmol/L)	572	2.516	0.447	604	2.517	0.460	1176	2.517	0.452	176	2.941	0.636	1147	2.975	0.593	1323	2.971	0.597
Free cholesterol (mmol/L)	572	1.062	0.179	604	1.079	0.186	1176	1.070	0.182	176	1.211	0.273	1147	1.260	0.239	1323	1.253	0.244
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	575	0.918	0.709	605	1.005	0.681	1180	0.962	0.696	177	1.686	0.809	1148	1.129	0.755	1325	1.190	0.782
Triglycerides in VLDL (mmol/L)*	575	0.582	0.885	605	0.648	0.830	1180	0.615	0.858	177	1.249	0.927	1148	0.694	0.945	1325	0.750	0.972
Triglycerides in LDL (mmol/L)	575	0.113	0.024	605	0.123	0.027	1180	0.118	0.026	177	0.153	0.040	1148	0.158	0.044	1325	0.157	0.043
Triglycerides in HDL (mmol/L)	575	0.129	0.030	605	0.136	0.030	1180	0.133	0.030	177	0.161	0.047	1148	0.151	0.040	1325	0.153	0.041
Total phosphoglycerides (mmol/L)	572	1.632	0.240	604	1.620	0.261	1176	1.626	0.250	176	1.865	0.354	1147	1.926	0.340	1323	1.918	0.342
Ratio of triglycerides to phosphoglycerides	572	0.526	0.253	604	0.569	0.277	1176	0.548	0.265	176	0.946	0.587	1147	0.580	0.279	1323	0.628	0.355
Phosphatidylcholine & other cholines (mmol/L)	572	1.691	0.240	604	1.687	0.267	1176	1.689	0.253	175	1.877	0.320	1147	1.978	0.336	1322	1.965	0.335
Sphingomyelins (mmol/L)	572	0.348	0.061	604	0.349	0.064	1176	0.348	0.062	175	0.370	0.070	1147	0.397	0.078	1322	0.394	0.077
Total cholines (mmol/L)	572	2.005	0.256	604	1.997	0.264	1176	2.001	0.259	175	2.185	0.334	1147	2.317	0.351	1322	2.299	0.351
Apolipoproteins																		
Apolipoprotein A1 (g/L)	575	1.509	0.159	605	1.484	0.151	1180	1.497	0.155	177	1.461	0.178	1148	1.589	0.205	1325	1.572	0.206
Apolipoprotein B (g/L)	575	0.682	0.135	604	0.706	0.136	1179	0.694	0.135	177	0.955	0.245	1148	0.812	0.196	1325	0.831	0.208
Ratio of apolipoprotein B to apolipoprotein A	575	0.455	0.097	604	0.479	0.098	1179	0.467	0.098	177	0.660	0.178	1148	0.518	0.136	1325	0.537	0.150
Fatty acids																		
Total fatty acids (mmol/L)	570	9.215	1.697	604	9.370	1.730	1174	9.294	1.709	173	11.850	2.723	1145	10.917	2.392	1318	11.034	2.446
Estimated degree of unsaturation	570	1.212	0.056	604	1.196	0.065	1174	1.204	0.061	173	1.179	0.070	1145	1.212	0.066	1318	1.208	0.068
22:6, docosahexaenoic acid (mmol/L)	570	0.078	0.028	604	0.074	0.029	1174	0.076	0.028	173	0.118	0.051	1145	0.111	0.039	1318	0.112	0.041
18:2, linoleic acid (mmol/L)	570	2.539	0.456	604	2.592	0.464	1174	2.566	0.459	173	2.919	0.567	1145	2.880	0.584	1318	2.885	0.580
Omega3 fatty acids (mmol/L)	570	0.309	0.086	604	0.296	0.083	1174	0.302	0.085	173	0.451	0.160	1145	0.400	0.117	1318	0.406	0.124
Omega6 fatty acids (mmol/L)	570	3.077	0.493	604	3.094	0.492	1174	3.086	0.491	173	3.553	0.648	1145	3.507	0.627	1318	3.513	0.628
Polyunsat. fatty acids (mmol/L)	570	3.386	0.565	604	3.390	0.561	1174	3.388	0.560	173	4.004	0.775	1145	3.907	0.721	1318	3.919	0.726
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	570	2.500	0.632	604	2.587	0.661	1174	2.544	0.646	173	3.520	1.058	1145	3.080	0.917	1318	3.135	0.943
Saturated fatty acids (mmol/L)	570	3.328	0.642	604	3.393	0.682	1174	3.362	0.661	173	4.325	1.088	1145	3.930	0.932	1318	3.979	0.958

Fatty acid ratios																			
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	570	0.841	0.239	604	0.790	0.269	1174	0.815	0.255	173	0.984	0.315	1145	1.023	0.284	1318	1.018	0.287
Ratio of 18:2 linoleic acid to total fatty acids (%)	570	27.741	3.127	604	27.867	3.274	1174	27.805	3.191	173	25.038	3.570	1145	26.656	3.417	1318	26.453	3.467
Ratio of omega3 fatty acids to total fatty acids (%)	570	3.329	0.579	604	3.150	0.600	1174	3.238	0.594	173	3.763	0.799	1145	3.659	0.697	1318	3.672	0.709
Ratio of omega6 fatty acids to total fatty acids (%)	570	33.650	3.047	604	33.308	3.228	1174	33.475	3.133	173	30.468	3.757	1145	32.502	3.311	1318	32.247	3.424
Ratio of polyunsat. fatty acids to total fatty acids (%)	570	36.979	3.235	604	36.459	3.514	1174	36.713	3.377	173	34.231	3.878	1145	36.161	3.588	1318	35.918	3.669
Ratio of monounsat. fatty acids to total fatty acids (%)	570	26.911	2.569	604	27.375	2.678	1174	27.148	2.625	173	29.366	2.968	1145	27.889	2.842	1318	28.075	2.891
Ratio of saturated fatty acids to total fatty acids (%)	570	36.110	1.675	604	36.167	1.802	1174	36.139	1.734	173	36.402	2.027	1145	35.950	2.032	1318	36.007	2.031
Glycolysis related																			
Glucose (mmol/L)*	574	1.350	0.115	605	1.336	0.105	1179	1.342	0.11	176	1.415	0.205	1148	1.334	0.176	1324	1.344	0.182
Lactate (mmol/L)	575	1.770	0.459	605	1.718	0.434	1180	1.743	0.446	177	1.696	0.472	1148	1.562	0.480	1325	1.580	0.480
Pyruvate (mmol/L)	574	0.100	0.024	605	0.098	0.023	1179	0.099	0.023	177	0.101	0.031	1147	0.093	0.034	1324	0.094	0.033
Citrate (mmol/L)	575	0.125	0.017	604	0.131	0.018	1179	0.128	0.018	177	0.110	0.016	1148	0.111	0.016	1325	0.111	0.016
Glycerol (mmol/L)#	240	0.078	0.021	283	0.083	0.022	523	0.081	0.021	84	0.073	0.021	470	0.071	0.023	554	0.071	0.023
Amino acids																			
Alanine (mmol/L)	575	0.387	0.061	605	0.396	0.060	1180	0.391	0.060	176	0.423	0.065	1147	0.399	0.060	1323	0.402	0.061
Glutamine (mmol/L)	575	0.474	0.050	605	0.497	0.051	1180	0.485	0.051	177	0.490	0.063	1148	0.456	0.066	1325	0.461	0.066
Glycine (mmol/L)	574	0.261	0.032	604	0.270	0.034	1178	0.265	0.033	176	0.243	0.029	1148	0.274	0.061	1324	0.270	0.059
Histidine (mmol/L)	574	0.065	0.009	605	0.065	0.008	1179	0.065	0.008	176	0.066	0.008	1148	0.065	0.009	1324	0.065	0.009
Isoleucine (mmol/L)	574	0.054	0.019	605	0.053	0.019	1179	0.054	0.019	174	0.072	0.021	1146	0.055	0.020	1320	0.057	0.021
Leucine (mmol/L)	575	0.073	0.019	605	0.071	0.019	1180	0.072	0.019	177	0.097	0.029	1148	0.074	0.021	1325	0.077	0.023
Valine (mmol/L)	575	0.162	0.037	604	0.156	0.035	1179	0.159	0.036	177	0.192	0.036	1147	0.162	0.042	1324	0.166	0.042
Phenylalanine (mmol/L)	575	0.068	0.009	605	0.066	0.009	1180	0.067	0.009	177	0.073	0.011	1148	0.072	0.011	1325	0.073	0.011
Tyrosine (mmol/L)	574	0.054	0.014	605	0.055	0.014	1179	0.055	0.014	176	0.060	0.013	1148	0.054	0.015	1324	0.055	0.015
Ketone bodies																			
Acetate (mmol/L)*	575	0.031	0.423	605	0.030	0.404	1180	0.030	0.413	177	0.037	0.655	1146	0.033	0.600	1323	0.034	0.609
Acetoacetate (mmol/L)*	575	0.025	1.310	605	0.023	1.429	1180	0.024	1.367	177	0.023	2.116	1147	0.024	1.278	1324	0.024	1.403
3hydroxybutyrate (mmol/L)*#	555	0.100	0.786	580	0.103	0.826	1135	0.101	0.805	170	0.104	0.669	1098	0.096	0.781	1268	0.097	0.769
Fluid balance																			
Albumin (signal area)	574	0.093	0.005	605	0.092	0.005	1179	0.093	0.005	177	0.090	0.005	1148	0.088	0.005	1325	0.089	0.005
Creatinine (mmol/L)	570	0.040	0.006	600	0.040	0.006	1170	0.040	0.006	173	0.066	0.015	1139	0.054	0.009	1312	0.055	0.010
Inflammation																			
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	575	1.170	0.191	605	1.173	0.186	1180	1.172	0.188	177	1.375	0.366	1148	1.242	0.233	1325	1.260	0.258

* geometric mean [relative SD] when skewed variable

Note: The presence of ethanol in a sample can affect quantification of glycerol and on some occasions 3hydroxybutyrate. Ethanol can be introduced in to a sample from disinfectants used during blood collection/processing of sample.

Supplementary table 2: Mean difference in metabolite levels in adults compared to children in absolute concentration unit

Metabolic subgroup	Differences by age (Adults - Child)				
	Estimate	95% CI	P-value	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.704	(0.519, 0.890)	<0.001	<0.001	2.534
Total lipids in very large VLDL (mmol/L)*	0.922	(0.700, 1.145)	<0.001	<0.001	3.031
Total lipids in large VLDL (mmol/L)*	0.648	(0.502, 0.795)	<0.001	<0.001	1.950
Total lipids in medium VLDL (mmol/L)	0.105	(0.080, 0.129)	<0.001	<0.001	0.348
Total lipids in small VLDL (mmol/L)	0.107	(0.094, 0.121)	<0.001	<0.001	0.199
Total lipids in very small VLDL (mmol/L)	0.093	(0.086, 0.099)	<0.001	<0.001	0.104
Total lipids in IDL (mmol/L)	0.181	(0.166, 0.196)	<0.001	<0.001	0.230
Total lipids in large LDL (mmol/L)	0.229	(0.211, 0.247)	<0.001	<0.001	0.286
Total lipids in medium LDL (mmol/L)	0.144	(0.132, 0.155)	<0.001	<0.001	0.178
Total lipids in small LDL (mmol/L)	0.089	(0.082, 0.096)	<0.001	<0.001	0.110
Total lipids in very large HDL (mmol/L)	0.012	(-0.003, 0.027)	0.128	0.132	0.217
Total lipids in large HDL (mmol/L)	0.032	(0.007, 0.057)	0.011	0.012	0.353
Total lipids in medium HDL (mmol/L)	0.076	(0.064, 0.089)	<0.001	<0.001	0.166
Total lipids in small HDL (mmol/L)	0.068	(0.058, 0.078)	<0.001	<0.001	0.137
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	-0.147	(-0.263, -0.031)	0.013	0.014	1.633
Mean diameter for LDL particles (nm)	-0.044	(-0.052, -0.037)	<0.001	<0.001	0.106
Mean diameter for HDL particles (nm)	-0.027	(-0.045, -0.010)	0.002	0.003	0.256
Cholesterol					
Serum total cholesterol (mmol/L)	0.670	(0.619, 0.721)	<0.001	<0.001	0.805
Total cholesterol in VLDL (mmol/L)	0.146	(0.129, 0.163)	<0.001	<0.001	0.249
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.261	(0.237, 0.284)	<0.001	<0.001	0.357
Total cholesterol in LDL (mmol/L)	0.327	(0.300, 0.354)	<0.001	<0.001	0.424
Total cholesterol in HDL (mmol/L)	0.082	(0.058, 0.106)	<0.001	<0.001	0.337
Total cholesterol in HDL2 (mmol/L)	0.064	(0.042, 0.086)	<0.001	<0.001	0.311
Total cholesterol in HDL3 (mmol/L)	0.018	(0.016, 0.020)	<0.001	<0.001	0.031
Esterified cholesterol (mmol/L)	0.474	(0.437, 0.511)	<0.001	<0.001	0.576
Free cholesterol (mmol/L)	0.195	(0.180, 0.210)	<0.001	<0.001	0.234
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	0.176	(0.145, 0.206)	<0.001	<0.001	0.443
Triglycerides in VLDL (mmol/L)*	0.140	(0.096, 0.183)	<0.001	<0.001	0.615
Triglycerides in LDL (mmol/L)	0.040	(0.038, 0.043)	<0.001	<0.001	0.042
Triglycerides in HDL (mmol/L)	0.020	(0.017, 0.022)	<0.001	<0.001	0.037
Total phosphoglycerides (mmol/L)	0.311	(0.290, 0.332)	<0.001	<0.001	0.337
Ratio of triglycerides to phosphoglycerides	0.049	(0.027, 0.071)	<0.001	<0.001	0.299
Phosphatidylcholine & other cholines (mmol/L)	0.295	(0.274, 0.316)	<0.001	<0.001	0.329
Sphingomyelins (mmol/L)	0.052	(0.047, 0.057)	<0.001	<0.001	0.075
Total cholines (mmol/L)	0.323	(0.302, 0.345)	<0.001	<0.001	0.347
Apolipoproteins					
Apolipoprotein A1 (g/L)	0.099	(0.086, 0.112)	<0.001	<0.001	0.191
Apolipoprotein B (g/L)	0.125	(0.113, 0.137)	<0.001	<0.001	0.182
Ratio of apolipoprotein B to apolipoprotein AI	0.055	(0.046, 0.064)	<0.001	<0.001	0.127
Fatty acids					
Total fatty acids (mmol/L)	1.738	(1.592, 1.885)	<0.001	<0.001	2.245
Estimated degree of unsaturation	0.005	(0.000, 0.009)	0.030	0.031	0.063
22:6, docosahexaenoic acid (mmol/L)	0.037	(0.035, 0.040)	<0.001	<0.001	0.040
18:2, linoleic acid (mmol/L)	0.347	(0.310, 0.384)	<0.001	<0.001	0.545
Omega3 fatty acids (mmol/L)	0.105	(0.098, 0.113)	<0.001	<0.001	0.118
Omega6 fatty acids (mmol/L)	0.453	(0.414, 0.492)	<0.001	<0.001	0.597
Polyunsat. fatty acids (mmol/L)	0.558	(0.513, 0.603)	<0.001	<0.001	0.695
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.568	(0.512, 0.625)	<0.001	<0.001	0.850
Saturated fatty acids (mmol/L)	0.612	(0.554, 0.669)	<0.001	<0.001	0.862

Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.209	(0.190, 0.228)	<0.001	<0.001	0.292
Ratio of 18:2 linoleic acid to total fatty acids (%)	-1.079	(-1.331, -0.827)	<0.001	<0.001	3.387
Ratio of omega3 fatty acids to total fatty acids (%)	0.446	(0.403, 0.490)	<0.001	<0.001	0.693
Ratio of omega6 fatty acids to total fatty acids (%)	-0.992	(-1.227, -0.757)	<0.001	<0.001	3.296
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.546	(-0.794, -0.298)	<0.001	<0.001	3.507
Ratio of monounsat. fatty acids to total fatty acids (%)	0.741	(0.547, 0.934)	<0.001	<0.001	2.797
Ratio of saturated fatty acids to total fatty acids (%)	-0.195	(-0.328, -0.062)	0.004	0.005	1.863
Glycolysis related					
Glucose (mmol/L)*	-0.002	(-0.014, 0.009)	0.700	0.709	0.147
Lactate (mmol/L)	-0.180	(-0.215, -0.144)	<0.001	<0.001	0.471
Pyruvate (mmol/L)	-0.007	(-0.009, -0.005)	<0.001	<0.001	0.029
Citrate (mmol/L)	-0.017	(-0.018, -0.016)	<0.001	<0.001	0.019
Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	<0.001	<0.001	0.023
Amino acids					
Alanine (mmol/L)	0.013	(0.009, 0.017)	<0.001	<0.001	0.060
Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	<0.001	<0.001	0.060
Glycine (mmol/L)	0.007	(0.003, 0.010)	<0.001	<0.001	0.049
Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	0.006	0.009
Isoleucine (mmol/L)	0.003	(0.002, 0.004)	<0.001	<0.001	0.019
Leucine (mmol/L)	0.004	(0.003, 0.006)	<0.001	<0.001	0.021
Valine (mmol/L)	0.009	(0.006, 0.011)	<0.001	<0.001	0.039
Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	<0.001	<0.001	0.010
Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	0.105	0.014
Ketone bodies					
Acetate (mmol/L)*	0.101	(0.084, 0.117)	<0.001	<0.001	0.235
Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	0.922	1.022
3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	0.001	0.493
Fluid balance					
Albumin (signal area)	-0.004	(-0.004, -0.004)	<0.001	<0.001	0.005
Creatinine (mmol/L)	0.016	(0.015, 0.017)	<0.001	0.001	0.012
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.062	(0.047, 0.078)	<0.001	<0.001	0.217

* Metabolite has been log transformed

^ Benjamini-Hochberg adjusted p-value

Associations in Figure 2 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor. Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Metabolic subgroup	Differences for children (Female - Male)				
	Estimate	95% CI	pvalue	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.737	(0.414, 1.059)	<0.001	<0.001	2.845
Total lipids in very large VLDL (mmol/L)*	0.744	(0.355, 1.134)	<0.001	0.001	3.428
Total lipids in large VLDL (mmol/L)*	0.663	(0.390, 0.936)	<0.001	<0.001	2.411
Total lipids in medium VLDL (mmol/L)	0.049	(0.018, 0.080)	0.002	0.004	0.269
Total lipids in small VLDL (mmol/L)	0.032	(0.015, 0.048)	<0.001	0.001	0.146
Total lipids in very small VLDL (mmol/L)	0.018	(0.010, 0.027)	<0.001	<0.001	0.074
Total lipids in IDL (mmol/L)	0.025	(0.004, 0.046)	0.017	0.035	0.182
Total lipids in large LDL (mmol/L)	0.020	(-0.006, 0.046)	0.132	0.187	0.227
Total lipids in medium LDL (mmol/L)	0.008	(-0.008, 0.024)	0.338	0.416	0.139
Total lipids in small LDL (mmol/L)	0.001	(-0.008, 0.011)	0.788	0.822	0.086
Total lipids in very large HDL (mmol/L)	-0.002	(-0.023, 0.020)	0.882	0.882	0.190
Total lipids in large HDL (mmol/L)	-0.033	(-0.066, -0.001)	0.044	0.074	0.283
Total lipids in medium HDL (mmol/L)	-0.045	(-0.059, -0.030)	<0.001	<0.001	0.131
Total lipids in small HDL (mmol/L)	-0.035	(-0.048, -0.022)	<0.001	<0.001	0.116
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	0.215	(0.035, 0.395)	0.019	0.038	1.580
Mean diameter for LDL particles (nm)	0.035	(0.023, 0.047)	<0.001	0.000	0.108
Mean diameter for HDL particles (nm)	0.003	(-0.023, 0.028)	0.847	0.870	0.226
Cholesterol					
Serum total cholesterol (mmol/L)	0.007	(-0.066, 0.079)	0.857	0.869	0.634
Total cholesterol in VLDL (mmol/L)	0.040	(0.019, 0.061)	<0.001	0.001	0.184
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.053	(0.023, 0.083)	0.001	0.002	0.265
Total cholesterol in LDL (mmol/L)	0.016	(-0.023, 0.054)	0.427	0.518	0.336
Total cholesterol in HDL (mmol/L)	-0.062	(-0.093, -0.031)	<0.001	<0.001	0.273
Total cholesterol in HDL2 (mmol/L)	-0.059	(-0.087, -0.030)	<0.001	<0.001	0.253
Total cholesterol in HDL3 (mmol/L)	-0.003	(-0.006, -0.001)	0.013	0.027	0.024
Esterified cholesterol (mmol/L)	-0.008	(-0.060, 0.044)	0.755	0.798	0.455
Free cholesterol (mmol/L)	0.013	(-0.008, 0.034)	0.211	0.284	0.184
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	0.101	(0.056, 0.145)	<0.001	<0.001	0.390
Triglycerides in VLDL (mmol/L)*	0.125	(0.062, 0.187)	<0.001	<0.001	0.551
Triglycerides in LDL (mmol/L)	0.009	(0.006, 0.012)	<0.001	<0.001	0.026
Triglycerides in HDL (mmol/L)	0.007	(0.004, 0.011)	<0.001	<0.001	0.029
Total phosphoglycerides (mmol/L)	-0.018	(-0.047, 0.010)	0.206	0.282	0.249
Ratio of triglycerides to phosphoglycerides	0.051	(0.020, 0.083)	0.001	0.003	0.274
Phosphatidylcholine & other cholines (mmol/L)	-0.011	(-0.040, 0.018)	0.447	0.534	0.251
Sphingomyelins (mmol/L)	-0.001	(-0.009, 0.006)	0.706	0.757	0.063
Total cholines (mmol/L)	-0.017	(-0.046, 0.013)	0.268	0.354	0.257
Apolipoproteins					
Apolipoprotein A1 (g/L)	-0.030	(-0.048, -0.013)	0.001	0.002	0.155
Apolipoprotein B (g/L)	0.027	(0.012, 0.042)	0.001	0.002	0.133
Ratio of apolipoprotein B to apolipoprotein A	0.027	(0.016, 0.038)	<0.001	<0.001	0.098
Fatty acids					
Total fatty acids (mmol/L)	0.200	(0.011, 0.389)	0.038	0.065	1.650
Estimated degree of unsaturation	-0.016	(-0.022, -0.009)	<0.001	<0.001	0.060
22:6, docosahexaenoic acid (mmol/L)	-0.004	(-0.007, -0.000)	0.033	0.059	0.028
18:2, linoleic acid (mmol/L)	0.068	(0.015, 0.120)	0.011	0.024	0.458
Omega3 fatty acids (mmol/L)	-0.008	(-0.018, 0.001)	0.083	0.128	0.084
Omega6 fatty acids (mmol/L)	0.031	(-0.024, 0.087)	0.271	0.352	0.485
Polyunsat. fatty acids (mmol/L)	0.023	(-0.041, 0.086)	0.483	0.567	0.553
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.105	(0.034, 0.176)	0.004	0.009	0.623
Saturated fatty acids (mmol/L)	0.073	(-0.000, 0.146)	0.052	0.085	0.638
Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	-0.049	(-0.078, -0.019)	0.001	0.003	0.255
Ratio of 18:2 linoleic acid to total fatty acids (%)	0.197	(-0.173, 0.567)	0.297	0.379	3.233
Ratio of omega3 fatty acids to total fatty acids (%)	-0.145	(-0.213, -0.078)	<0.001	0.000	0.593
Ratio of omega6 fatty acids to total fatty acids (%)	-0.302	(-0.658, 0.054)	0.096	0.142	3.109
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.447	(-0.830, -0.065)	0.022	0.042	3.346
Ratio of monounsat. fatty acids to total fatty acids (%)	0.499	(0.202, 0.796)	0.001	0.003	2.606
Ratio of saturated fatty acids to total fatty acids (%)	-0.051	(-0.251, 0.148)	0.614	0.689	1.743
Glycolysis related					
Glucose (mmol/L)*	-0.013	(-0.026, 0.001)	0.061	0.098	0.118
Lactate (mmol/L)	-0.045	(-0.097, 0.007)	0.088	0.133	0.456
Pyruvate (mmol/L)	-0.001	(-0.004, 0.002)	0.524	0.606	0.024
Citrate (mmol/L)	0.007	(0.005, 0.009)	<0.001	<0.001	0.018
Glycerol (mmol/L)	0.006	(0.002, 0.010)	0.004	0.009	0.023
Amino acids					
Alanine (mmol/L)	0.011	(0.004, 0.017)	0.002	0.004	0.058
Glutamine (mmol/L)	0.023	(0.018, 0.029)	<0.001	<0.001	0.051
Glycine (mmol/L)	0.010	(0.006, 0.014)	<0.001	<0.001	0.032
Histidine (mmol/L)	0.001	(-0.000, 0.002)	0.075	0.118	0.008
Isoleucine (mmol/L)	0.000	(-0.003, 0.002)	0.637	0.693	0.018

Leucine (mmol/L)	-0.002	(-0.005, -0.000)	0.022	0.041	0.018
Valine (mmol/L)	-0.007	(-0.011, -0.003)	0.001	0.003	0.036
Phenylalanine (mmol/L)	-0.002	(-0.003, -0.001)	0.003	0.007	0.009
Tyrosine (mmol/L)	0.000	(-0.001, 0.002)	0.582	0.663	0.014
Ketone bodies					
Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.005	0.166
Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.393	0.992
3hydroxybutyrate (mmol/L)*	0.041	(-0.019, 0.101)	0.178	0.248	0.513
Fluid balance					
Albumin (signal area)	-0.001	(-0.001, -0.000)	0.037	0.064	0.005
Creatinine (mmol/L)	0.000	(-0.001, 0.001)	0.624	0.690	0.007
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.017	(-0.004, 0.038)	0.104	0.151	0.183

* Metabolite has been log transformed
^ Benjamini-Hochberg adjusted p-value
# Associations for children in Figure 3 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure in children) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.
Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Metabolic subgroup	Differences for adults (Female - Male)				
	Estimate	95% CI	pvalue	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	-0.930	(-1.271, -0.589)	<0.001	<0.001	2.173
Total lipids in very large VLDL (mmol/L)*	-1.217	(-1.617, -0.818)	<0.001	<0.001	2.555
Total lipids in large VLDL (mmol/L)*	-0.900	(-1.107, -0.693)	<0.001	<0.001	1.343
Total lipids in medium VLDL (mmol/L)	-0.325	(-0.385, -0.264)	<0.001	<0.001	0.398
Total lipids in small VLDL (mmol/L)	-0.167	(-0.201, -0.133)	<0.001	<0.001	0.223
Total lipids in very small VLDL (mmol/L)	-0.022	(-0.039, -0.005)	0.013	0.018	0.107
Total lipids in IDL (mmol/L)	0.014	(-0.023, 0.051)	0.465	0.530	0.236
Total lipids in large LDL (mmol/L)	-0.011	(-0.058, 0.035)	0.634	0.671	0.293
Total lipids in medium LDL (mmol/L)	-0.028	(-0.057, 0.001)	0.058	0.076	0.182
Total lipids in small LDL (mmol/L)	-0.017	(-0.035, 0.001)	0.061	0.079	0.113
Total lipids in very large HDL (mmol/L)	0.195	(0.158, 0.231)	<0.001	<0.001	0.239
Total lipids in large HDL (mmol/L)	0.395	(0.334, 0.455)	<0.001	<0.001	0.405
Total lipids in medium HDL (mmol/L)	0.129	(0.101, 0.158)	<0.001	<0.001	0.184
Total lipids in small HDL (mmol/L)	0.002	(-0.021, 0.025)	0.850	0.874	0.145
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	-1.414	(-1.669, -1.159)	<0.001	<0.001	1.678
Mean diameter for LDL particles (nm)	0.081	(0.066, 0.096)	<0.001	<0.001	0.100
Mean diameter for HDL particles (nm)	0.278	(0.236, 0.320)	<0.001	<0.001	0.279
Cholesterol					
Serum total cholesterol (mmol/L)	0.112	(-0.018, 0.241)	0.091	0.116	0.817
Total cholesterol in VLDL (mmol/L)	-0.187	(-0.230, -0.145)	<0.001	<0.001	0.275
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	-0.184	(-0.244, -0.124)	<0.001	<0.001	0.383
Total cholesterol in LDL (mmol/L)	-0.048	(-0.117, 0.021)	0.175	0.213	0.437
Total cholesterol in HDL (mmol/L)	0.344	(0.286, 0.401)	<0.001	<0.001	0.382
Total cholesterol in HDL2 (mmol/L)	0.324	(0.271, 0.378)	<0.001	<0.001	0.354
Total cholesterol in HDL3 (mmol/L)	0.019	(0.014, 0.025)	<0.001	<0.001	0.034
Esterified cholesterol (mmol/L)	0.070	(-0.023, 0.163)	0.139	0.172	0.586
Free cholesterol (mmol/L)	0.046	(0.009, 0.084)	0.016	0.023	0.238
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	-0.344	(-0.416, -0.273)	<0.001	<0.001	0.468
Triglycerides in VLDL (mmol/L)*	-0.530	(-0.630, -0.429)	<0.001	<0.001	0.659
Triglycerides in LDL (mmol/L)	0.008	(0.001, 0.014)	0.033	0.044	0.044
Triglycerides in HDL (mmol/L)	-0.004	(-0.010, 0.002)	0.228	0.272	0.040
Total phosphoglycerides (mmol/L)	0.106	(0.052, 0.159)	<0.001	<0.001	0.340
Ratio of triglycerides to phosphoglycerides	-0.289	(-0.337, -0.241)	<0.001	<0.001	0.318
Phosphatidylcholine & other cholines (mmol/L)	0.138	(0.086, 0.190)	<0.001	<0.001	0.331
Sphingomyelins (mmol/L)	0.032	(0.020, 0.045)	<0.001	<0.001	0.078
Total cholines (mmol/L)	0.170	(0.115, 0.224)	<0.001	<0.001	0.349
Apolipoproteins					
Apolipoprotein A1 (g/L)	0.146	(0.114, 0.178)	<0.001	<0.001	0.209
Apolipoprotein B (g/L)	-0.115	(-0.146, -0.084)	<0.001	<0.001	0.198
Ratio of apolipoprotein B to apolipoprotein AI	-0.126	(-0.148, -0.105)	<0.001	<0.001	0.144
Fatty acids					
Total fatty acids (mmol/L)	-0.711	(-1.091, -0.330)	<0.001	<0.001	2.388
Estimated degree of unsaturation	0.031	(0.021, 0.042)	<0.001	<0.001	0.066
22:6, docosahexaenoic acid (mmol/L)	-0.002	(-0.008, 0.005)	0.622	0.667	0.041
18:2, linoleic acid (mmol/L)	0.004	(-0.087, 0.094)	0.934	0.947	0.566
Omega3 fatty acids (mmol/L)	-0.035	(-0.054, -0.016)	<0.001	0.001	0.122
Omega6 fatty acids (mmol/L)	0.004	(-0.094, 0.102)	0.936	0.936	0.612
Polyunsat. fatty acids (mmol/L)	-0.031	(-0.144, 0.082)	0.592	0.644	0.708
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	-0.372	(-0.520, -0.225)	<0.001	<0.001	0.930
Saturated fatty acids (mmol/L)	-0.307	(-0.455, -0.159)	<0.001	<0.001	0.932
Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.064	(0.018, 0.110)	0.006	0.009	0.288
Ratio of 18:2 linoleic acid to total fatty acids (%)	1.527	(0.984, 2.070)	<0.001	<0.001	3.430
Ratio of omega3 fatty acids to total fatty acids (%)	-0.038	(-0.152, 0.075)	0.508	0.570	0.710
Ratio of omega6 fatty acids to total fatty acids (%)	1.882	(1.351, 2.412)	<0.001	<0.001	3.376
Ratio of polyunsat. fatty acids to total fatty acids (%)	1.843	(1.272, 2.414)	<0.001	<0.001	3.621
Ratio of monounsat. fatty acids to total fatty acids (%)	-1.456	(-1.914, -0.998)	<0.001	<0.001	2.904
Ratio of saturated fatty acids to total fatty acids (%)	-0.387	(-0.700, -0.074)	0.015	0.022	1.959
Glycolysis related					
Glucose (mmol/L)*	-0.071	(-0.097, -0.044)	<0.001	<0.001	0.168
Lactate (mmol/L)	-0.178	(-0.252, -0.105)	<0.001	<0.001	0.469
Pyruvate (mmol/L)	-0.007	(-0.013, -0.002)	0.004	0.006	0.032

Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.387	0.016
Glycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.328	0.022
Amino acids					
Alanine (mmol/L)	-0.020	(-0.030, -0.011)	<0.001	<0.001	0.060
Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	<0.001	<0.001	0.065
Glycine (mmol/L)	0.029	(0.020, 0.038)	<0.001	<0.001	0.059
Histidine (mmol/L)	-0.001	(-0.003, 0.000)	0.116	0.146	0.009
Isoleucine (mmol/L)	-0.016	(-0.019, -0.013)	<0.001	<0.001	0.021
Leucine (mmol/L)	-0.019	(-0.022, -0.016)	<0.001	<0.001	0.022
Valine (mmol/L)	-0.029	(-0.035, -0.022)	<0.001	<0.001	0.042
Phenylalanine (mmol/L)	0.000	(-0.002, 0.001)	0.576	0.637	0.010
Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	<0.001	<0.001	0.014
Ketone bodies					
Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.001	0.273
Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	0.863	1.048
3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.035	0.472
Fluid balance					
Albumin (signal area)	-0.002	(-0.002, -0.001)	<0.001	<0.001	0.005
Creatinine (mmol/L)	-0.013	(-0.015, -0.012)	<0.001	<0.001	0.010
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	<0.001	0.239

* Metabolite has been log transformed

^ Benjamini-Hochberg adjusted p-value

Associations for parents in Figure 3 are presented in SD-units. The conversion factor provided (unweighted standard deviaton of each metabolite measure in adults/parents) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.

Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Metabolic subgroup	Mother																	
	Boys						Girls						All Children					
	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	433	0.23	0.14 - 0.31	518	0.24	0.16 - 0.32	476	0.21	0.12 - 0.29	987	0.23	0.17 - 0.29	909	0.22	0.16 - 0.28
Total lipids in very large VLDL (mmol/L)*	469	0.25	0.16 - 0.33	433	0.25	0.16 - 0.34	518	0.22	0.14 - 0.30	476	0.20	0.12 - 0.29	987	0.24	0.18 - 0.29	909	0.23	0.17 - 0.29
Total lipids in large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	433	0.24	0.15 - 0.33	518	0.23	0.14 - 0.31	476	0.22	0.14 - 0.31	987	0.22	0.16 - 0.28	909	0.23	0.17 - 0.29
Total lipids in medium VLDL (mmol/L)	469	0.26	0.17 - 0.34	433	0.28	0.19 - 0.36	518	0.29	0.21 - 0.37	476	0.30	0.21 - 0.38	987	0.28	0.22 - 0.34	909	0.29	0.23 - 0.35
Total lipids in small VLDL (mmol/L)	469	0.26	0.18 - 0.35	433	0.28	0.19 - 0.36	518	0.30	0.21 - 0.37	476	0.30	0.21 - 0.38	987	0.29	0.23 - 0.34	909	0.29	0.23 - 0.35
Total lipids in very small VLDL (mmol/L)	469	0.22	0.14 - 0.31	433	0.21	0.12 - 0.30	518	0.26	0.17 - 0.34	476	0.26	0.18 - 0.35	987	0.25	0.19 - 0.30	909	0.25	0.18 - 0.31
Total lipids in IDL (mmol/L)	469	0.27	0.18 - 0.35	433	0.23	0.14 - 0.32	518	0.29	0.21 - 0.37	476	0.31	0.23 - 0.39	987	0.29	0.23 - 0.34	909	0.28	0.22 - 0.34
Total lipids in large LDL (mmol/L)	469	0.28	0.19 - 0.36	433	0.24	0.15 - 0.33	518	0.30	0.22 - 0.37	476	0.32	0.24 - 0.40	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Total lipids in medium LDL (mmol/L)	469	0.28	0.20 - 0.36	433	0.24	0.15 - 0.33	518	0.29	0.21 - 0.37	476	0.32	0.24 - 0.40	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Total lipids in small LDL (mmol/L)	469	0.28	0.19 - 0.36	433	0.24	0.15 - 0.32	518	0.29	0.21 - 0.37	476	0.32	0.23 - 0.40	987	0.28	0.23 - 0.34	909	0.28	0.22 - 0.34
Total lipids in very large HDL (mmol/L)	469	0.30	0.22 - 0.38	433	0.30	0.21 - 0.38	518	0.32	0.24 - 0.39	476	0.30	0.21 - 0.38	987	0.31	0.25 - 0.36	909	0.30	0.24 - 0.36
Total lipids in large HDL (mmol/L)	469	0.31	0.23 - 0.39	433	0.31	0.23 - 0.40	518	0.28	0.20 - 0.36	476	0.26	0.18 - 0.34	987	0.30	0.24 - 0.35	909	0.29	0.23 - 0.35
Total lipids in medium HDL (mmol/L)	469	0.22	0.13 - 0.30	433	0.20	0.11 - 0.29	518	0.12	0.03 - 0.20	476	0.13	0.04 - 0.22	987	0.17	0.11 - 0.23	909	0.17	0.10 - 0.23
Total lipids in small HDL (mmol/L)	469	0.23	0.14 - 0.31	433	0.22	0.13 - 0.31	518	0.20	0.12 - 0.29	476	0.20	0.11 - 0.28	987	0.21	0.15 - 0.27	909	0.21	0.15 - 0.27
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	469	0.30	0.22 - 0.38	433	0.32	0.23 - 0.40	518	0.27	0.19 - 0.35	476	0.25	0.16 - 0.33	987	0.29	0.23 - 0.35	909	0.28	0.22 - 0.34
Mean diameter for LDL particles (nm)	469	0.22	0.13 - 0.31	433	0.20	0.11 - 0.29	518	0.30	0.22 - 0.38	476	0.32	0.24 - 0.40	987	0.26	0.20 - 0.31	909	0.26	0.20 - 0.32
Mean diameter for HDL particles (nm)	469	0.32	0.23 - 0.40	433	0.32	0.23 - 0.40	518	0.33	0.26 - 0.41	476	0.31	0.23 - 0.39	987	0.33	0.27 - 0.38	909	0.31	0.25 - 0.37
Cholesterol																		
Serum total cholesterol (mmol/L)	469	0.27	0.19 - 0.35	433	0.23	0.14 - 0.32	518	0.32	0.24 - 0.39	476	0.34	0.26 - 0.42	987	0.30	0.24 - 0.35	909	0.30	0.24 - 0.35
Total cholesterol in VLDL (mmol/L)	469	0.25	0.17 - 0.34	433	0.27	0.18 - 0.36	518	0.29	0.21 - 0.36	476	0.29	0.21 - 0.37	987	0.28	0.22 - 0.33	909	0.29	0.23 - 0.35
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	469	0.25	0.17 - 0.34	433	0.26	0.17 - 0.34	518	0.29	0.21 - 0.37	476	0.31	0.22 - 0.39	987	0.28	0.22 - 0.34	909	0.29	0.23 - 0.35
Total cholesterol in LDL (mmol/L)	469	0.28	0.20 - 0.36	433	0.24	0.15 - 0.33	518	0.29	0.21 - 0.37	476	0.32	0.23 - 0.40	987	0.29	0.23 - 0.34	909	0.29	0.23 - 0.35
Total cholesterol in HDL (mmol/L)	469	0.30	0.22 - 0.38	433	0.30	0.21 - 0.39	518	0.25	0.16 - 0.33	476	0.23	0.15 - 0.32	987	0.28	0.22 - 0.33	909	0.27	0.20 - 0.33
Total cholesterol in HDL2 (mmol/L)	469	0.30	0.22 - 0.38	433	0.31	0.22 - 0.39	518	0.25	0.16 - 0.32	476	0.23	0.15 - 0.32	987	0.28	0.22 - 0.33	909	0.27	0.21 - 0.33
Total cholesterol in HDL3 (mmol/L)	469	0.25	0.16 - 0.33	433	0.23	0.14 - 0.32	518	0.25	0.16 - 0.33	476	0.24	0.16 - 0.33	987	0.25	0.19 - 0.31	909	0.24	0.17 - 0.30
Esterified cholesterol (mmol/L)	465	0.28	0.19 - 0.36	430	0.23	0.14 - 0.32	518	0.32	0.24 - 0.39	476	0.34	0.26 - 0.42	983	0.30	0.24 - 0.35	906	0.29	0.23 - 0.35
Free cholesterol (mmol/L)	465	0.26	0.18 - 0.34	430	0.22	0.13 - 0.31	518	0.32	0.24 - 0.40	476	0.35	0.27 - 0.43	983	0.30	0.24 - 0.35	906	0.30	0.24 - 0.35
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	469	0.28	0.20 - 0.36	433	0.30	0.21 - 0.38	518	0.29	0.21 - 0.37	476	0.29	0.20 - 0.37	987	0.29	0.23 - 0.35	909	0.30	0.24 - 0.36
Triglycerides in VLDL (mmol/L)*	469	0.30	0.22 - 0.38	433	0.31	0.23 - 0.40	518	0.28	0.20 - 0.36	476	0.27	0.18 - 0.35	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Triglycerides in LDL (mmol/L)	469	0.19	0.10 - 0.28	433	0.18	0.09 - 0.27	518	0.26	0.18 - 0.34	476	0.27	0.18 - 0.35	987	0.23	0.17 - 0.29	909	0.23	0.17 - 0.29
Triglycerides in HDL (mmol/L)	469	0.21	0.12 - 0.30	433	0.23	0.14 - 0.32	518	0.26	0.18 - 0.34	476	0.26	0.18 - 0.34	987	0.24	0.18 - 0.30	909	0.25	0.19 - 0.31
Total phosphoglycerides (mmol/L)	465	0.26	0.17 - 0.34	430	0.23	0.14 - 0.32	518	0.26	0.17 - 0.34	476	0.27	0.18 - 0.35	983	0.26	0.20 - 0.32	906	0.25	0.18 - 0.31
Ratio of triglycerides to phosphoglycerides	465	0.23	0.15 - 0.32	430	0.26	0.16 - 0.34	518	0.26	0.18 - 0.34	476	0.27	0.19 - 0.35	983	0.25	0.20 - 0.31	906	0.27	0.20 - 0.33
Phosphatidylcholine & other cholines (mmol/L)	465	0.27	0.18 - 0.35	430	0.24	0.14 - 0.32	518	0.25	0.17 - 0.33	476	0.27	0.18 - 0.35	983	0.26	0.20 - 0.32	906	0.25	0.19 - 0.31
Sphingomyelins (mmol/L)	465	0.23	0.15 - 0.32	430	0.22	0.12 - 0.30	518	0.29	0.21 - 0.37	476	0.31	0.23 - 0.39	983	0.27	0.21 - 0.32	906	0.27	0.21 - 0.33
Total cholines (mmol/L)	465	0.27	0.18 - 0.35	430	0.23	0.14 - 0.32	518	0.28	0.20 - 0.35	476	0.28	0.20 - 0.37	983	0.27	0.21 - 0.33	906	0.26	0.20 - 0.32
Apolipoproteins																		
Apolipoprotein A1 (g/L)	469	0.28	0.20 - 0.36	433	0.26	0.17 - 0.35	518	0.26	0.18 - 0.34	476	0.26	0.17 - 0.34	987	0.27	0.21 - 0.33	909	0.26	0.20 - 0.32
Apolipoprotein B (g/L)	469	0.26	0.18 - 0.35	433	0.27	0.18 - 0.35	517	0.30	0.22 - 0.38	475	0.32	0.24 - 0.40	986	0.29	0.23 - 0.35	908	0.31	0.25 - 0.36
Ratio of apolipoprotein B to apolipoprotein AI	469	0.28	0.20 - 0.36	433	0.30	0.21 - 0.38	517	0.25	0.17 - 0.33	475	0.25	0.16 - 0.33	986	0.27	0.21 - 0.33	908	0.28	0.22 - 0.34
Fatty acids																		
Total fatty acids (mmol/L)	462	0.22	0.13 - 0.30	427	0.22	0.13 - 0.31	517	0.31	0.23 - 0.39	475	0.33	0.25 - 0.41	979	0.27	0.22 - 0.33	902	0.29	0.22 - 0.34
Estimated degree of unsaturation	462	0.30	0.22 - 0.38	427	0.32	0.23 - 0.41	517	0.27	0.19 - 0.35	475	0.24	0.16 - 0.33	979	0.29	0.23 - 0.34	902	0.28	0.22 - 0.34
22:6, docosahexaenoic acid (mmol/L)	462	0.20	0.11 - 0.29	427	0.18	0.09 - 0.27	517	0.34	0.26 - 0.41	475	0.32	0.24 - 0.40	979	0.27	0.21 - 0.32	902	0.25	0.19 - 0.31
18:2, linoleic acid (mmol/L)	462	0.22	0.13 - 0.31	427	0.22	0.13 - 0.31	517	0.27	0.19 - 0.35	475	0.30	0.21 - 0.38	979	0.25	0.19 - 0.31	902	0.27	0.21 - 0.33
Omega3 fatty acids (mmol/L)	462	0.20	0.11 - 0.29	427	0.19	0.09 - 0.28	517	0.34	0.27 - 0.42	475	0.34	0.26 - 0.42	979	0.27	0.21 - 0.33	902	0.26	0.20 - 0.32
Omega6 fatty acids (mmol/L)	462	0.23	0.14 - 0.32	427	0.22	0.13 - 0.31	517	0.31	0.23 - 0.38	475	0.33	0.25 - 0.41	979	0.27	0.21 - 0.33	902	0.28	0.22 - 0.34
Polyunsat. fatty acids (mmol/L)	462	0.23	0.14 - 0.31	427	0.22	0.12 - 0.30	517	0.32	0.24 - 0.39	475	0.34	0.26 - 0.42	979	0.28	0.22 - 0.33	902	0.28	0.22 - 0.34
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	462	0.24	0.15 - 0.33	427	0.25	0.16 - 0.34	517	0.33	0.25 - 0.40	475	0.33	0.25 - 0.41	979	0.29	0.24 - 0.35	902	0.30	0.24 - 0.36
Saturated fatty acids (mmol/L)	462	0.21	0.12 - 0.29	427	0.21	0.12 - 0.30	517	0.29	0.21 - 0.37	475	0.30	0.21 - 0.38	979	0.26	0.20 - 0.32	902	0.26	0.20 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	462	0.31	0.22 - 0.39	427	0.29	0.20 - 0.38	517	0.35	0.27 - 0.42	475	0.32	0.24 - 0.40	979	0.33	0.27 - 0.39	902	0.31	0.25 - 0.36
Ratio of 18:2 linoleic acid to total fatty acids (%)	462	0.13	0.04 - 0.22	427	0.15	0.06 - 0.24	517	0.20	0.11 - 0.28	475	0.16	0.08 - 0.25	979	0.17	0.11 - 0.23	902	0.16	0.09 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	462	0.32	0.24 - 0.40	427	0.32	0.23 - 0.40	517	0.40	0.33 - 0.47	475	0.37	0.29 - 0.44	979	0.36	0.31 - 0.41	902	0.34	0.28 - 0.39
Ratio of omega6 fatty acids to total fatty acids (%)	462	0.23	0.15 - 0.32	427	0.26	0.17 - 0.35	517	0.25	0.17 - 0.33	475	0.22	0.13 - 0.30	979	0.24	0.18 - 0.30	902	0.24	0.17 - 0.30
Ratio of polyunsat. fatty acids to total fatty acids (%)	462	0.27	0.19 - 0.36	427	0.30	0.21 - 0.38	517	0.28	0.20 - 0.36	475	0.24	0.16 - 0.33	979	0.28	0.22 - 0.33	902	0.27	0.20 - 0.33
Ratio of monounsat. fatty acids to total fatty acids (%)	462	0.29	0.21 - 0.38	427	0.30	0.22 - 0.39	517	0.33	0.25 - 0.40	475	0.32	0.23 - 0.40	979	0.32	0.26 - 0.37	902	0.31	0.25 - 0.37
Ratio of saturated fatty acids to total fatty acids (%)	462	0.20	0.11 - 0.29	427	0.21	0.12 - 0.30	517	0.26	0.18 - 0.34	475	0.24	0.16 - 0.33	979	0.23	0.17 - 0.29	902	0.23	0.17 - 0.29
Glycolysis related																		
Glucose (mmol/L)*	468	0.06	-0.03 - 0.15	432	0.05	-0.04 - 0.14	518	0.14	0.06 - 0.23	476	0.14	0.05 - 0.23	986	0.11	0.05 - 0.17	908	0.10	0.04 - 0.17
Lactate (mmol/L)	469	0.04	-0.05 - 0.13	433	0.06	-0.04 - 0.15	518	0.11	0.02 - 0.19	476	0.12	0.03 - 0.20	987	0.07	0.00 - 0.13	909	0.08	0.01 - 0.14
Pyruvate (mmol/L)	467	0.12	0.03 - 0.21	431	0.13	0.04 - 0.23	518	0.23	0.14 - 0.31	476	0.23	0.14 - 0.31	985	0.17	0.11 - 0.23	907	0.17	0.11 - 0.24
Citrate (mmol/L)	469	0.24	0.15 - 0.32	433	0.25	0.16 - 0.34	517	0.16	0.07 - 0.24	475	0.16	0.07 - 0.25	986	0.18	0.12 - 0.24	908	0.19	0.13 - 0.26
Glycerol (mmol/L)	124	0.25	0.07 - 0.41	113	0.24	0.06 - 0.41	155	0.10	-0.05 - 0.26	137	0.08	-0.09 - 0.24	279	0.16	0.05 - 0.28	250	0.16	0.04 - 0.28
Amino acids																		
Alanine (mmol/L)	469	0.22	0.13 - 0.31	433	0.21	0.12 - 0.30	517	0.32	0.24 - 0.40	475	0.30	0.21 - 0.38	986	0.28	0.22 - 0.33	908	0.26	0.20 - 0.32
Glutamine (mmol/L)	469	0.28	0.20 - 0.36	433	0.27	0.18 - 0.35	518	0.19	0.10 - 0.27	476	0.18	0.09 - 0.27	987	0.23	0.17 - 0.29	909	0.22	0.16 - 0.28
Glycine (mmol/L)	468	0.22	0.13 - 0.30	432	0.25	0.15 - 0.33	517	0.19	0.10 - 0.27	475	0.18	0.09 - 0.27	985	0.20	0.14 - 0.26	907	0.21	0.15 - 0.27
Histidine (mmol/L)	468	0.20	0.11 - 0.29	432	0.20	0.11 - 0.29	518	0.21	0.13 - 0.29	476	0.20	0.11 - 0.28	986	0.21	0.15 - 0.27	908	0.20	0.13 - 0.26
Isoleucine (mmol/L)	466	0.39	0.31 - 0.47	431	0.40	0.32 - 0.47	518	0.33	0.25 - 0.40	476	0.30	0.22 - 0.38	984	0.36	0.30 - 0.41	907	0.34	0.28 - 0.40
Leucine (mmol/L)	469	0.34	0.25 - 0.42	433	0.35	0.26 - 0.43	518	0.28	0.20 - 0.35	476	0.25	0.16 - 0.33	987	0.30	0.25 - 0.36	909	0.29	0.23 - 0.35
Valine (mmol/L)	469	0.39	0.31 - 0.46	433	0.38	0.30 - 0.46	516	0.27	0.19 - 0.35	474	0.26	0.17 - 0.34	985	0.33	0.27 - 0.38	907	0.32	0.26 - 0.38
Phenylalanine (mmol/L)	469	0.32	0.23 - 0.39	433	0.31	0.22 - 0.39	518	0.29	0.21 - 0.37	476	0.27	0.18 - 0.35	987	0.30	0.24 - 0.36	909	0.29	0.23 - 0.35
Tyrosine (mmol/L)	468	0.33	0.25 - 0.41	432	0.32	0.24 - 0.41	518	0.26	0.17 - 0.34	476	0.21	0.13 - 0.30	986	0.29	0.23 - 0.35	908	0.27	0.21 - 0.33
Ketone bodies																		
Acetate (mmol/L)*	468	0.11	0.02 - 0.20	433	0.08	-0.02 - 0.17	517	0.14	0.06 - 0.22	475	0.14	0.06 - 0.23	985	0.13	0.07 - 0.19	908	0.12	0.05 - 0.18
Acetoacetate (mmol/L)*	469	0.13	0.04 - 0.22	433	0.14	0.04 - 0.23	517	0.03	-0.05 - 0.12	475	0.01	-0.08 - 0.10	986	0.08	0.01 - 0.14	908	0.07	0.00 - 0.13
3hydroxybutyrate (mmol/L)*	437	0.23	0.14 - 0.32	403	0.18	0.08 - 0.27	486	0.34	0.26 - 0.42	445	0.27	0.19 - 0.36	923	0.28	0.22 - 0.34	848	0.22	0.16 - 0.29
Fluid balance																		
Albumin (signal area)	468	0.29	0.20 - 0.37	432	0.26	0.17 - 0.34	518	0.28	0.20 - 0.36	476	0.28	0.19 - 0.36	986	0.28	0.22 - 0.34	908	0.27	0.21 - 0.33
Creatinine (mmol/L)	458	0.31	0.23 - 0.39	423	0.30	0.21 - 0.39	514	0.25	0.17 - 0.33	473	0.26	0.17 - 0.34	972	0.28	0.22 - 0.34	896	0.28	0.22 - 0.34
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	469	0.23	0.15 - 0.32	433	0.23	0.14 - 0.32	518	0.28	0.19 - 0.35	476	0.28	0.19 - 0.36	987	0.26	0.20 - 0.32	909	0.26	0.19 - 0.32

* log transformation has been applied to metabolite
# adjusted for child and parent age, disadvantage index, fasting time, and processing lag time (and for child sex where appropriate).

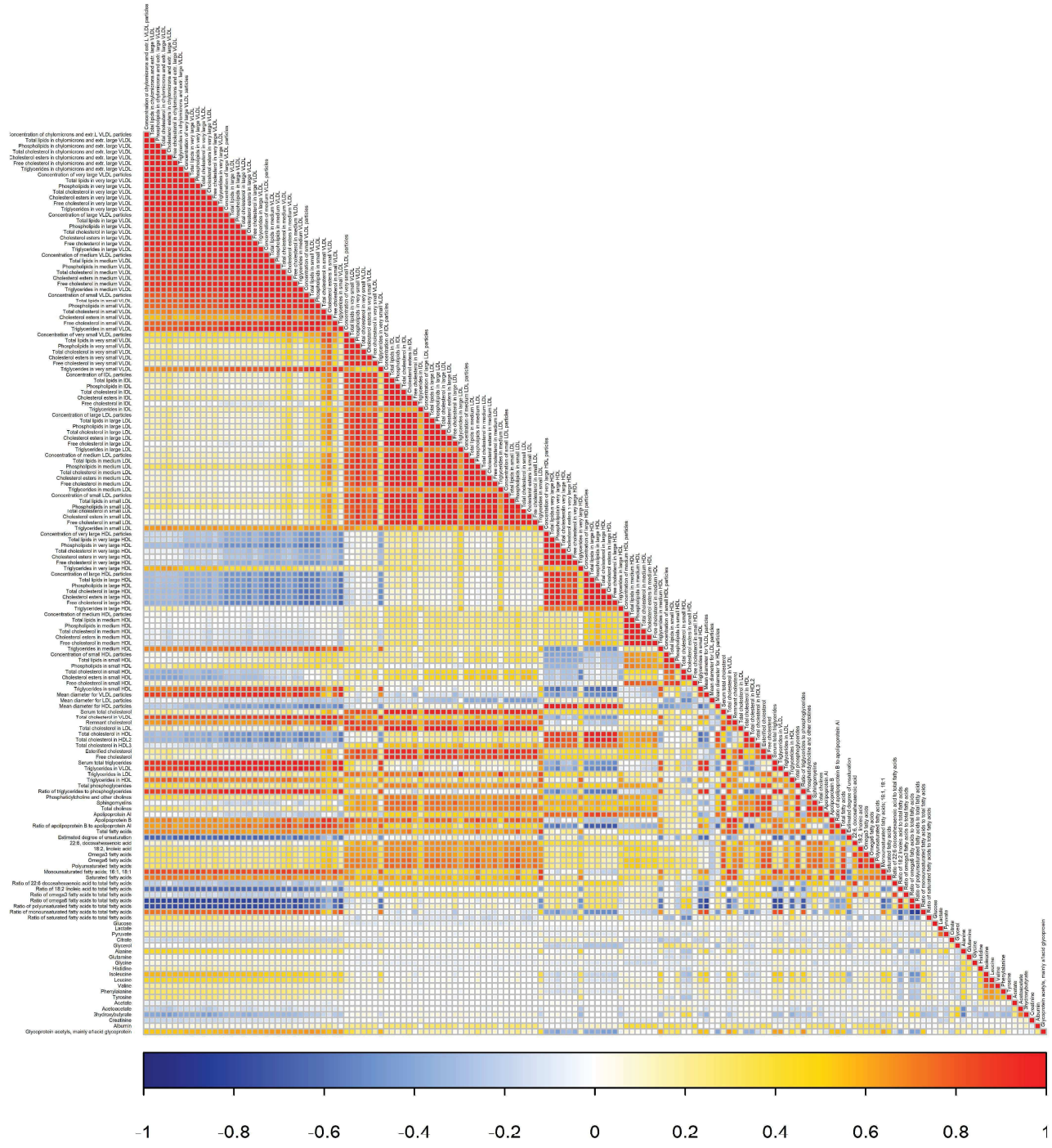
Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

Metabolic subgroup	All Parents																	
	Male Child						Female Child							All Children				
	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	547	0.22	0.14 - 0.30	505	0.23	0.14 - 0.31	586	0.20	0.12 - 0.28	544	0.17	0.08 - 0.25	1133	0.22	0.16 - 0.27	1049	0.20	0.15 - 0.26
Total lipids in very large VLDL (mmol/L)*	547	0.23	0.15 - 0.31	505	0.23	0.15 - 0.31	586	0.17	0.10 - 0.25	544	0.16	0.08 - 0.24	1133	0.21	0.15 - 0.26	1049	0.20	0.14 - 0.26
Total lipids in large VLDL (mmol/L)*	547	0.20	0.12 - 0.28	505	0.22	0.13 - 0.30	586	0.20	0.12 - 0.28	544	0.19	0.11 - 0.27	1133	0.20	0.15 - 0.26	1049	0.21	0.15 - 0.26
Total lipids in medium VLDL (mmol/L)	547	0.27	0.19 - 0.35	505	0.28	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.24	0.16 - 0.32	1133	0.26	0.21 - 0.31	1049	0.26	0.20 - 0.32
Total lipids in small VLDL (mmol/L)	547	0.27	0.19 - 0.34	505	0.27	0.19 - 0.35	586	0.26	0.18 - 0.33	544	0.26	0.17 - 0.33	1133	0.26	0.21 - 0.32	1049	0.26	0.21 - 0.32
Total lipids in very small VLDL (mmol/L)	547	0.23	0.15 - 0.31	505	0.22	0.13 - 0.30	586	0.25	0.17 - 0.33	544	0.26	0.18 - 0.34	1133	0.25	0.19 - 0.30	1049	0.24	0.19 - 0.30
Total lipids in IDL (mmol/L)	547	0.28	0.20 - 0.35	505	0.25	0.16 - 0.33	586	0.29	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.28	0.23 - 0.34
Total lipids in large LDL (mmol/L)	547	0.28	0.20 - 0.36	505	0.25	0.17 - 0.33	586	0.29	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.29	0.23 - 0.34
Total lipids in medium LDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.25	0.17 - 0.33	586	0.28	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.28	0.23 - 0.34	1049	0.29	0.23 - 0.34
Total lipids in small LDL (mmol/L)	547	0.28	0.20 - 0.35	505	0.24	0.16 - 0.32	586	0.28	0.20 - 0.35	544	0.31	0.23 - 0.38	1133	0.28	0.22 - 0.33	1049	0.28	0.22 - 0.33
Total lipids in very large HDL (mmol/L)	547	0.29	0.21 - 0.37	505	0.28	0.20 - 0.36	586	0.29	0.21 - 0.36	544	0.27	0.19 - 0.35	1133	0.29	0.24 - 0.34	1049	0.28	0.22 - 0.33
Total lipids in large HDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.28	0.19 - 0.36	586	0.24	0.17 - 0.32	544	0.22	0.14 - 0.30	1133	0.27	0.21 - 0.32	1049	0.25	0.19 - 0.30
Total lipids in medium HDL (mmol/L)	547	0.15	0.07 - 0.24	505	0.13	0.04 - 0.21	586	0.10	0.02 - 0.18	544	0.10	0.02 - 0.19	1133	0.12	0.07 - 0.18	1049	0.11	0.05 - 0.17
Total lipids in small HDL (mmol/L)	547	0.19	0.10 - 0.27	505	0.16	0.08 - 0.25	586	0.18	0.10 - 0.26	544	0.17	0.09 - 0.25	1133	0.18	0.12 - 0.23	1049	0.17	0.11 - 0.22
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	547	0.29	0.21 - 0.36	505	0.30	0.22 - 0.38	586	0.22	0.14 - 0.29	544	0.19	0.11 - 0.27	1133	0.25	0.20 - 0.31	1049	0.24	0.19 - 0.30
Mean diameter for LDL particles (nm)	547	0.19	0.11 - 0.27	505	0.17	0.08 - 0.25	586	0.27	0.19 - 0.34	544	0.29	0.21 - 0.36	1133	0.23	0.17 - 0.28	1049	0.23	0.17 - 0.28
Mean diameter for HDL particles (nm)	547	0.31	0.23 - 0.38	505	0.30	0.22 - 0.38	586	0.30	0.22 - 0.37	544	0.27	0.19 - 0.35	1133	0.30	0.25 - 0.35	1049	0.29	0.23 - 0.34
Cholesterol																		
Serum total cholesterol (mmol/L)	547	0.28	0.20 - 0.36	505	0.24	0.16 - 0.32	586	0.31	0.24 - 0.38	544	0.33	0.26 - 0.41	1133	0.30	0.24 - 0.35	1049	0.29	0.24 - 0.35
Total cholesterol in VLDL (mmol/L)	547	0.26	0.18 - 0.34	505	0.27	0.19 - 0.35	586	0.26	0.18 - 0.33	544	0.26	0.18 - 0.34	1133	0.26	0.21 - 0.32	1049	0.27	0.21 - 0.32
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	547	0.27	0.19 - 0.34	505	0.26	0.18 - 0.34	586	0.28	0.20 - 0.35	544	0.29	0.21 - 0.37	1133	0.27	0.22 - 0.33	1049	0.28	0.22 - 0.34
Total cholesterol in LDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.26	0.17 - 0.34	586	0.28	0.21 - 0.35	544	0.30	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.28	0.23 - 0.34
Total cholesterol in HDL (mmol/L)	547	0.27	0.19 - 0.35	505	0.25	0.17 - 0.33	586	0.22	0.14 - 0.29	544	0.20	0.12 - 0.28	1133	0.24	0.19 - 0.30	1049	0.22	0.17 - 0.28
Total cholesterol in HDL2 (mmol/L)	547	0.27	0.19 - 0.34	505	0.25	0.17 - 0.33	586	0.21	0.14 - 0.29	544	0.20	0.11 - 0.28	1133	0.24	0.18 - 0.29	1049	0.22	0.16 - 0.28
Total cholesterol in HDL3 (mmol/L)	547	0.25	0.17 - 0.33	505	0.22	0.14 - 0.30	586	0.23	0.15 - 0.31	544	0.23	0.14 - 0.30	1133	0.24	0.19 - 0.30	1049	0.22	0.16 - 0.28
Esterified cholesterol (mmol/L)	543	0.28	0.20 - 0.36	502	0.24	0.15 - 0.32	584	0.31	0.24 - 0.38	542	0.33	0.25 - 0.40	1127	0.30	0.24 - 0.35	1044	0.29	0.23 - 0.34
Free cholesterol (mmol/L)	543	0.27	0.19 - 0.34	502	0.23	0.14 - 0.31	584	0.32	0.24 - 0.39	542	0.34	0.26 - 0.41	1127	0.29	0.24 - 0.35	1044	0.29	0.23 - 0.34
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	547	0.28	0.20 - 0.35	505	0.29	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.24	0.16 - 0.32	1133	0.26	0.21 - 0.32	1049	0.26	0.21 - 0.32
Triglycerides in VLDL (mmol/L)*	547	0.28	0.20 - 0.36	505	0.29	0.21 - 0.37	586	0.23	0.15 - 0.30	544	0.21	0.13 - 0.29	1133	0.26	0.20 - 0.31	1049	0.25	0.20 - 0.31
Triglycerides in LDL (mmol/L)	547	0.20	0.12 - 0.28	505	0.19	0.10 - 0.27	586	0.27	0.19 - 0.34	544	0.28	0.20 - 0.35	1133	0.24	0.18 - 0.29	1049	0.24	0.18 - 0.29
Triglycerides in HDL (mmol/L)	547	0.25	0.17 - 0.33	505	0.26	0.18 - 0.34	586	0.27	0.20 - 0.35	544	0.28	0.20 - 0.35	1133	0.26	0.21 - 0.32	1049	0.27	0.21 - 0.32
Total phosphoglycerides (mmol/L)	543	0.28	0.20 - 0.36	502	0.24	0.16 - 0.32	584	0.27	0.19 - 0.34	542	0.29	0.21 - 0.36	1127	0.27	0.22 - 0.33	1044	0.26	0.20 - 0.32
Ratio of triglycerides to phosphoglycerides	543	0.23	0.15 - 0.31	502	0.24	0.16 - 0.32	584	0.21	0.13 - 0.28	542	0.20	0.12 - 0.28	1127	0.22	0.16 - 0.27	1044	0.22	0.16 - 0.28
Phosphatidylcholine & other cholines (mmol/L)	542	0.28	0.20 - 0.36	501	0.23	0.15 - 0.32	584	0.26	0.19 - 0.34	542	0.28	0.20 - 0.36	1126	0.27	0.21 - 0.32	1043	0.26	0.20 - 0.31
Sphingomyelins (mmol/L)	542	0.23	0.15 - 0.31	501	0.20	0.12 - 0.29	584	0.28	0.21 - 0.36	542	0.30	0.22 - 0.37	1126	0.26	0.20 - 0.31	1043	0.26	0.20 - 0.31
Total cholines (mmol/L)	542	0.27	0.19 - 0.35	501	0.23	0.14 - 0.31	584	0.29	0.21 - 0.36	542	0.30	0.22 - 0.37	1126	0.28	0.22 - 0.33	1043	0.26	0.20 - 0.32
Apolipoproteins																		
Apolipoprotein A1 (g/L)	547	0.26	0.18 - 0.34	505	0.23	0.14 - 0.31	586	0.25	0.17 - 0.33	544	0.25	0.17 - 0.32	1133	0.25	0.20 - 0.31	1049	0.23	0.18 - 0.29
Apolipoprotein B (g/L)	547	0.27	0.19 - 0.35	505	0.27	0.19 - 0.35	585	0.28	0.20 - 0.35	543	0.30	0.22 - 0.37	1132	0.28	0.23 - 0.33	1048	0.29	0.23 - 0.34
Ratio of apolipoprotein B to apolipoprotein AI	547	0.26	0.18 - 0.33	505	0.27	0.18 - 0.34	585	0.21	0.13 - 0.29	543	0.20	0.12 - 0.28	1132	0.23	0.18 - 0.29	1048	0.24	0.18 - 0.29
Fatty acids																		
Total fatty acids (mmol/L)	537	0.26	0.18 - 0.33	496	0.25	0.17 - 0.33	583	0.30	0.22 - 0.37	541	0.31	0.23 - 0.39	1120	0.28	0.23 - 0.33	1037	0.29	0.23 - 0.34
Estimated degree of unsaturation	537	0.30	0.22 - 0.37	496	0.32	0.23 - 0.39	583	0.24	0.17 - 0.32	541	0.22	0.14 - 0.30	1120	0.27	0.21 - 0.32	1037	0.26	0.20 - 0.32
22:6, docosahexaenoic acid (mmol/L)	537	0.23	0.15 - 0.31	496	0.21	0.13 - 0.30	583	0.33	0.26 - 0.40	541	0.33	0.25 - 0.40	1120	0.28	0.23 - 0.34	1037	0.27	0.21 - 0.32
18:2, linoleic acid (mmol/L)	537	0.24	0.16 - 0.32	496	0.25	0.16 - 0.33	583	0.27	0.19 - 0.34	541	0.29	0.21 - 0.37	1120	0.26	0.21 - 0.32	1037	0.28	0.22 - 0.33
Omega3 fatty acids (mmol/L)	537	0.24	0.16 - 0.32	496	0.22	0.14 - 0.31	583	0.34	0.27 - 0.41	541	0.34	0.27 - 0.41	1120	0.29	0.23 - 0.34	1037	0.28	0.22 - 0.33
Omega6 fatty acids (mmol/L)	537	0.26	0.18 - 0.33	496	0.25	0.16 - 0.33	583	0.30	0.23 - 0.38	541	0.33	0.25 - 0.40	1120	0.28	0.23 - 0.34	1037	0.29	0.24 - 0.35
Polyunsat. fatty acids (mmol/L)	537	0.26	0.17 - 0.33	496	0.24	0.16 - 0.33	583	0.31	0.24 - 0.39	541	0.34	0.26 - 0.41	1120	0.29	0.23 - 0.34	1037	0.29	0.24 - 0.35
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	537	0.27	0.19 - 0.35	496	0.28	0.19 - 0.36	583	0.30	0.22 - 0.37	541	0.30	0.23 - 0.38	1120	0.29	0.24 - 0.34	1037	0.29	0.24 - 0.35
Saturated fatty acids (mmol/L)	537	0.25	0.17 - 0.33	496	0.25	0.16 - 0.33	583	0.28	0.20 - 0.35	541	0.28	0.20 - 0.36	1120	0.27	0.21 - 0.32	1037	0.27	0.21 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	537	0.31	0.23 - 0.38	496	0.30	0.21 - 0.37	583	0.33	0.26 - 0.40	541	0.31	0.23 - 0.38	1120	0.32	0.27 - 0.37	1037	0.30	0.24 - 0.36
Ratio of 18:2 linoleic acid to total fatty acids (%)	537	0.15	0.07 - 0.23	496	0.17	0.08 - 0.25	583	0.18	0.10 - 0.26	541	0.16	0.08 - 0.24	1120	0.17	0.11 - 0.23	1037	0.16	0.10 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	537	0.32	0.24 - 0.39	496	0.32	0.23 - 0.39	583	0.40	0.33 - 0.47	541	0.38	0.31 - 0.45	1120	0.36	0.31 - 0.41	1037	0.35	0.29 - 0.40
Ratio of omega6 fatty acids to total fatty acids (%)	537	0.24	0.16 - 0.32	496	0.26	0.18 - 0.34	583	0.23	0.15 - 0.30	541	0.20	0.12 - 0.28	1120	0.24	0.18 - 0.29	1037	0.23	0.17 - 0.28
Ratio of polyunsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.35	496	0.29	0.21 - 0.37	583	0.26	0.18 - 0.33	541	0.23	0.15 - 0.31	1120	0.26	0.21 - 0.32	1037	0.25	0.20 - 0.31
Ratio of monounsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.34	496	0.27	0.19 - 0.35	583	0.30	0.23 - 0.37	541	0.29	0.21 - 0.37	1120	0.29	0.23 - 0.34	1037	0.28	0.22 - 0.34
Ratio of saturated fatty acids to total fatty acids (%)	537	0.22	0.14 - 0.30	496	0.23	0.14 - 0.31	583	0.26	0.19 - 0.34	541	0.25	0.17 - 0.33	1120	0.24	0.19 - 0.30	1037	0.24	0.19 - 0.30
Glycolysis related																		
Glucose (mmol/L)*	545	0.05	-0.04 - 0.13	503	0.04	-0.05 - 0.12	586	0.14	0.06 - 0.21	544	0.14	0.05 - 0.22	1131	0.1	0.04 - 0.15	1047	0.09	0.03 - 0.15
Lactate (mmol/L)	547	0.02	-0.07 - 0.10	505	0.03	-0.06 - 0.12	586	0.13	0.05 - 0.21	544	0.14	0.05 - 0.22	1133	0.07	0.01 - 0.12	1049	0.08	0.01 - 0.13
Pyruvate (mmol/L)	545	0.11	0.03 - 0.20	503	0.13	0.04 - 0.21	586	0.22	0.15 - 0.30	544	0.22	0.14 - 0.30	1131	0.17	0.11 - 0.22	1047	0.17	0.11 - 0.23
Citrate (mmol/L)	547	0.23	0.15 - 0.31	505	0.25	0.16 - 0.33	585	0.14	0.06 - 0.22	543	0.14	0.05 - 0.22	1132	0.17	0.12 - 0.23	1048	0.18	0.12 - 0.24
Glycerol (mmol/L)	151	0.30	0.15 - 0.44	137	0.28	0.12 - 0.43	175	0.12	-0.03 - 0.26	157	0.08	-0.08 - 0.23	326	0.20	0.09 - 0.30	294	0.19	0.07 - 0.29
Amino acids																		
Alanine (mmol/L)	546	0.24	0.16 - 0.31	504	0.22	0.14 - 0.31	585	0.32	0.25 - 0.39	543	0.30	0.22 - 0.37	1131	0.28	0.23 - 0.34	1047	0.27	0.21 - 0.32
Glutamine (mmol/L)	547	0.30	0.22 - 0.37	505	0.29	0.21 - 0.37	586	0.20	0.12 - 0.27	544	0.19	0.11 - 0.27	1133	0.24	0.19 - 0.30	1049	0.24	0.18 - 0.29
Glycine (mmol/L)	545	0.21	0.13 - 0.29	503	0.24	0.15 - 0.32	585	0.19	0.11 - 0.27	543	0.19	0.11 - 0.27	1130	0.20	0.15 - 0.26	1046	0.21	0.15 - 0.27
Histidine (mmol/L)	545	0.21	0.13 - 0.29	503	0.21	0.12 - 0.29	586	0.22	0.14 - 0.29	544	0.20	0.12 - 0.28	1131	0.21	0.16 - 0.27	1047	0.21	0.15 - 0.26
Isoleucine (mmol/L)	541	0.36	0.29 - 0.44	500	0.37	0.29 - 0.44	586	0.29	0.22 - 0.36	544	0.26	0.18 - 0.34	1127	0.33	0.27 - 0.38	1044	0.31	0.25 - 0.36
Leucine (mmol/L)	547	0.32	0.24 - 0.39	505	0.33	0.25 - 0.40	586	0.24	0.16 - 0.32	544	0.21	0.13 - 0.29	1133	0.28	0.23 - 0.33	1049	0.27	0.21 - 0.32
Valine (mmol/L)	547	0.38	0.31 - 0.45	505	0.38	0.31 - 0.45	584	0.27	0.19 - 0.34	542	0.25	0.17 - 0.32	1131	0.33	0.27 - 0.38	1047	0.32	0.26 - 0.37
Phenylalanine (mmol/L)	547	0.29	0.22 - 0.37	505	0.29	0.21 - 0.37	586	0.30	0.22 - 0.37	544	0.28	0.20 - 0.36	1133	0.29	0.24 - 0.35	1049	0.28	0.23 - 0.34
Tyrosine (mmol/L)	545	0.31	0.24 - 0.39	503	0.31	0.22 - 0.38	586	0.27	0.19 - 0.34	544	0.23	0.15 - 0.31	1131	0.29	0.24 - 0.34	1047	0.27	0.21 - 0.32
Ketone bodies																		
Acetate (mmol/L)*	546	0.09	0.01 - 0.17	505	0.05	-0.03 - 0.14	585	0.15	0.06 - 0.22	543	0.15	0.07 - 0.23	1131	0.12	0.06 - 0.17	1048	0.11	0.05 - 0.17
Acetoacetate (mmol/L)*	547	0.11	0.03 - 0.20	505	0.11	0.03 - 0.20	585	0.04	-0.04 - 0.12	543	0.04	-0.05 - 0.12	1132	0.07	0.02 - 0.13	1048	0.07	0.01 - 0.13
3hydroxybutyrate (mmol/L)*	511	0.22	0.13 - 0.30	472	0.16	0.07 - 0.24	551	0.34	0.26 - 0.41	510	0.28	0.20 - 0.36	1062	0.27	0.21 - 0.33	982	0.21	0.15 - 0.27
Fluid balance																		
Albumin (signal area)	546	0.32	0.24 - 0.39	504	0.29	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.25	0.17 - 0.33	1132	0.28	0.23 - 0.33	1048	0.27	0.21 - 0.32
Creatinine (mmol/L)	534	0.29	0.21 - 0.37	493	0.27	0.19 - 0.35	580	0.27	0.19 - 0.34	539	0.27	0.19 - 0.35	1114	0.28	0.22 - 0.33	1032	0.27	0.22 - 0.33
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	547	0.24	0.16 - 0.32	505	0.24	0.16 - 0.32	586	0.24	0.17 - 0.32	544	0.25	0.16 - 0.32	1133	0.24	0.19 - 0.30	1049	0.24	0.18 - 0.30

* log transformation has been applied to metabolite

adjusted for child and parent age, disadvantage index, fasting time, and processing lag time (and for child and parent sex where appropriate).

Supplementary Figure 1: Correlations between Child Health CheckPoint metabolites - children

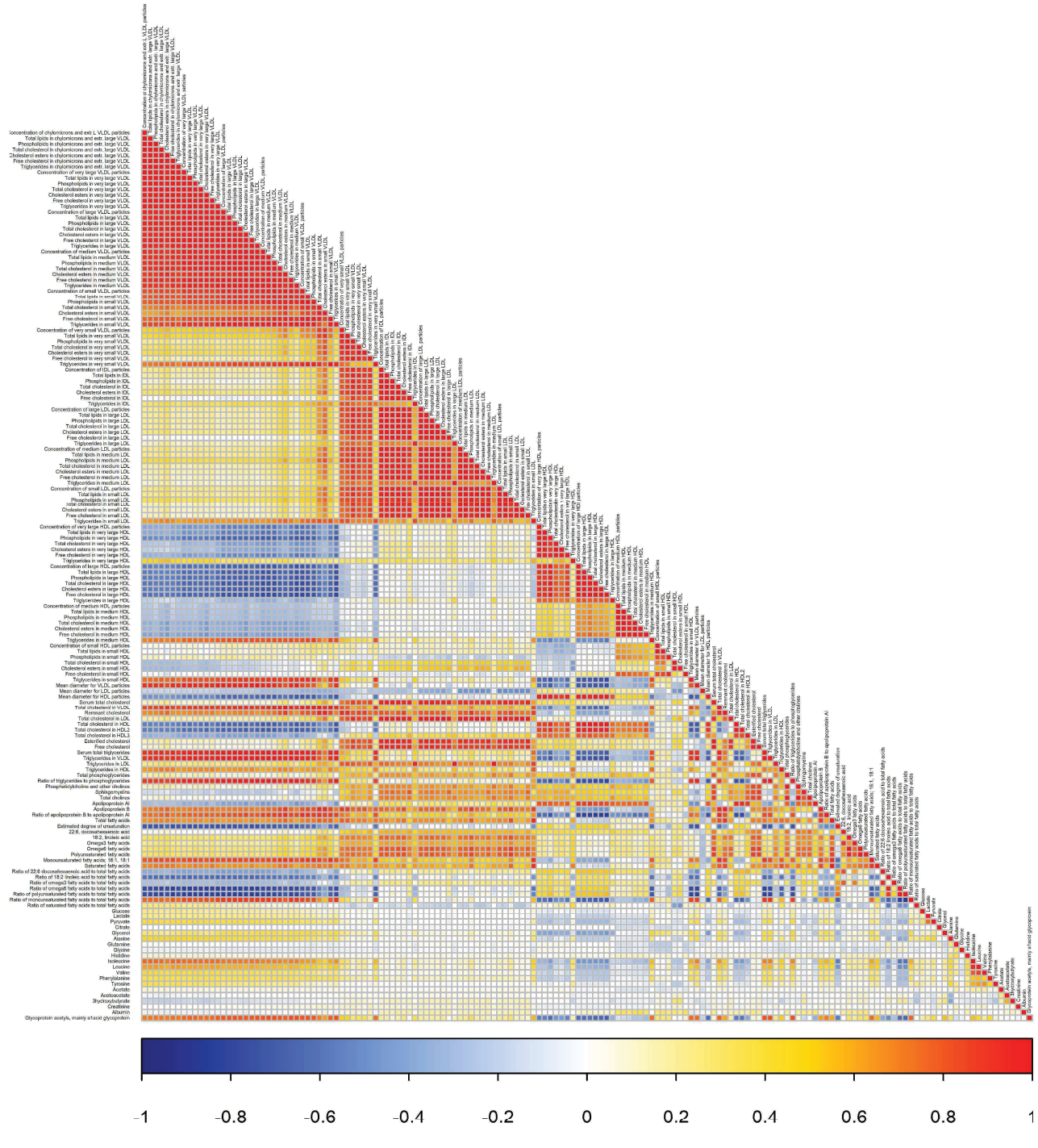


Note: Correlations (spearmans) between metabolites for the CheckPoint child metabolomics data

Supplementary figure 1: Correlation of NMR measures in children.

Heatmap showing the correlation between metabolite measures in children. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary Figure 2: Correlations between Child Health CheckPoint metabolites - parents

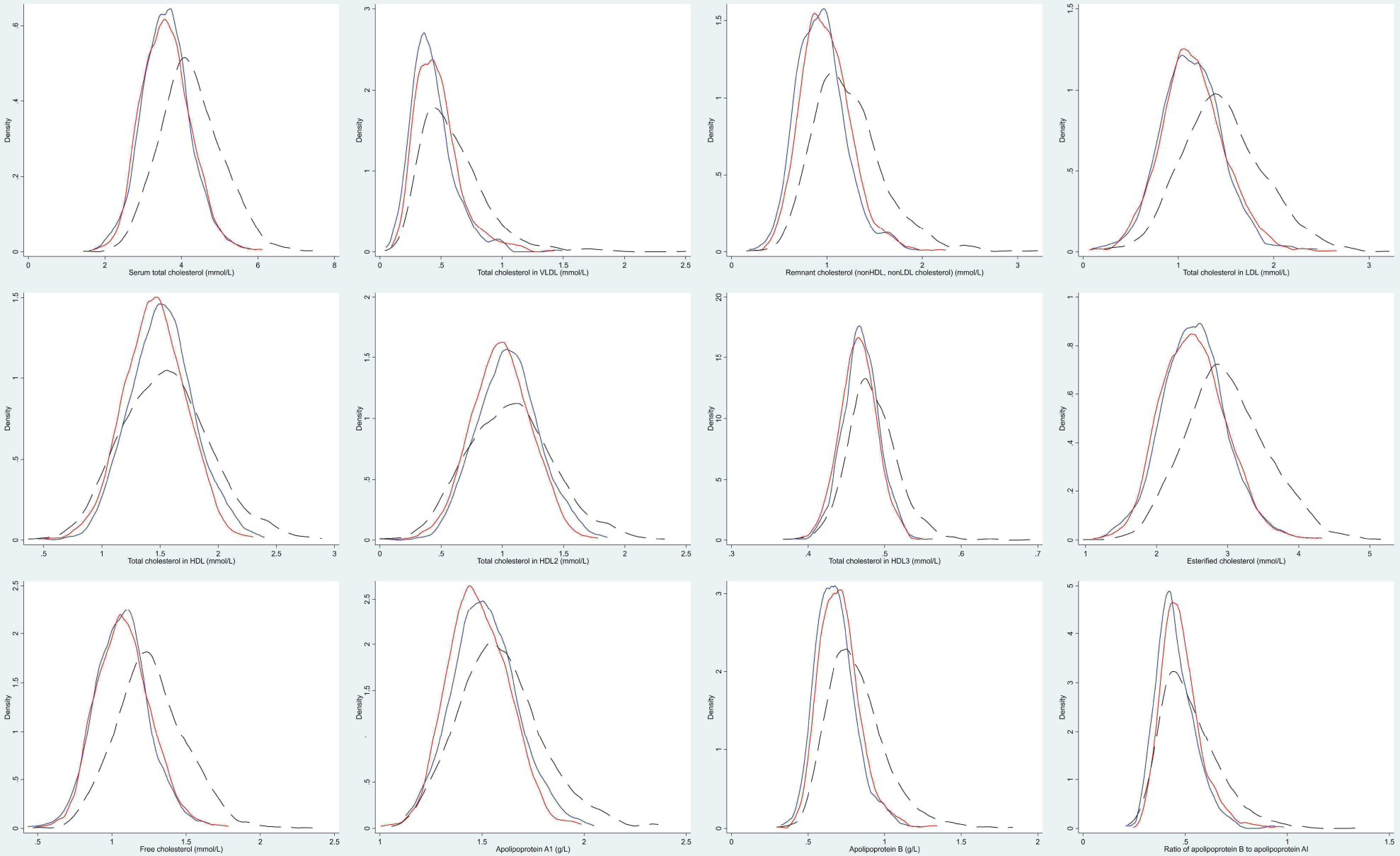


Note: Correlations (spearmans) between metabolites for the CheckPoint parent metabolomics data

Supplementary figure 2: Correlation of NMR metabolite measures in parents.

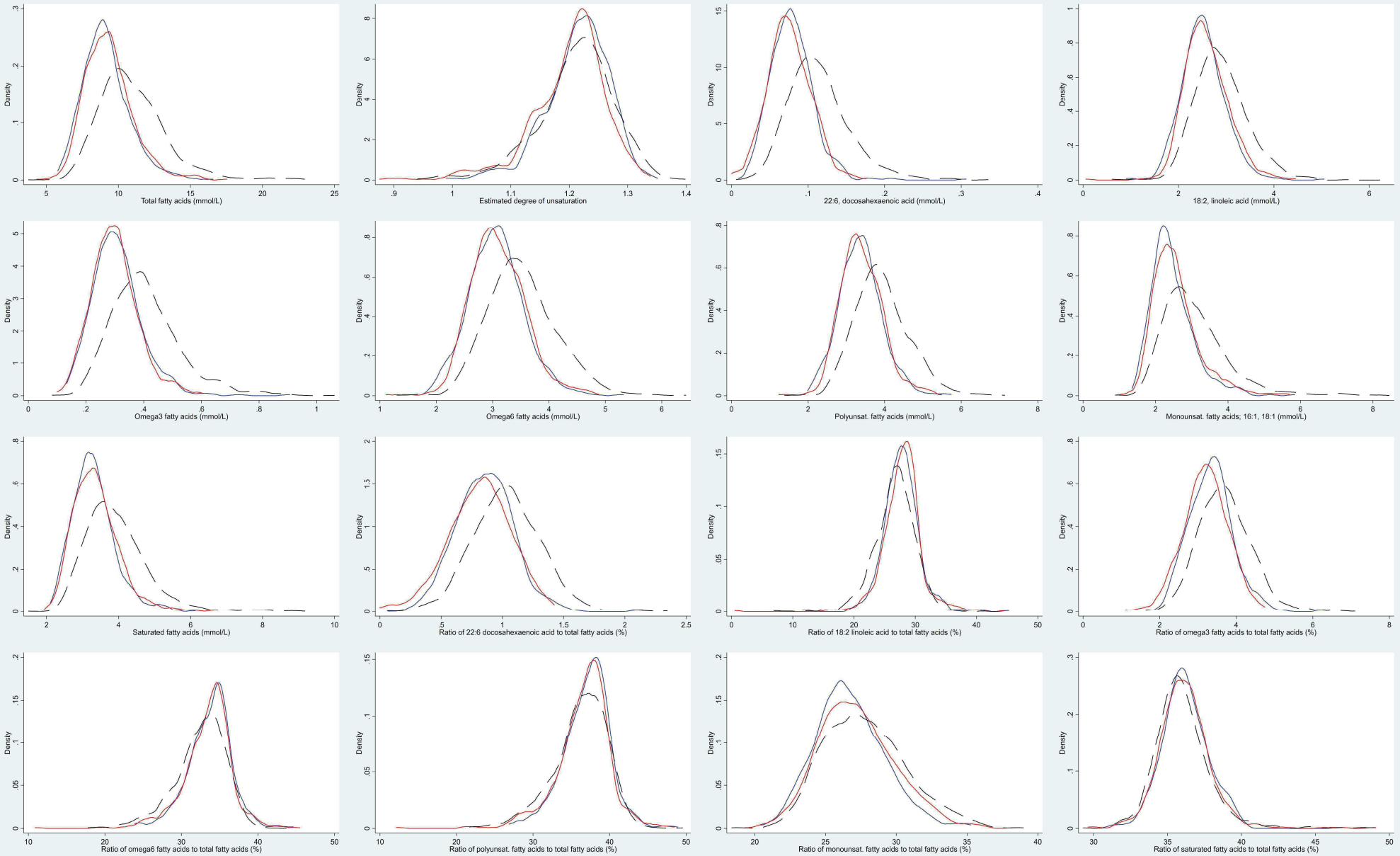
Heatmap showing the correlation between metabolite measures in parents. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.



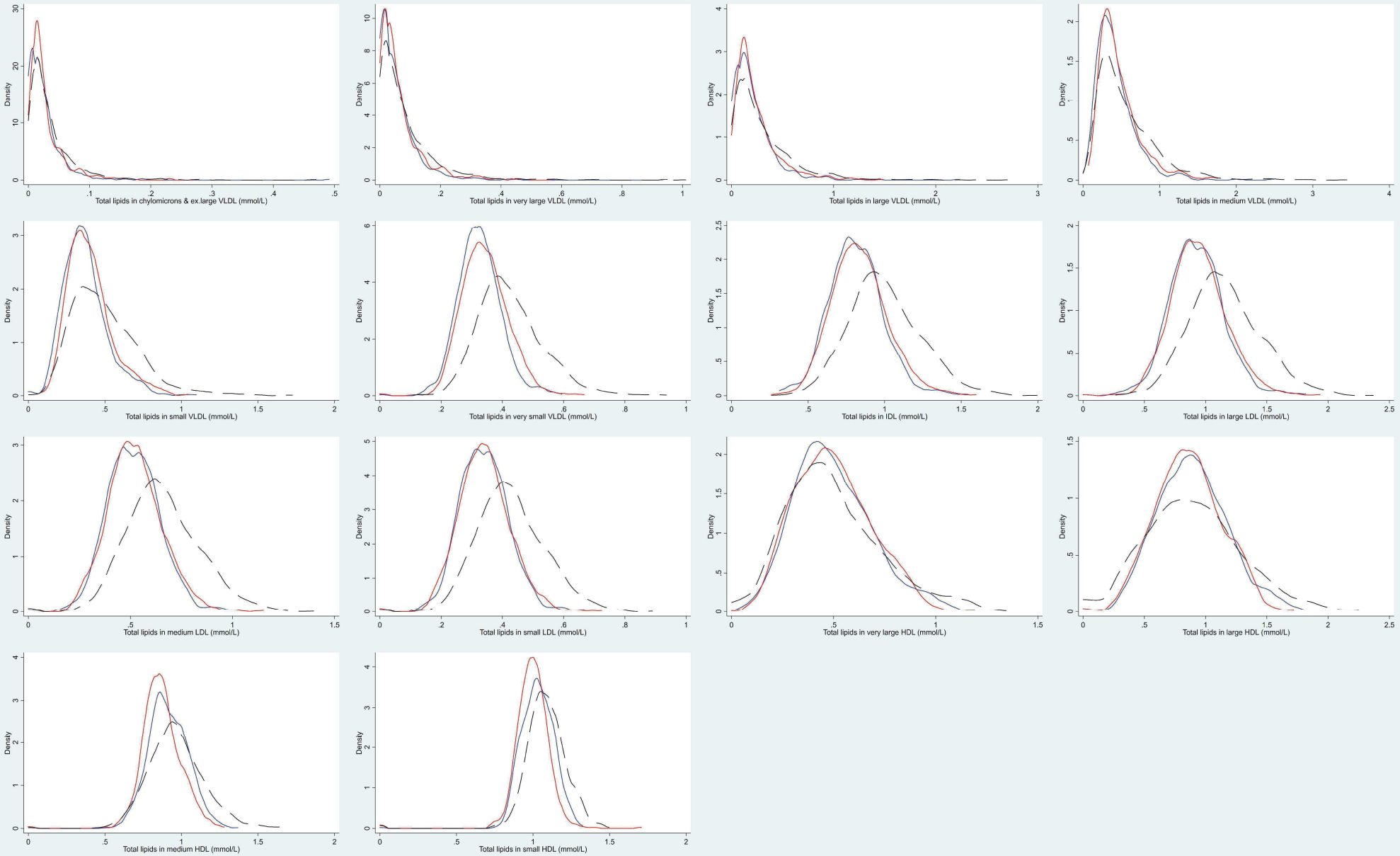
Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each cholesterol and apolipoprotein measure.

Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.



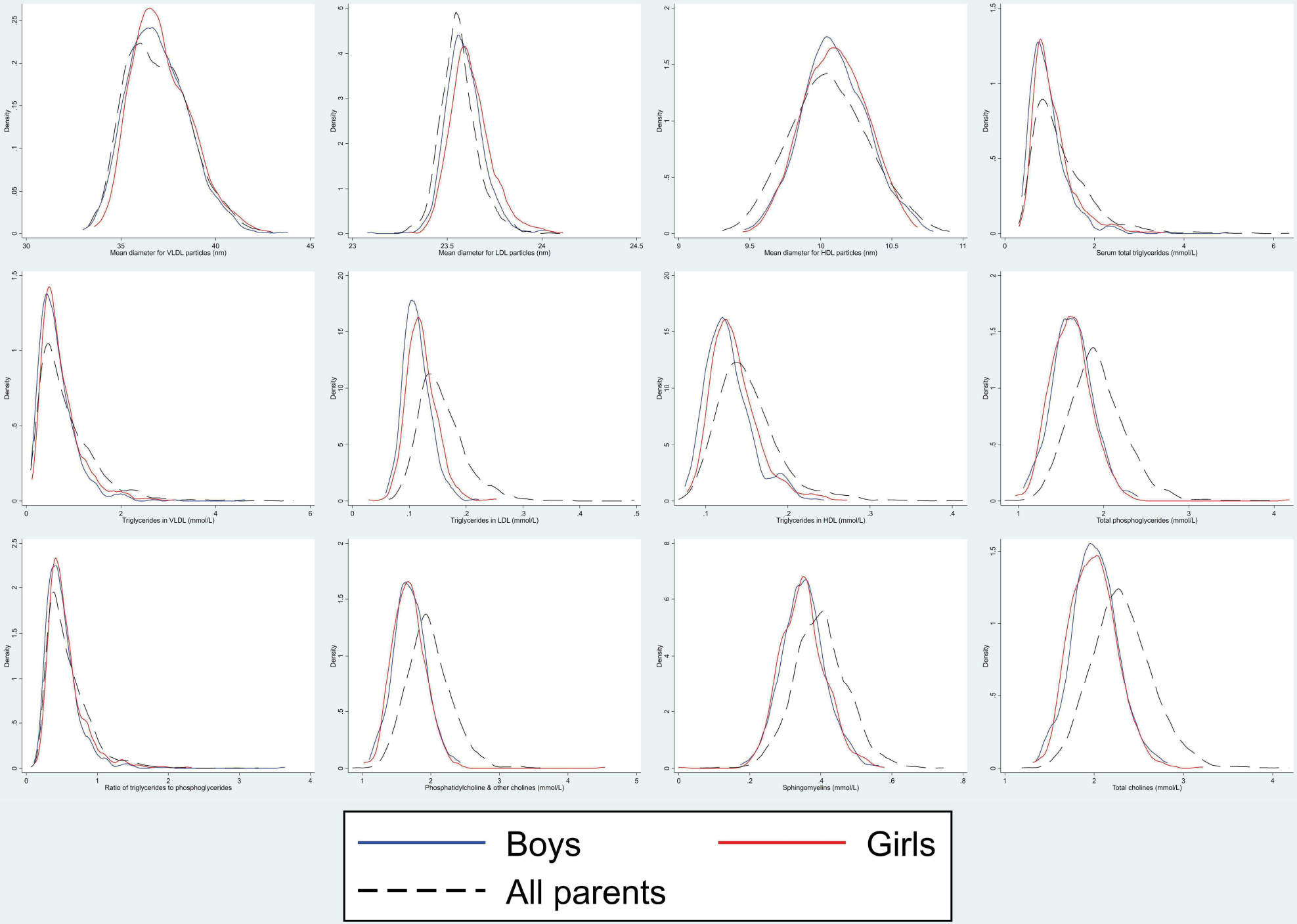
Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each fatty acid and fatty acid ratio measure.

Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass particles.



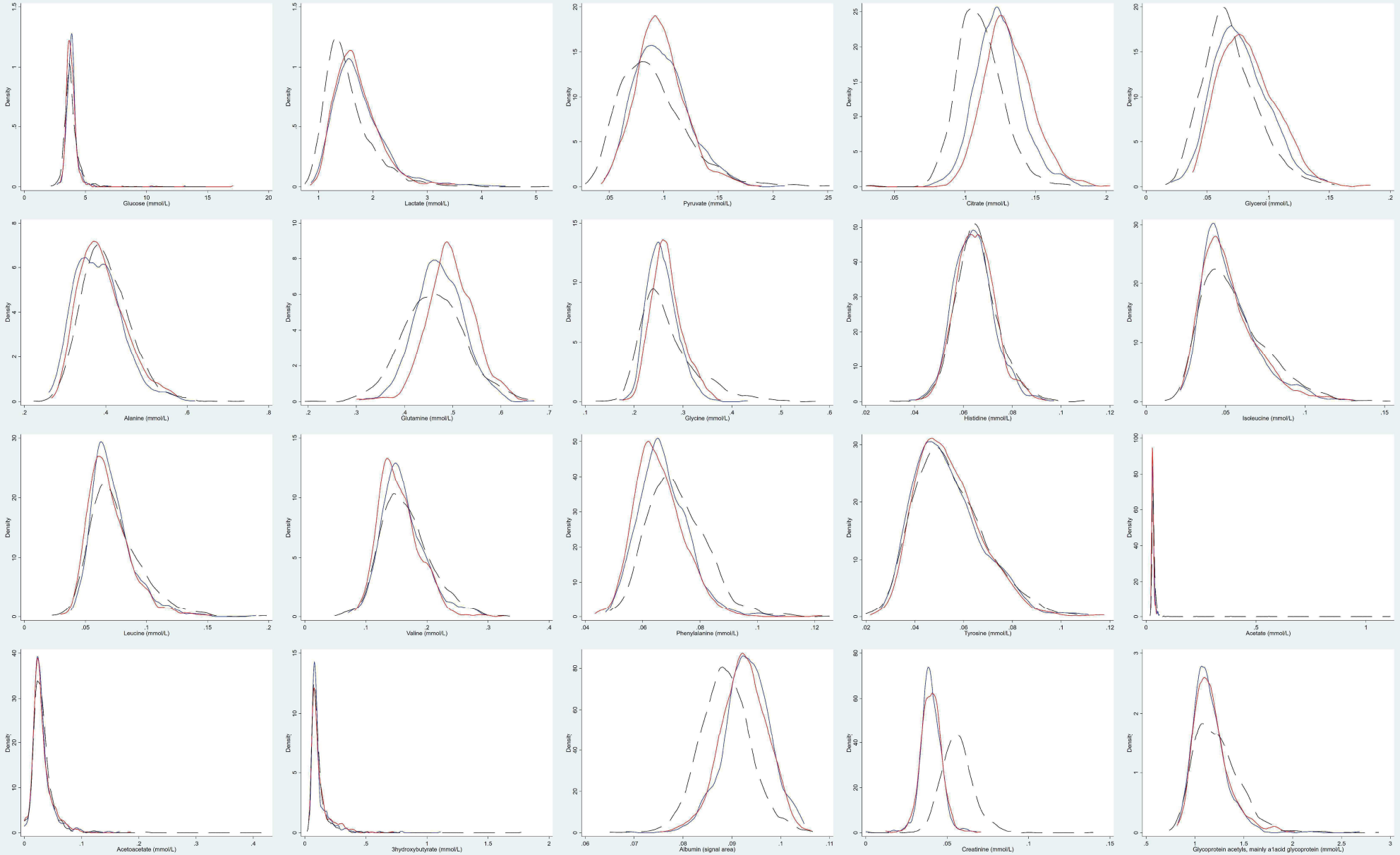
Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for total lipids within each of the 14 lipoprotein subclass particles.

Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride measures.



Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for lipoprotein particle sizes and triglyceride measures.

Supplementary figure 7: Density plots for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.



Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.

STROBE Statement—checklist of items that should be included in reports of observational studies

Paper title: Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

Person completing checklist: Susan Ellul

	Item No	Recommendation	Page number	Line number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2	14-15
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	9-13 25-32
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5	Pg 4 (3-9 ,21-22, 31-32, 33-35) Pg 5 (5-7)
Objectives	3	State specific objectives, including any prespecified hypotheses	5	8-11
Methods				
Study design	4	Present key elements of study design early in the paper	5	14-22
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6	Pg 5 (17-20) Pg 6 (3-32)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ~~*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale~~ for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	23-31
		~~(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed~~ ~~*Case-control study*—For matched studies, give matching criteria and the number of controls per case~~		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9	Pg 7 (4-17) Pg 8 (table 1, 9-16) Pg 9 (1-24)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9	Pg 7 (4-17) Pg 8 (table 1, 9-16) Pg 9 (1-24)
Bias	9	Describe any efforts to address potential sources of bias	9 -10	Pg 9 (31-35) Pg 10 (16-18)

Study size	10	Explain how the study size was arrived at	11	5-14, figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-10	Pg 9 (28-35) Pg 10 (3-15)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10	Pg 9 (28-35) Pg 10 (3-15)
		(*b*) Describe any methods used to examine subgroups and interactions	9-10	Pg 9 (28-35) Pg 10 (3-15)
		(*c*) Explain how missing data were addressed	9 -10	Pg 9 (31-35) Pg 10 (16-18)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed ~~*Case-control study*—If applicable, explain how matching of cases and controls was addressed~~	9	31-35
		(*e*) Describe any sensitivity analyses	10	16-18

Results			**Page number**	**Line number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11 and figure 1	5-12
		(b) Give reasons for non-participation at each stage	11 6 figure 1	8-12 7-11
		(c) Consider use of a flow diagram	figure 1	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12 (table 2)	2
		(b) Indicate number of participants with missing data for each variable of interest	figure 1 table 2 sup Table 1	2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	sup table 1	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13 supp tables 2-6	21-24
		(*b*) Report category boundaries when continuous variables were categorized	NA	

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	supp tables 2-6	
Discussion				
Key results	18	Summarise key results with reference to study objectives	14	3-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14	18-28
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-17	Pg 14 (30-32) Pg 15 (all) Pg 16 (all) Pg 17 (1-2)
Generalisability	21	Discuss the generalisability (external validity) of the study results	14	18-28
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18-19	Pg 18 (21-33) Pg 19 (1-2)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords:	Metabolomics, Lipids, Reference values, Children, Inheritance patterns, Epidemiologic studies

Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords: Metabolomics; lipids; inflammation; reference values; parents; children; inheritance patterns; correlation studies; epidemiologic studies; cross-sectional studies.

Word count: 4328

Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched chain amino acid; BD: Becton Dickinson; CDC: Centers for Disease Prevention and Control; CVD: Cardiovascular disease; CPS1: Carbamoyl-phosphate synthase 1; DHA: Docosahexaenoic acid; DOB: Date of birth; EDTA: Ethylenediaminetetraacetic acid; FDR: False discovery rate; GlycA: Glycoprotein acetyls; HbA1c: Haemoglobin A1c; HDL: High-density lipoprotein; HOMA: Homeostatic model assessment; IDL: Intermediate density lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein; LiH: Lithium Heparin; LSAC:

Longitudinal Study of Australian Children; MUFA: Monounsaturated fatty acid; NMR: Nuclear magnetic resonance; PCOS: Polycystic Ovary Syndrome; PUFA: Polyunsaturated fatty acid; REDCap: Research Electronic Data Capture; SFA: Saturated fatty acids; SST: serum separating tubes; T2D: Type 2 diabetes; T2DM: Type 2 diabetes mellitus; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

ABSTRACT

Objectives: Nuclear Magnetic Resonance (NMR) metabolomics is high throughput and cost effective, with the potential to improve the understanding of disease and risk. We examine the circulating metabolic profile by quantitative NMR metabolomics of a sample of Australian 11-12 year old children and their parents, describe differences by age and sex, and explore correlation of metabolites in parent-child dyads.

Design: The population-based cross-sectional Child Health CheckPoint study nested within the Longitudinal Study of Australian Children.

Setting: Blood samples collected from CheckPoint participants at assessment centres in seven Australian cities and eight regional towns; February 2015-March 2016.

Participants: 1180 children and 1325 parents provided a blood sample and had metabolomics data available. This included 1133 parent-child dyads (518 mother-daughter, 469 mother-son, 68 father-daughter, and 78 father-son).

Outcome measures: 228 metabolic measures were obtained for each participant. We focused on 74 biomarkers including amino acid species, lipoprotein subclass measures, lipids, fatty acids, measures related to fatty acid saturation, and composite markers of inflammation and energy homeostasis.

Results: We identified differences in the concentration of specific metabolites between childhood and adulthood and in metabolic profiles in children and adults by sex. In general, metabolite concentrations were higher in adults than children and sex differences were larger in adults than in children. Positive correlations were observed for the majority of metabolites including isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total cholesterol (CC 0.30, 95% CI 0.24 to 0.35) and omega 6 fatty acids (CC 0.28, 95% CI 0.23 to 0.34) in parent-child comparisons.

Conclusions: We describe the serum metabolite profiles from mid-childhood and adulthood in a population-based sample, together with parent-child concordance. Differences in profiles by age and sex were observed. These data will be informative for investigation of the childhood origins of adult non-communicable diseases and for comparative studies in other populations.

Strengths and limitations of this study:

- In a large population-based cohort, venous blood was collected for children and their attending parent on the same day using the same methods
- Rapidly processed, high quality serum samples with standardised metabolomic data generated as a single batch
- Cross-sectional design does not enable longitudinal analysis of specific metabolite species over short term or longer periods of time
- Assessment of paternal associations with offspring metabolite measures is limited by a relatively small sample size compared to mother-child pairs, reducing the precision of estimates
- Factors known to influence metabolomic profile (such as body mass index) were not considered as the aim was to describe the distribution of metabolites in children and their parents.

INTRODUCTION

Metabolomics involves the quantitative analysis of a large number of metabolites and lipids involved in a diverse range of biochemical pathways.[1] Genetic/gene expression and environmental exposures are associated with specific metabolic changes across many tissues and body fluids.[2 3] As such, metabolomics is recognised as a powerful top-down approach to understanding genetic and environmental influences on health and disease. Metabolomic profiling also has considerable potential to identify clinically relevant biomarkers for risk stratification and disease monitoring.

Recent advances in nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry have enabled the simultaneous quantitative measurement of hundreds of metabolites. These approaches are sufficiently cost effective and high throughput to be applicable to large cohort studies. For example, NMR metabolomics of serum from the Cardiovascular Risk in Young Finns Study identified many biomarkers from multiple metabolic pathways reflective of fatty liver disease.[4] These were also predictive of risk 10 years prior to diagnosis, indicating that metabolic disruptions precede overt phenotype. Similar population and disease-specific studies have identified metabolomic profiles associated with a range of exposures and health outcomes with potential to reveal clinically important biomarkers and information on disease mechanisms.[5] In addition, specific serum metabolites can also be considered 'intermediate phenotypes' linking genetic risk with disease outcomes.[6 7]

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.[8 9] However, in adults sex appears to be a major driver of variation in metabolite profile, potentially interacting with age. For example, the effects of sex appeared to be greater in younger (age 25-35) than older Japanese adults.[10] A study of 26,000 Northern European adults identified many sex-specific metabolic species at the population level.[9] In men, several lipid measures begin to rise at early middle age whereas a similar increase is only observed in females post menopause. This pattern is consistent for all non-HDL cholesterol measures – very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) subclass particle concentrations - as well as for triglycerides.[9] Physiological states such as pregnancy also have consistent and measurable influence on serum metabolome.[11] However, it remains unclear how the serum metabolome differs in adults compared to children and by sex particularly in childhood.

Moreover, factors regulating the metabolic trajectory from early life to adulthood, the role of metabolomic profile in health at the population level and the extent to which blood metabolomic profiles are concordant for parents and children have not been fully explored. One small study has reported correlations between parents (n=179) and their offspring

(n=255) for a range of cardiometabolic risk factors including standard lipid profile measured using conventional methods; this proved stronger for total cholesterol and LDL cholesterol than for high density lipoprotein (HDL) cholesterol or triglycerides.[12] Considerable evidence exists that the metabolomic profile is regulated, at least in part, by genetic factors[13 14] and is also influenced by dietary and lifestyle factors. Each of these influences is likely to be shared between parents and their offspring to varying degrees, however, parent-child correlations of metabolites from NMR-based platforms have not been reported previously.

Here, we describe (1) the distribution of NMR-based metabolite measures in a population-based cohort of 11-12 year old children and their parents, differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults; and (4) report sex-specific parent-child concordance.

METHODS

Study Design: Details of the initial Longitudinal Study of Australian Children (LSAC) study design and recruitment are outlined elsewhere.[15 16] The LSAC commenced in 2004, when two cohorts (the 'B' and 'K' cohorts, of which the B cohort only was included in the present study) were recruited who have since been followed biennially. The Child Health CheckPoint comprised a detailed cross-sectional assessment of physical health and biomarkers in a population-based national sample of children (age 11-12 years) and their parents between February 2015 to March 2016. The CheckPoint was nested between waves 6 (2014) and 7 (2016) of the LSAC. Further details regarding the CheckPoint study design and methods are available elsewhere. [17 18]

Participants: Of the 8,921 families contacted to be part of the LSAC B cohort 5,107 families (57%) agreed to take part in the first wave of data collection in 2004; 4,484 families were retained for Wave 6 in 2014. During the Wave 6 LSAC home visit, B cohort families were introduced to the upcoming Child Health CheckPoint and asked to consent to their contact details being shared with the CheckPoint team. A total of 3,513 families provided permission to receive an information pack by mail and an information and recruitment phone call regarding the CheckPoint study (78% of Wave 6 cohort, 69% of original cohort). Of the families agreeing to receive information about the CheckPoint study, 1874 families took part (53% of eligible participants, 42% of Wave 6 cohort and 37% of original cohort).

Ethics and consent: The CheckPoint data collection protocol was approved by The Royal Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26). The attending parent/caregiver provided written informed consent for themselves and their child to

participate in the study, and asked to provide optional consent for the collection and use of biological samples.

Procedure: The specialised CheckPoint assessment centre sequentially visited seven Australian cities and eight regional towns between February 2015 and March 2016.[18] Each participating child attended the centre with one parent or caregiver (usually the biological mother) at which both participated in a wide range of measures relevant to non-communicable disease. Those families who could not attend a centre were offered a home visit. Participants were included in the current analyses if metabolomic data from CheckPoint were available (figure 1). Venous blood was not available for home-visit participants, but was collected at all city and most regional assessment centres. Participant pairs were excluded from the concordance analyses in this study if the attending parent was not the biological parent.

An experienced phlebotomist collected approximately 28mL of blood from the brachial vein of the non-dominant arm of semi-reclining, semi-fasted participants (at the time of collection, participants reported when they last ate or drank). Blood was collected sequentially into four Becton Dickinson (BD) Vacutainer® tubes using a butterfly needle so only a single venepuncture was required. Order of collection was (i) 2.7mL EDTA, (ii) 9mL EDTA, (iii) 9mL serum, (iv) 7.5mL Lithium Heparin. The latter two tubes were immediately inverted 6 times to ensure mixing with anticoagulant, and all tubes were transferred to the on-site laboratory. Time of collection was scheduled earlier in the visit for parents than for children.

Collection tube barcodes were linked to the participant and samples were immediately transported to an on-site laboratory where they were processed within two hours. Blood clotting was allowed at room temperature for at least 30 minutes after collection. The sample tubes were spun at 550g relative centrifugal force for 10 minutes at room temperature and distributed into 0.5mL aliquots of plasma, serum, buffy coat (lymphocytes), whole blood and/or an aliquot tube containing a blood clot (1.0mL FluidX screwcap tubes, Cheshire, UK) and stored immediately at -80˚C (Thermo Fisher Scientific, Waltham, USA). Each FluidX tube contained a unique 2D barcode linked to the original collection tube and participant. As each assessment centre closed, samples were shipped on dry ice to the Melbourne Children's Bioresource Centre for long term storage at -80˚C (serum, whole blood, plasma, blood clot) or vapour phase liquid nitrogen (lymphocytes). At a later date, single 0.5ml serum aliquot was removed for every CheckPoint participant and the combined aliquots were shipped in a single batch to Nightingale Health (Helsinki, Finland) on dry ice for NMR metabolomics.

Measures

Metabolomic profiling

The Nightingale® NMR metabolomics platform (Helsinki, Finland) was used to obtain metabolomics for children and parents using the 2016-version quantification algorithm. Details of this platform and methodology have been extensively described elsewhere,[6 19] and epidemiological applications were recently reviewed.[20] Briefly, metabolites were measured from 0.35mL of serum using a single high-throughput experimental setup for the simultaneous quantification of routine lipids, lipoprotein subclass distributions, particle size and composition, fatty acids, and other low–molecular weight metabolites such as amino acids and glycolysis-related metabolites. This generated data on 228 serum metabolite measures in absolute concentration units (eg millimoles per liter) and ratios (summarised in Table 1). Whilst widely used for epidemiological research, the NMR-based quantification has not been certified for clinical diagnostics. Further analytical validation of the quantification protocols for the biomarker subset routinely used in clinical settings (eg established cholesterol measures and creatinine) is expected to lead to recalibration of certain metabolite concentrations to better match clinical gold standards.[20]

Table 1. Summary of biomarkers and derived variables obtained via high-throughput NMR

Metabolic group	Species and derived measures
Amino acids	Alanine, Glutamine, Glycine, Histidine
	Branched chain: Isoleucine, Leucine, Valine
	Aromatic: Phenylalanine, Tyrosine
Cholesterol	VLDL, LDL, HDL, HDL2, HDL3, Total, Free, Esterified, Remnant
Triglycerides and phospholipids	Triglycerides (VLDL, LDL, HDL, total)
	Phosphoglycerides
	Ratio of triglycerides to phosphoglycerides*
	Phosphatidylcholine
	Sphingomyelins
	Total cholines
Apolipoproteins	Apolipoprotein A-1 (ApoA-1)
	Apolipoprotein B (Apo B)
	Ratio of Apolipoprotein B to Apolipoprotein A-1 (ApoB/Apo A-1)*
Fatty acids (FA)	Total, Omega-3, Omega-6, Polyunsaturated (PUFA), Saturated (SFA)
	Monounsaturated (MUFA), Docosahexaenoic acid (DHA), Linoleic (LA)
	Estimated degree of unsaturation
Fatty acid ratios	Omega-3/total FA*, Omega-6/total FA*, PUFA/total FA* (all %)
	SFA/total FA*, MUFA/total FA*, DHA/total FA*, LA/total FA* (all %)
Lipoprotein subclasses*	12 lipid measures in each of 14 subclasses VLDL (XXL, XL, L, M, S, XS), IDL, LDL (L,M,S), HDL (XL, L, M,S): Particle concentration, Total lipids, Esterified cholesterol, Total cholesterol, Phospholipids, Free cholesterol, Triglycerides and Esterified cholesterol/Total lipids (%), Free cholesterol/Total lipids (%), Total cholesterol/Total lipids (%), Triglycerides/Total lipids (%) and Phospholipids/Total lipids (%).
Lipoprotein size*	Mean diameter of VLDL, LDL and HDL particles
Ketone bodies	Acetate, Acetoacetate, 3-hydroxybutyrate
Glycolysis related	Glucose, Lactate, Pyruvate, Citrate, Glycerol
Fluid balance	Creatinine, Albumin
Inflammation	Glycoprotein acetyls (GlycA)

Information obtained from https://nightingalehealth.com/science/biomarkers

* ratio; ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; DHA: Docosahexaenoic acid; GlycA: Glycoprotein acetyls; HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; L: Large; LDL: Low-density lipoprotein; LA: Linoleic acid; M: Medium; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; S: Small; SFA: Saturated fatty acids; VLDL: Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very small.

Many of the 228 metabolomics measures correlate substantially both in adults (supplementary figure 1) and children (supplementary figure 2) and the pattern of correlations were similar for adults and children. For clarity, we therefore focused on a subset of 74 metabolites in analyses. We eliminated the 5 ratio measures for each of the 14 lipoprotein subclass particles. In addition, the 7 other measures within each of the lipoproteins (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, total lipids and particle concentration) are all highly correlated and therefore we only reported total lipids for each of the lipoprotein subclass particles.

Other measures and sample characteristics

Age and sex: For children, LSAC provided date of birth (DOB) and sex, which was originally exported from the Medicare Australia database. In parents, DOB and sex was self-reported in the CheckPoint questionnaire, which was administered on an iPad using the Research Electronic Data Capture (REDCap) tool.[21] Age in years was calculated as the difference between date of the CheckPoint assessment and DOB divided by 365.

Disadvantage index: LSAC provided contact details of families consenting to be contacted by CheckPoint. The family's residential postcode was confirmed during the CheckPoint recruitment phone call and updated, if required. The disadvantage index score of postcode was used to summarise neighbourhood socioeconomic position. Generated by the ABS from the 2011 national Census, the index numerically summarises the social and economic conditions of Australian neighbourhoods; national mean 1000, standard deviation 100; higher scores indicate less disadvantage.[22]

Time of blood collection, processing and fasting time: Time of blood collection and start of laboratory processing were recorded. When missing, collection time was estimated using the midpoint between the time the CheckPoint visit began and time that processing of the sample commenced. Processing lag time was calculated as the minutes between blood collection and the processing commencement. Most samples were processed within two hours.

Fasting time was calculated as the hours between last eating/drinking to time of blood collection. The last time of eating/drinking was cross-checked against when the participant was taking part in other CheckPoint stations (and known not to be eating) as well as sleep and wake times from accelerometry data (to identify usual activity, and therefore likely eating patterns) when available. Further details of cleaning processes for the time of last eat/drink can be found elsewhere.[23]

Statistical analysis

Sample Characteristics

Continuous descriptive variables and metabolite measures were summarised using means and standard deviations (SD) for children and adults separately, by sex and overall. For skewed metabolites, geometric means and relative SD were reported. To provide visual comparisons of distributions of metabolites by age and sex, density plots were used. Population summary statistics were estimated by applying survey weights and survey procedures that corrected for sampling, participation and non-response biases, and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[24] More detail on the calculation of weights is provided elsewhere.[25]

Differences in metabolite concentration by age (adults compared to children) and by sex (adults, children)

Skewed metabolites (skewness greater or equal to 2) were log-transformed. We used two-sided paired and unpaired t-tests (as appropriate) to assess differences in mean metabolite concentrations between adults and children in parent-child dyads, and between males and females for adults and children separately. P-values were adjusted using Benjamini-Hochberg (B-H) with a false discovery rate (FDR) of 10% to account for multiple comparisons.

Parent-Child concordance

Concordance between parents and children was assessed by 1) Pearson's correlation coefficients (CC) with 95% confidence intervals, and 2) partial correlation coefficients (PCC), adjusting for child and parent age, disadvantage index, fasting time, processing lag time (and for child and parent sex where appropriate). Scatterplots of parent versus child metabolites (log-transformed where needed as above) were examined to check for outliers and to ensure assumptions were met.

The analyses were repeated using weighted multi-level survey analyses and compared to unweighted analyses. As there appeared to be no major effect of response patterns on results we reported results from unweighted analyses. Analyses were undertaken using Stata version 14.2 (StataCorp, College Station, TX) and R version 3.3.2. [26]

Patient and Public Involvement: Because LSAC is a population-based longitudinal study, no patient groups were involved in its design or conduct. To our knowledge, the public was not involved in the study design, recruitment or conduct of LSAC study or its CheckPoint module. Parents received a summary health report for their child and themselves at or soon after the assessment visit. They consented to take part knowing that they would not otherwise receive individual results about themselves or their child.

RESULTS

Sample characteristics

The recruitment and retention of participants in the Child Health CheckPoint are described elsewhere.[18] Of the 1874 families who participated in CheckPoint assessment centres, blood serum samples of analysable quality from 1180 children and 1325 parents (figure 1) were sent for NMR quantification of metabolites. The majority of excluded families undertook home visits or attended a regional centre, where blood samples could not be collected (n=385, 20.5%), while some participants declined a blood sample (children, n=150, 8.0%; adults, n=108, 5.8%). Few data were lost due to insufficient volume or poor quality samples at the assessment centre (figure 1). The sample characteristics of parents and children are outlined in table 2. Summary statistics for our main child and parent metabolite measures are presented in supplementary table 1. Supplementary figures 3-7 show density plots comparing the distributions of metabolites for boys, girls and adults.

Table 2: Sample characteristics; values are weighted mean (standard deviation)

Characteristic	All	Male	Female
Child			
n	1152-1180	558-575	594-605
Age, years	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)
BMI, (kg/m2)	19.4 (3.5)	19.2 (3.4)	19.6 (3.7)
BMI z-score	0.38 (1.0)	0.40 (1.0)	0.37 (1.0)
Disadvantage Index	1012 (63)	1011 (65)	1014 (61)
Fasting time (hours)	4.2 (1.2)	4.3 (1.3)	4.2 (1.1)
Time of day - blood collection	14.16 (2.0)	14.12 (2.0)	14.20 (2.1)
Processing lag time (hours)	1.16 (0.5)	1.18 (0.5)	1.14 (0.5)
Parent			
n	1272-1325	174-177	1098-1148
Age, years	43.9 (5.6)	46.9 (6.9)	43.4 (5.2)
BMI, (kg/m2)	28.4 (6.4)	28.9 (4.7)	28.4 (6.6)
Fasting time (hours)	3.3 (1.6)	3.6 (2.0)	3.2 (1.5)
Time of day - blood collection	13.10 (2.0)	13.18 (2.1)	13.09 (2.0)
Processing lag time (hours)	1.26 (0.5)	1.31 (0.5)	1.26 (0.5)

Disadvantage Index: Index of Relative Socioeconomic Disadvantage; n: number of participants in cohort with this measure.

Differences in metabolite levels - adults compared to children

Figure 2 shows mean differences in metabolite levels for adults relative to children in standard deviation (SD) units. Most concentrations were higher in adults than children. Values that were similar in adults and children included total lipids in very large HDL lipoprotein subclass particles, acetoacetate, tyrosine and glucose. Levels in children were higher than those of adults for the majority of glycolysis related measures (lactate, pyruvate, citrate and glycerol), the ketone body 3-hydroxybutrate, the amino acid glutamine, many fatty acid ratios and all lipoprotein particle sizes. Supplementary table 2 lists the corresponding estimates in absolute concentration units.

Sex differences in metabolite levels in children and adults

Figure 3 shows differences in mean metabolite levels by sex for children and adults separately in SD units, with estimates in absolute concentration units listed in supplementary table 3 and 4.

In general, sex differences were more pronounced in adulthood, resulting in distinct overall patterns for children and adults. Children generally showed smaller differences by sex than adults. Of note, sex differences for apolipoproteins and fatty acid measures showed different patterns in children compared to adults.

Girls had lower levels of apolipoprotein-A-1 (ApoA-1) and higher ApoB than boys. In adults, the opposite pattern was observed with females having higher ApoA-1 and lower ApoB than males. In children, some fatty acid concentrations were higher in girls than boys. In contrast, many adult fatty acid measures were higher in males. There was no evidence of a difference in the level of inflammation (GlycA) by sex in children, while in adults, GlycA levels tended to be higher in males than females.

For some metabolites, sex differences in children mirrored (but were smaller in magnitude than) those of adults, particularly for the ketone body acetate and some key amino acids. At both ages, the amino acid glycine was higher in females but the branched-chain amino acids leucine and valine tended to be higher in males.

Parent-child concordance

Figure 4 shows the correlations between metabolite measures for all children with all parents, and for boys and girls with mothers (but not with the 177 fathers, given the small numbers). The corresponding correlation coefficients and partial correlation coefficients are listed in supplementary tables 5 and 6.

Correlations for all parents and all children showed similar patterns to that observed for mother and child by sex. While there was little suggestion of substantial correlation within parent-child dyads for some metabolites (eg glucose, acetate) a positive correlation was found for many metabolite measures irrespective of child sex. For example, positive correlations were observed for isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total serum cholesterol (CC 0.30, 95% CI 0.24 to 0.35) and omega 6 fatty acids (CC 0.28, 95% CI 0.23 to 0.34) in parent-child comparisons. Additional adjustment for factors that potentially influence metabolite levels (age, socioeconomic status, fasting time and processing lag time) had little effect on the degree of correlation in any comparison (supplementary tables 5 and 6).

DISCUSSION

Principal findings

Here we present age and sex differences, describing the distribution of detailed/NMR-based metabolite measures in Australian 11-12 year old children and their parents, and demonstrate that many metabolite measures have moderate parent-child concordance and in general there is a high level of agreement in the magnitude of concordance across metabolites. In accord with previous studies, we observed major differences in metabolite levels between childhood and adulthood and also differences by sex in both childhood and adulthood. We also observed variability in the magnitude of differences by sex for several metabolites in childhood compared to adulthood and identified a complex interplay of correlations of specific metabolites between parents and their children according to parent-child sex relationships.

Strengths and weaknesses

This is the first major cohort study to report both sex- and cross-generational differences in metabolomic concentrations in mid-childhood to adulthood utilising the NMR platform. Further strengths include the large number of parent-child dyads representing a wide range of parent ages, the national population-based sample and the state-of-the-art measurements. Replication studies exploring sex differences at earlier and later stages of childhood and adolescence would strengthen findings.

An important limitation is that paternal factors were not fully represented, as most parental samples were from mothers (a well-documented problem in longitudinal cohort studies). This also limited sex-specific parental contribution analysis; further studies including more fathers are warranted. Additional limitations are that, without samples from both parents for each child, we could not estimate heritability, and our results might not apply to mid-life adults who are not parents (although we see no good reason why these would differ greatly). The original uptake of just over 50% and subsequent attrition within LSAC and then the CheckPoint has led to a relatively advantaged sample, but nonetheless participants varied widely on key potential confounders (eg disadvantage, age) and this was at least partly offset by application or consideration of survey weights. Given the large number of metabolites and modest sample size, considerable uncertainty remains in any ranking of the various effects across metabolites. In addition, given the descriptive aims of the paper, additional factors and potential confounders not considered could explain some of the results observed.

Meaning and implications for clinicians and policymakers

Overall, we found a difference in metabolite profile between children and their parents. This was apparent for specific metabolite measures (such as some amino acids) as well as the distribution of metabolites (such as lipid composition of lipoproteins of different density). Some measures were higher in adults, some similar, while a minority were lower. Previous studies, largely in adults, have identified a range of specific metabolite changes with age, particularly from mid to late adulthood.[27] This includes a general decrease in several amino acid species, which contrasts with our findings from childhood to mid adulthood.[8] Only the amino acid glutamine showed this pattern in our dataset.

Differences in children by sex (±0.2 SD) were generally much smaller than in adults (±0.8 SD). Large metabolomic studies using alternative platforms have previously reported reproducible, sex-specific signatures in circulating metabolite profile in adults.[28] [29] This includes differences in amino acid and lipid serum concentrations, potentially influenced by sex-specific effects of genetic polymorphisms on metabolite levels. [29] [30] As in our study, most amino acids have usually been reported to be higher in men than women.[29] [31] For example, in a recent study of 507 metabolic markers in 1756 individuals (903 female and 853 male aged ~60 years), one third of metabolites showed significant sexual dimorphism. These were predominantly related to pathways of steroid metabolism, fatty acids, other lipids, and a large proportion of amino acids.[31] Of particular note, branched chain amino acids (BCAAs) and their related metabolic products were amongst the most differentially represented, with much higher isoleucine, leucine and valine in males. A similar finding of higher leucine and valine was also noted in the Cooperative Health Research in the Region of Augsburg (KORA) follow-ups 3 (F3) and 4 (F4) analysis of >3000 adults,[29] consistent with our observations in adulthood.

In children, we found that sex differences for leucine and valine were smaller but in the same direction as adults. Several lines of evidence implicate BCAA metabolism with metabolic risk in humans. For example, three candidate genes for obesity and/or type 2 diabetes mellitus (T2DM) are involved in the BCAA metabolic pathway.[32] In a recent large meta-analysis of metabolomics in diabetes, a >30% higher risk of type 2 diabetes was found per SD increase in isoleucine, leucine, valine or tyrosine, whereas glycine and glutamine were inversely associated with risk.[32] Several clinical studies have also reported that BCAAs positively correlate with insulin resistance, homeostatic model assessment (HOMA) index and levels of haemoglobin A1c (HbA1c), while longitudinal studies have reported that

increased blood BCAAs are predictive of future insulin resistance and type 2 diabetes (T2D).[33] It is intriguing to speculate that the higher BCAA in males from early life could contribute to the well-described increasing prevalence of T2D in men. Levels of BCAA are elevated in females with Polycystic Ovary Syndrome (PCOS), potentially contributing to the associated insulin resistance.[34] However, it remains unclear whether BCAA are on the causal pathway to T2D or result from adverse metabolic health. Our demonstration that the sex differences in BCAA possibly arise early in life offers potential to track their association with sex-specific measures of metabolic health from an early age to help clarify where they lie on the causal pathway.

In accord with previous adult studies[29], we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of ~10,000 adults with cardiovascular disease (CVD), with replication in >53,000 subjects, identified a genetic variant in carbamoyl-phosphate synthase 1 (*CPS1)* (linked to plasma glycine levels) to be strongly associated with a reduced risk of CVD in women ($p=6.3x10^{-5}$) but not men (p=0.95), suggesting a direct link between glycine levels and CVD risk, although whether this is a causal association remains unclear.[35] It will be interesting in the future to explore the link between variants in *CPS1* and circulating glycine levels from early life to adulthood in relation to markers of cardiovascular health in females.

The small sex-differences of HDL cholesterol and ApoA-1 in children compared to adults is consistent with modest differences in children, whereas substantial differences in adulthood have previously been reported.[36] ApoA-1 was more abundant in boys, while ApoB was higher in girls, leading to a higher ApoB/ApoA-1 ratio in girls. The opposite pattern was found in our limited sample of fathers relative to mothers. These data are surprising and differ from a similarly sized study of slightly older European adolescent children (mean age 15 years) that found higher ApoA-1 and ApoB in girls relative to boys.[37] Interestingly, a higher ApoB/ApoA-1 ratio has been strongly linked to increased coronary risk in adults,[38-40] suggesting that sex differences may alter with increasing age, in keeping with the increased CVD risk in adult males. ApoA-1 is the main protein component of HDL cholesterol[41] thus the differences in trajectories in lipids and HDL cholesterol for boys and girls across childhood that have been reported[42 43] could partially explain this observation.

These are the first data on the mother-child or parent-child correlations of NMR metabolites. Smaller studies have reported positive correlations between parents and children for a limited range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL

cholesterol and triglycerides measured using conventional methods. We found positive correlations between parents and children for the same lipid measures (although measured using NMR) consistent with previously reported findings. One study reported a positive association between the serum lipid levels of 4 year old children (n=127) and their parents (122 mothers and 118 fathers) [44] while another study of children aged 6-18 (n=255) and their parents (n=179) found that the age of the child influenced the degree of correlation of several lipid measures, with older (10-18 years) children more similar to their parents in terms of triglyceride levels than younger individuals (6-9 years).[12]

Unanswered questions and future research

The temporal and sex specific dynamism of the metabolomics data we describe here offer considerable opportunities for identification of biomarkers of risk for a range of non-communicable diseases early in life, to inform targeted interventions and monitor their efficacy. Combining metabolomics with other 'omics data (such as genetics), as is increasingly reported from large adult studies, offers considerable promise in understanding the causal pathways that link early life exposures, genetics and intermediate phenotypes with later onset chronic disease, and in identifying clinically relevant biomarkers.

In conclusion, we describe the metabolite profile from mid-childhood and adulthood in a population-based sample, together with parent-child concordance and differences by sex in children and adults. In this descriptive paper, distinct differences in profiles were observed by age and sex, as well as considerable evidence of correlation between parent and child measures. These data will be informative for investigation of the childhood origins of adult non-communicable diseases and for comparative studies across populations.

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REDCap (Research Electronic Data Capture) tools were used in this study. More information about this software can be found at: www.project-redcap.org.

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COMPETING INTERESTS

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and decision to submit the manuscript for publication. Research at the MCRI is supported by the Victorian Government's Operational Infrastructure Support Program.

CONTRIBUTIONS

DB, RS and JC conceptualised and developed the Metabolomics Checkpoint study. SE and JC undertook all aspects of data analysis. SAC coordinated the acquisition of metabolomics data and provided critical review of this manuscript. MW, the Principal Investigator of the Child Health CheckPoint, planned the analyses and provided critical review of this manuscript. SE and RS drafted the manuscript. PW, MJ, TD, KL, JC, DB provided critical expert advice and critical review of this manuscript.

DATA SHARING STATEMENT

Dataset and technical documents available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au

FIGURE CAPTIONS AND FOOTNOTES

Figure 1: Participant flow chart.

n=number of families, c=number of children, p=number of attending adults,

MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,

LSAC=Longitudinal Study of Australian Children

*Unable to analyse due to insufficient volume or poor quality sample

^Data from 6 non-biological child-parent pairs excluded from concordance analyses

Figure 2: Differences in metabolite levels between children and adults.

Association measures are SD difference in metabolite concentration for adults compared to children. Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 3: Sex differences in metabolite levels in childhood and adulthood.

Association measures are SD difference in metabolite concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

Figure 4: Parent:child correlation for metabolite measures.

Pearson's correlation coefficients for all children with all parents (A); and for boys (blue) with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence intervals. Correlation coefficients with associated 95% confidence intervals are listed in

supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

SUPPLEMENTARY DOCUMENTS

Supplementary figure 1: Correlation of NMR measures in children.

Heatmap showing the correlation between metabolite measures in children. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 2: Correlation of NMR metabolite measures in parents.

Heatmap showing the correlation between metabolite measures in parents. The correlations shown are Spearman's correlation coefficients with blue cells representing negative correlations and red cells representing positive correlations.

Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each cholesterol and apolipoprotein measure.

Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each fatty acid and fatty acid ratio measure.

Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass particles.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for total lipids within each of the 14 lipoprotein subclass particles.

Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for lipoprotein particle sizes and triglyceride measures.

Supplementary figure 7: Density plots for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.

Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.

Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.

Supplementary table 2: Mean difference in metabolite levels in adults compared to children in absolute concentration units.

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

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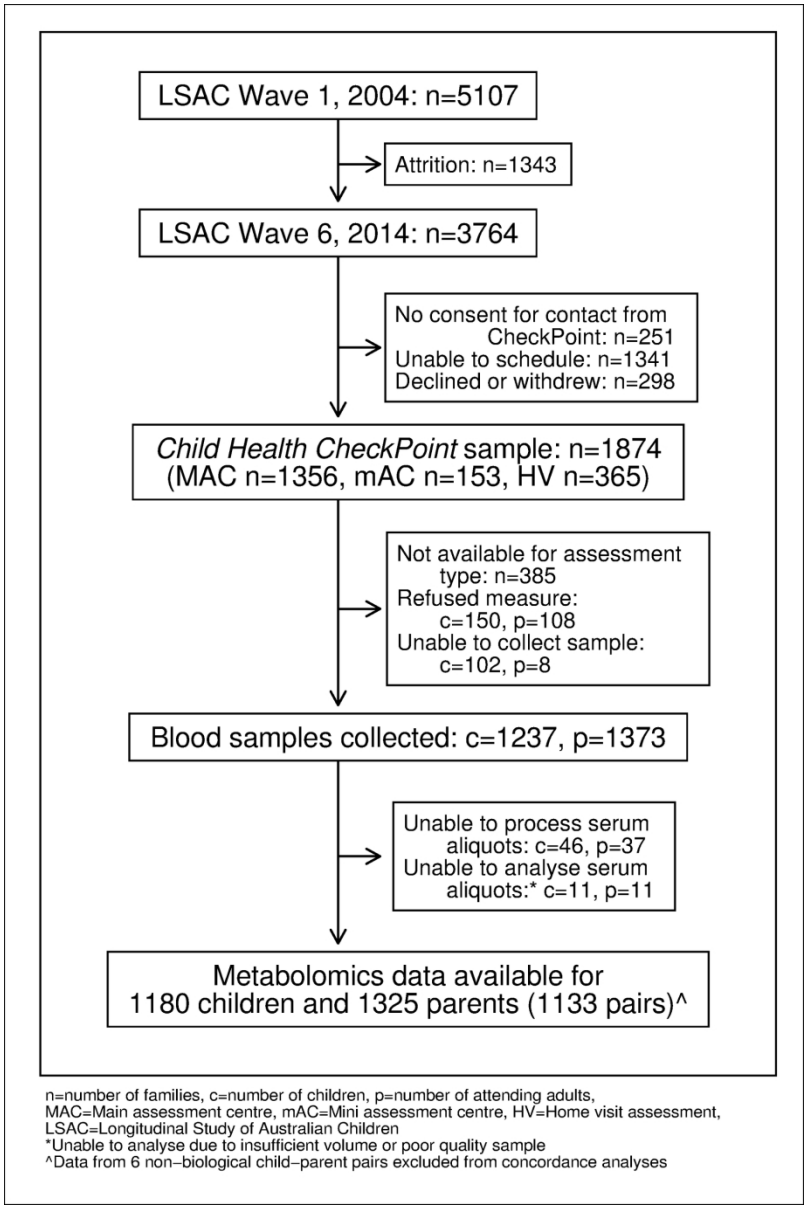

Figure 1: Participant flow chart.
n=number of families, c=number of children, p=number of attending adults,
MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,
LSAC=Longitudinal Study of Australian Children
*Unable to analyse due to insufficient volume or poor quality sample
^Data from 6 non-biological child-parent pairs excluded from concordance analyses

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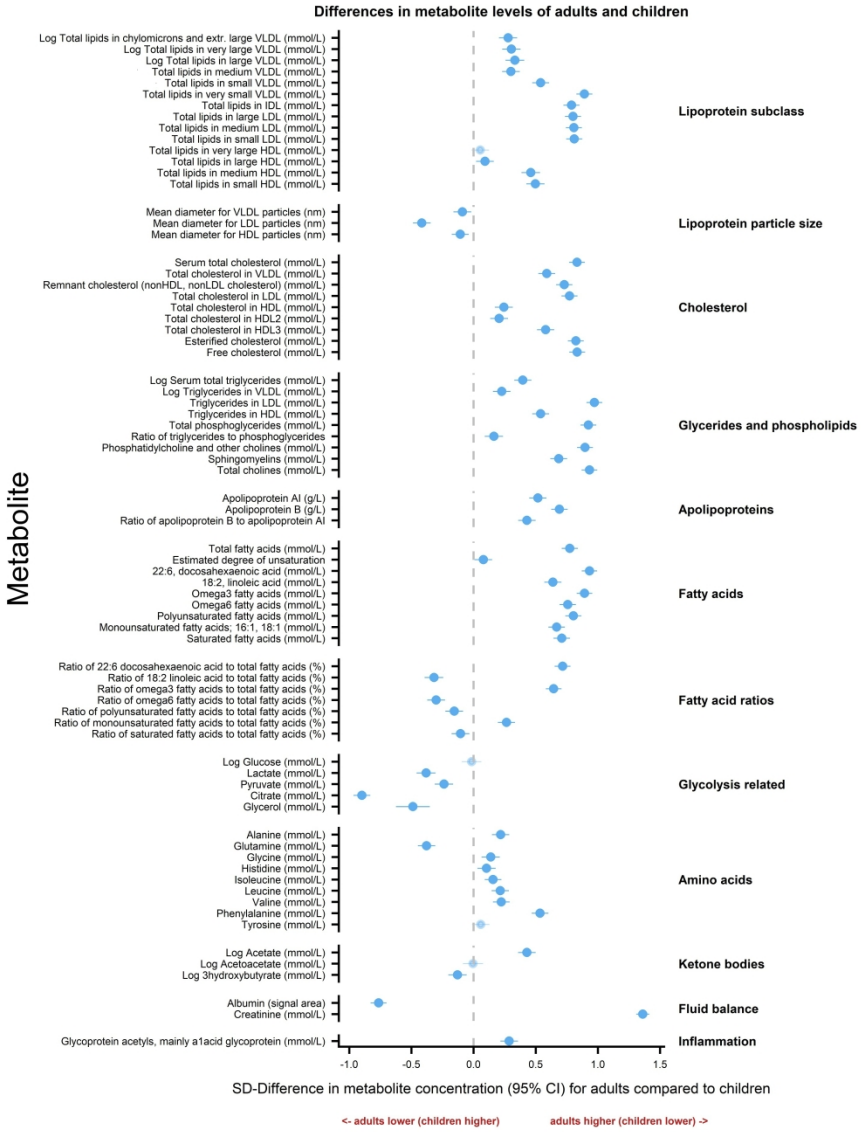

Figure 2: Differences in metabolite levels between children and adults.
Association measures are SD difference in metabolite concentration for adults compared to children. Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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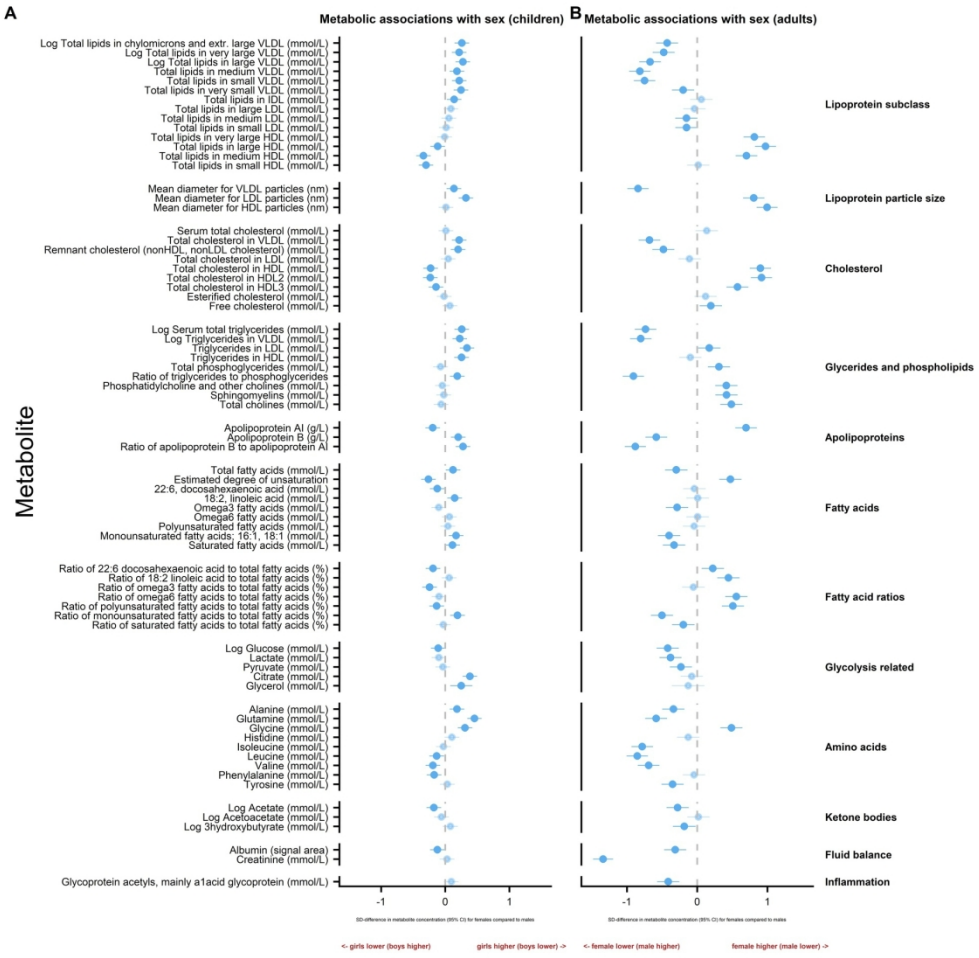

Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood.
Association measures are SD difference in metabolite concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals and associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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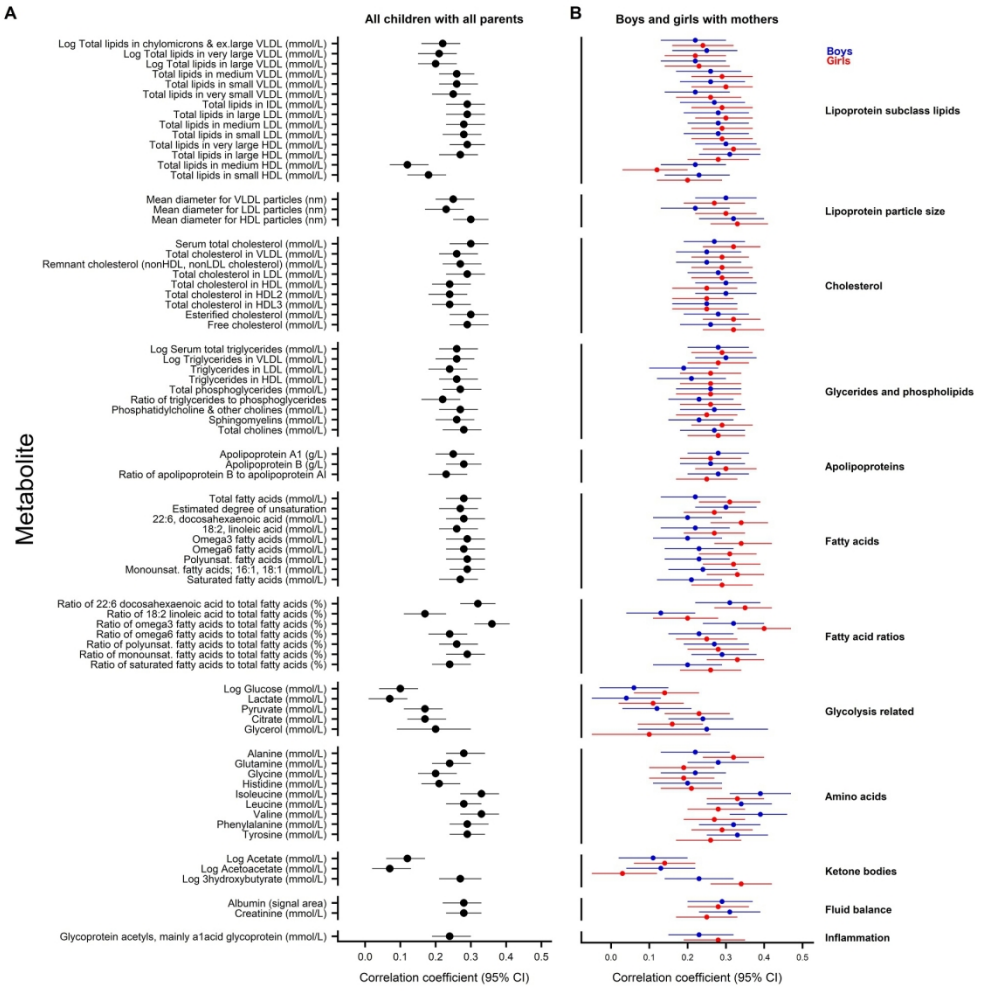

Figure 4: Parent:child correlation for metabolite measures. Pearson's correlation coefficients for all children with all parents (A); and for boys (blue) with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence intervals. Correlation coefficients with associated 95% confidence intervals are listed in supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.

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Supplementary table 1: Weighted mean (SD) of metabolite measures in children and parents.*

Metabolic subgroup	Children									Adults								
	Male			Female			All			Male			Female			All		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	575	0.005	4.826	605	0.011	3.260	1180	0.007	4.054	177	0.040	2.101	1148	0.013	2.822	1325	0.015	2.777
Total lipids in very large VLDL (mmol/L)*	575	0.006	6.246	605	0.014	4.800	1180	0.010	5.521	177	0.087	2.419	1148	0.022	3.458	1325	0.027	3.398
Total lipids in large VLDL (mmol/L)*	575	0.059	3.945	605	0.121	2.488	1180	0.085	3.234	177	0.423	1.287	1148	0.161	1.666	1325	0.182	1.665
Total lipids in medium VLDL (mmol/L)	575	0.441	0.270	605	0.478	0.272	1180	0.460	0.271	177	0.959	0.630	1148	0.548	0.366	1325	0.602	0.432
Total lipids in small VLDL (mmol/L)	575	0.381	0.153	605	0.405	0.146	1180	0.393	0.149	177	0.690	0.279	1148	0.494	0.216	1325	0.520	0.234
Total lipids in very small VLDL (mmol/L)	575	0.325	0.070	605	0.342	0.076	1180	0.334	0.073	177	0.451	0.111	1148	0.426	0.110	1325	0.429	0.110
Total lipids in IDL (mmol/L)	575	0.804	0.176	605	0.834	0.183	1180	0.819	0.180	177	0.985	0.261	1148	0.999	0.240	1325	0.997	0.242
Total lipids in large LDL (mmol/L)	575	0.917	0.220	605	0.941	0.228	1180	0.929	0.224	177	1.162	0.327	1148	1.155	0.298	1325	1.156	0.301
Total lipids in medium LDL (mmol/L)	575	0.511	0.136	605	0.519	0.140	1180	0.515	0.138	177	0.676	0.214	1148	0.655	0.185	1325	0.658	0.189
Total lipids in small LDL (mmol/L)	575	0.338	0.083	605	0.340	0.087	1180	0.339	0.085	177	0.439	0.135	1148	0.425	0.114	1325	0.427	0.117
Total lipids in very large HDL (mmol/L)	575	0.482	0.196	605	0.495	0.184	1180	0.488	0.189	177	0.320	0.189	1148	0.497	0.229	1325	0.474	0.232
Total lipids in large HDL (mmol/L)	575	0.874	0.291	605	0.859	0.275	1180	0.866	0.282	177	0.509	0.335	1148	0.900	0.382	1325	0.849	0.399
Total lipids in medium HDL (mmol/L)	575	0.917	0.127	605	0.871	0.126	1180	0.894	0.128	177	0.828	0.241	1148	0.971	0.175	1325	0.952	0.191
Total lipids in small HDL (mmol/L)	575	1.039	0.103	605	0.997	0.115	1180	1.018	0.111	177	1.055	0.254	1148	1.085	0.138	1325	1.081	0.157
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	575	37.063	1.633	605	37.238	1.557	1180	37.152	1.591	177	38.527	1.737	1148	36.943	1.599	1325	37.152	1.701
Mean diameter for LDL particles (nm)	575	23.587	0.103	605	23.628	0.109	1180	23.608	0.107	177	23.487	0.093	1148	23.573	0.100	1325	23.562	0.104
Mean diameter for HDL particles (nm)	575	10.081	0.233	605	10.102	0.221	1180	10.092	0.226	177	9.798	0.244	1148	10.068	0.262	1325	10.032	0.275
Cholesterol																		
Serum total cholesterol (mmol/L)	575	3.576	0.620	605	3.596	0.643	1180	3.586	0.629	177	4.161	0.885	1148	4.234	0.828	1325	4.225	0.835
Total cholesterol in VLDL (mmol/L)	575	0.438	0.188	605	0.472	0.189	1180	0.455	0.189	177	0.826	0.395	1148	0.592	0.265	1325	0.623	0.295
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	575	0.944	0.269	605	0.994	0.271	1180	0.970	0.270	177	1.452	0.472	1148	1.219	0.383	1325	1.250	0.402
Total cholesterol in LDL (mmol/L)	575	1.130	0.330	605	1.149	0.341	1180	1.139	0.334	177	1.492	0.498	1148	1.460	0.442	1325	1.464	0.449
Total cholesterol in HDL (mmol/L)	575	1.503	0.274	605	1.453	0.266	1180	1.477	0.270	177	1.217	0.350	1148	1.556	0.363	1325	1.511	0.379
Total cholesterol in HDL2 (mmol/L)	575	1.035	0.254	605	0.988	0.246	1180	1.011	0.250	177	0.751	0.327	1148	1.072	0.335	1325	1.030	0.351
Total cholesterol in HDL3 (mmol/L)	575	0.468	0.024	605	0.466	0.024	1180	0.467	0.024	177	0.466	0.035	1148	0.483	0.033	1325	0.481	0.034
Esterified cholesterol (mmol/L)	572	2.516	0.447	604	2.517	0.460	1176	2.517	0.452	176	2.941	0.636	1147	2.975	0.593	1323	2.971	0.597
Free cholesterol (mmol/L)	572	1.062	0.179	604	1.079	0.186	1176	1.070	0.182	176	1.211	0.273	1147	1.260	0.239	1323	1.253	0.244
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	575	0.918	0.709	605	1.005	0.681	1180	0.962	0.696	177	1.686	0.809	1148	1.129	0.755	1325	1.190	0.782
Triglycerides in VLDL (mmol/L)*	575	0.582	0.885	605	0.648	0.830	1180	0.615	0.858	177	1.249	0.927	1148	0.694	0.945	1325	0.750	0.972
Triglycerides in LDL (mmol/L)	575	0.113	0.024	605	0.123	0.027	1180	0.118	0.026	177	0.153	0.040	1148	0.158	0.044	1325	0.157	0.043
Triglycerides in HDL (mmol/L)	575	0.129	0.030	605	0.136	0.030	1180	0.133	0.030	177	0.161	0.047	1148	0.151	0.040	1325	0.153	0.041
Total phosphoglycerides (mmol/L)	572	1.632	0.240	604	1.620	0.261	1176	1.626	0.250	176	1.865	0.354	1147	1.926	0.340	1323	1.918	0.342
Ratio of triglycerides to phosphoglycerides	572	0.526	0.253	604	0.569	0.277	1176	0.548	0.265	176	0.946	0.587	1147	0.580	0.279	1323	0.628	0.355
Phosphatidylcholine & other cholines (mmol/L)	572	1.691	0.240	604	1.687	0.267	1176	1.689	0.253	175	1.877	0.320	1147	1.978	0.336	1322	1.965	0.335
Sphingomyelins (mmol/L)	572	0.348	0.061	604	0.349	0.064	1176	0.348	0.062	175	0.370	0.070	1147	0.397	0.078	1322	0.394	0.077
Total cholines (mmol/L)	572	2.005	0.256	604	1.997	0.264	1176	2.001	0.259	175	2.185	0.334	1147	2.317	0.351	1322	2.299	0.351
Apolipoproteins																		
Apolipoprotein A1 (g/L)	575	1.509	0.159	605	1.484	0.151	1180	1.497	0.155	177	1.461	0.178	1148	1.589	0.205	1325	1.572	0.206
Apolipoprotein B (g/L)	575	0.682	0.135	604	0.706	0.136	1179	0.694	0.135	177	0.955	0.245	1148	0.812	0.196	1325	0.831	0.208
Ratio of apolipoprotein B to apolipoprotein A	575	0.455	0.097	604	0.479	0.098	1179	0.467	0.098	177	0.660	0.178	1148	0.518	0.136	1325	0.537	0.150
Fatty acids																		
Total fatty acids (mmol/L)	570	9.215	1.697	604	9.370	1.730	1174	9.294	1.709	173	11.850	2.723	1145	10.917	2.392	1318	11.034	2.446
Estimated degree of unsaturation	570	1.212	0.056	604	1.196	0.065	1174	1.204	0.061	173	1.179	0.070	1145	1.212	0.066	1318	1.208	0.068
22:6, docosahexaenoic acid (mmol/L)	570	0.078	0.028	604	0.074	0.029	1174	0.076	0.028	173	0.118	0.051	1145	0.111	0.039	1318	0.112	0.041
18:2, linoleic acid (mmol/L)	570	2.539	0.456	604	2.592	0.464	1174	2.566	0.459	173	2.919	0.567	1145	2.880	0.584	1318	2.885	0.580
Omega3 fatty acids (mmol/L)	570	0.309	0.086	604	0.296	0.083	1174	0.302	0.085	173	0.451	0.160	1145	0.400	0.117	1318	0.406	0.124
Omega6 fatty acids (mmol/L)	570	3.077	0.493	604	3.094	0.492	1174	3.086	0.491	173	3.553	0.648	1145	3.507	0.627	1318	3.513	0.628
Polyunsat. fatty acids (mmol/L)	570	3.386	0.565	604	3.390	0.561	1174	3.388	0.560	173	4.004	0.775	1145	3.907	0.721	1318	3.919	0.726
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	570	2.500	0.632	604	2.587	0.661	1174	2.544	0.646	173	3.520	1.058	1145	3.080	0.917	1318	3.135	0.943
Saturated fatty acids (mmol/L)	570	3.328	0.642	604	3.393	0.682	1174	3.362	0.661	173	4.325	1.088	1145	3.930	0.932	1318	3.979	0.958

Fatty acid ratios																			
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	570	0.841	0.239	604	0.790	0.269	1174	0.815	0.255	173	0.984	0.315	1145	1.023	0.284	1318	1.018	0.287
Ratio of 18:2 linoleic acid to total fatty acids (%)	570	27.741	3.127	604	27.867	3.274	1174	27.805	3.191	173	25.038	3.570	1145	26.656	3.417	1318	26.453	3.467
Ratio of omega3 fatty acids to total fatty acids (%)	570	3.329	0.579	604	3.150	0.600	1174	3.238	0.594	173	3.763	0.799	1145	3.659	0.697	1318	3.672	0.709
Ratio of omega6 fatty acids to total fatty acids (%)	570	33.650	3.047	604	33.308	3.228	1174	33.475	3.133	173	30.468	3.757	1145	32.502	3.311	1318	32.247	3.424
Ratio of polyunsat. fatty acids to total fatty acids (%)	570	36.979	3.235	604	36.459	3.514	1174	36.713	3.377	173	34.231	3.878	1145	36.161	3.588	1318	35.918	3.669
Ratio of monounsat. fatty acids to total fatty acids (%)	570	26.911	2.569	604	27.375	2.678	1174	27.148	2.625	173	29.366	2.968	1145	27.889	2.842	1318	28.075	2.891
Ratio of saturated fatty acids to total fatty acids (%)	570	36.110	1.675	604	36.167	1.802	1174	36.139	1.734	173	36.402	2.027	1145	35.950	2.032	1318	36.007	2.031
Glycolysis related																			
Glucose (mmol/L)*	574	1.350	0.115	605	1.336	0.105	1179	1.342	0.11	176	1.415	0.205	1148	1.334	0.176	1324	1.344	0.182
Lactate (mmol/L)	575	1.770	0.459	605	1.718	0.434	1180	1.743	0.446	177	1.696	0.472	1148	1.562	0.480	1325	1.580	0.480
Pyruvate (mmol/L)	574	0.100	0.024	605	0.098	0.023	1179	0.099	0.023	177	0.101	0.031	1147	0.093	0.034	1324	0.094	0.033
Citrate (mmol/L)	575	0.125	0.017	604	0.131	0.018	1179	0.128	0.018	177	0.110	0.016	1148	0.111	0.016	1325	0.111	0.016
Glycerol (mmol/L)#	240	0.078	0.021	283	0.083	0.022	523	0.081	0.021	84	0.073	0.021	470	0.071	0.023	554	0.071	0.023
Amino acids																			
Alanine (mmol/L)	575	0.387	0.061	605	0.396	0.060	1180	0.391	0.060	176	0.423	0.065	1147	0.399	0.060	1323	0.402	0.061
Glutamine (mmol/L)	575	0.474	0.050	605	0.497	0.051	1180	0.485	0.051	177	0.490	0.063	1148	0.456	0.066	1325	0.461	0.066
Glycine (mmol/L)	574	0.261	0.032	604	0.270	0.034	1178	0.265	0.033	176	0.243	0.029	1148	0.274	0.061	1324	0.270	0.059
Histidine (mmol/L)	574	0.065	0.009	605	0.065	0.008	1179	0.065	0.008	176	0.066	0.008	1148	0.065	0.009	1324	0.065	0.009
Isoleucine (mmol/L)	574	0.054	0.019	605	0.053	0.019	1179	0.054	0.019	174	0.072	0.021	1146	0.055	0.020	1320	0.057	0.021
Leucine (mmol/L)	575	0.073	0.019	605	0.071	0.019	1180	0.072	0.019	177	0.097	0.029	1148	0.074	0.021	1325	0.077	0.023
Valine (mmol/L)	575	0.162	0.037	604	0.156	0.035	1179	0.159	0.036	177	0.192	0.036	1147	0.162	0.042	1324	0.166	0.042
Phenylalanine (mmol/L)	575	0.068	0.009	605	0.066	0.009	1180	0.067	0.009	177	0.073	0.011	1148	0.072	0.011	1325	0.073	0.011
Tyrosine (mmol/L)	574	0.054	0.014	605	0.055	0.014	1179	0.055	0.014	176	0.060	0.013	1148	0.054	0.015	1324	0.055	0.015
Ketone bodies																			
Acetate (mmol/L)*	575	0.031	0.423	605	0.030	0.404	1180	0.030	0.413	177	0.037	0.655	1146	0.033	0.600	1323	0.034	0.609
Acetoacetate (mmol/L)*	575	0.025	1.310	605	0.023	1.429	1180	0.024	1.367	177	0.023	2.116	1147	0.024	1.278	1324	0.024	1.403
3hydroxybutyrate (mmol/L)*#	555	0.100	0.786	580	0.103	0.826	1135	0.101	0.805	170	0.104	0.669	1098	0.096	0.781	1268	0.097	0.769
Fluid balance																			
Albumin (signal area)	574	0.093	0.005	605	0.092	0.005	1179	0.093	0.005	177	0.090	0.005	1148	0.088	0.005	1325	0.089	0.005
Creatinine (mmol/L)	570	0.040	0.006	600	0.040	0.006	1170	0.040	0.006	173	0.066	0.015	1139	0.054	0.009	1312	0.055	0.010
Inflammation																			
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	575	1.170	0.191	605	1.173	0.186	1180	1.172	0.188	177	1.375	0.366	1148	1.242	0.233	1325	1.260	0.258

* geometric mean [relative SD] when skewed variable

Note: The presence of ethanol in a sample can affect quantification of glycerol and on some occasions 3hydroxybutyrate. Ethanol can be introduced in to a sample from disinfectants used during blood collection/processing of sample.

Supplementary table 2: Mean difference in metabolite levels in adults compared to children in absolute concentration unit

Metabolic subgroup	Differences by age (Adults - Child)				
	Estimate	95% CI	P-value	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.704	(0.519, 0.890)	<0.001	<0.001	2.534
Total lipids in very large VLDL (mmol/L)*	0.922	(0.700, 1.145)	<0.001	<0.001	3.031
Total lipids in large VLDL (mmol/L)*	0.648	(0.502, 0.795)	<0.001	<0.001	1.950
Total lipids in medium VLDL (mmol/L)	0.105	(0.080, 0.129)	<0.001	<0.001	0.348
Total lipids in small VLDL (mmol/L)	0.107	(0.094, 0.121)	<0.001	<0.001	0.199
Total lipids in very small VLDL (mmol/L)	0.093	(0.086, 0.099)	<0.001	<0.001	0.104
Total lipids in IDL (mmol/L)	0.181	(0.166, 0.196)	<0.001	<0.001	0.230
Total lipids in large LDL (mmol/L)	0.229	(0.211, 0.247)	<0.001	<0.001	0.286
Total lipids in medium LDL (mmol/L)	0.144	(0.132, 0.155)	<0.001	<0.001	0.178
Total lipids in small LDL (mmol/L)	0.089	(0.082, 0.096)	<0.001	<0.001	0.110
Total lipids in very large HDL (mmol/L)	0.012	(-0.003, 0.027)	0.128	0.132	0.217
Total lipids in large HDL (mmol/L)	0.032	(0.007, 0.057)	0.011	0.012	0.353
Total lipids in medium HDL (mmol/L)	0.076	(0.064, 0.089)	<0.001	<0.001	0.166
Total lipids in small HDL (mmol/L)	0.068	(0.058, 0.078)	<0.001	<0.001	0.137
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	-0.147	(-0.263, -0.031)	0.013	0.014	1.633
Mean diameter for LDL particles (nm)	-0.044	(-0.052, -0.037)	<0.001	<0.001	0.106
Mean diameter for HDL particles (nm)	-0.027	(-0.045, -0.010)	0.002	0.003	0.256
Cholesterol					
Serum total cholesterol (mmol/L)	0.670	(0.619, 0.721)	<0.001	<0.001	0.805
Total cholesterol in VLDL (mmol/L)	0.146	(0.129, 0.163)	<0.001	<0.001	0.249
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.261	(0.237, 0.284)	<0.001	<0.001	0.357
Total cholesterol in LDL (mmol/L)	0.327	(0.300, 0.354)	<0.001	<0.001	0.424
Total cholesterol in HDL (mmol/L)	0.082	(0.058, 0.106)	<0.001	<0.001	0.337
Total cholesterol in HDL2 (mmol/L)	0.064	(0.042, 0.086)	<0.001	<0.001	0.311
Total cholesterol in HDL3 (mmol/L)	0.018	(0.016, 0.020)	<0.001	<0.001	0.031
Esterified cholesterol (mmol/L)	0.474	(0.437, 0.511)	<0.001	<0.001	0.576
Free cholesterol (mmol/L)	0.195	(0.180, 0.210)	<0.001	<0.001	0.234
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	0.176	(0.145, 0.206)	<0.001	<0.001	0.443
Triglycerides in VLDL (mmol/L)*	0.140	(0.096, 0.183)	<0.001	<0.001	0.615
Triglycerides in LDL (mmol/L)	0.040	(0.038, 0.043)	<0.001	<0.001	0.042
Triglycerides in HDL (mmol/L)	0.020	(0.017, 0.022)	<0.001	<0.001	0.037
Total phosphoglycerides (mmol/L)	0.311	(0.290, 0.332)	<0.001	<0.001	0.337
Ratio of triglycerides to phosphoglycerides	0.049	(0.027, 0.071)	<0.001	<0.001	0.299
Phosphatidylcholine & other cholines (mmol/L)	0.295	(0.274, 0.316)	<0.001	<0.001	0.329
Sphingomyelins (mmol/L)	0.052	(0.047, 0.057)	<0.001	<0.001	0.075
Total cholines (mmol/L)	0.323	(0.302, 0.345)	<0.001	<0.001	0.347
Apolipoproteins					
Apolipoprotein A1 (g/L)	0.099	(0.086, 0.112)	<0.001	<0.001	0.191
Apolipoprotein B (g/L)	0.125	(0.113, 0.137)	<0.001	<0.001	0.182
Ratio of apolipoprotein B to apolipoprotein A1	0.055	(0.046, 0.064)	<0.001	<0.001	0.127
Fatty acids					
Total fatty acids (mmol/L)	1.738	(1.592, 1.885)	<0.001	<0.001	2.245
Estimated degree of unsaturation	0.005	(0.000, 0.009)	0.030	0.031	0.063
22:6, docosahexaenoic acid (mmol/L)	0.037	(0.035, 0.040)	<0.001	<0.001	0.040
18:2, linoleic acid (mmol/L)	0.347	(0.310, 0.384)	<0.001	<0.001	0.545
Omega3 fatty acids (mmol/L)	0.105	(0.098, 0.113)	<0.001	<0.001	0.118
Omega6 fatty acids (mmol/L)	0.453	(0.414, 0.492)	<0.001	<0.001	0.597
Polyunsat. fatty acids (mmol/L)	0.558	(0.513, 0.603)	<0.001	<0.001	0.695
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.568	(0.512, 0.625)	<0.001	<0.001	0.850
Saturated fatty acids (mmol/L)	0.612	(0.554, 0.669)	<0.001	<0.001	0.862

Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.209	(0.190, 0.228)	<0.001	<0.001	0.292
Ratio of 18:2 linoleic acid to total fatty acids (%)	-1.079	(-1.331, -0.827)	<0.001	<0.001	3.387
Ratio of omega3 fatty acids to total fatty acids (%)	0.446	(0.403, 0.490)	<0.001	<0.001	0.693
Ratio of omega6 fatty acids to total fatty acids (%)	-0.992	(-1.227, -0.757)	<0.001	<0.001	3.296
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.546	(-0.794, -0.298)	<0.001	<0.001	3.507
Ratio of monounsat. fatty acids to total fatty acids (%)	0.741	(0.547, 0.934)	<0.001	<0.001	2.797
Ratio of saturated fatty acids to total fatty acids (%)	-0.195	(-0.328, -0.062)	0.004	0.005	1.863
Glycolysis related					
Glucose (mmol/L)*	-0.002	(-0.014, 0.009)	0.700	0.709	0.147
Lactate (mmol/L)	-0.180	(-0.215, -0.144)	<0.001	<0.001	0.471
Pyruvate (mmol/L)	-0.007	(-0.009, -0.005)	<0.001	<0.001	0.029
Citrate (mmol/L)	-0.017	(-0.018, -0.016)	<0.001	<0.001	0.019
Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	<0.001	<0.001	0.023
Amino acids					
Alanine (mmol/L)	0.013	(0.009, 0.017)	<0.001	<0.001	0.060
Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	<0.001	<0.001	0.060
Glycine (mmol/L)	0.007	(0.003, 0.010)	<0.001	<0.001	0.049
Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	0.006	0.009
Isoleucine (mmol/L)	0.003	(0.002, 0.004)	<0.001	<0.001	0.019
Leucine (mmol/L)	0.004	(0.003, 0.006)	<0.001	<0.001	0.021
Valine (mmol/L)	0.009	(0.006, 0.011)	<0.001	<0.001	0.039
Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	<0.001	<0.001	0.010
Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	0.105	0.014
Ketone bodies					
Acetate (mmol/L)*	0.101	(0.084, 0.117)	<0.001	<0.001	0.235
Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	0.922	1.022
3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	0.001	0.493
Fluid balance					
Albumin (signal area)	-0.004	(-0.004, -0.004)	<0.001	<0.001	0.005
Creatinine (mmol/L)	0.016	(0.015, 0.017)	<0.001	0.001	0.012
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.062	(0.047, 0.078)	<0.001	<0.001	0.217

* Metabolite has been log transformed

^ Benjamini-Hochberg adjusted p-value

Associations in Figure 2 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor. Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Metabolic subgroup	Differences for children (Female - Male)				
	Estimate	95% CI	pvalue	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.737	(0.414, 1.059)	<0.001	<0.001	2.845
Total lipids in very large VLDL (mmol/L)*	0.744	(0.355, 1.134)	<0.001	0.001	3.428
Total lipids in large VLDL (mmol/L)*	0.663	(0.390, 0.936)	<0.001	<0.001	2.411
Total lipids in medium VLDL (mmol/L)	0.049	(0.018, 0.080)	0.002	0.004	0.269
Total lipids in small VLDL (mmol/L)	0.032	(0.015, 0.048)	<0.001	0.001	0.146
Total lipids in very small VLDL (mmol/L)	0.018	(0.010, 0.027)	<0.001	<0.001	0.074
Total lipids in IDL (mmol/L)	0.025	(0.004, 0.046)	0.017	0.035	0.182
Total lipids in large LDL (mmol/L)	0.020	(-0.006, 0.046)	0.132	0.187	0.227
Total lipids in medium LDL (mmol/L)	0.008	(-0.008, 0.024)	0.338	0.416	0.139
Total lipids in small LDL (mmol/L)	0.001	(-0.008, 0.011)	0.788	0.822	0.086
Total lipids in very large HDL (mmol/L)	-0.002	(-0.023, 0.020)	0.882	0.882	0.190
Total lipids in large HDL (mmol/L)	-0.033	(-0.066, -0.001)	0.044	0.074	0.283
Total lipids in medium HDL (mmol/L)	-0.045	(-0.059, -0.030)	<0.001	<0.001	0.131
Total lipids in small HDL (mmol/L)	-0.035	(-0.048, -0.022)	<0.001	<0.001	0.116
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	0.215	(0.035, 0.395)	0.019	0.038	1.580
Mean diameter for LDL particles (nm)	0.035	(0.023, 0.047)	<0.001	0.000	0.108
Mean diameter for HDL particles (nm)	0.003	(-0.023, 0.028)	0.847	0.870	0.226
Cholesterol					
Serum total cholesterol (mmol/L)	0.007	(-0.066, 0.079)	0.857	0.869	0.634
Total cholesterol in VLDL (mmol/L)	0.040	(0.019, 0.061)	<0.001	0.001	0.184
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.053	(0.023, 0.083)	0.001	0.002	0.265
Total cholesterol in LDL (mmol/L)	0.016	(-0.023, 0.054)	0.427	0.518	0.336
Total cholesterol in HDL (mmol/L)	-0.062	(-0.093, -0.031)	<0.001	<0.001	0.273
Total cholesterol in HDL2 (mmol/L)	-0.059	(-0.087, -0.030)	<0.001	<0.001	0.253
Total cholesterol in HDL3 (mmol/L)	-0.003	(-0.006, -0.001)	0.013	0.027	0.024
Esterified cholesterol (mmol/L)	-0.008	(-0.060, 0.044)	0.755	0.798	0.455
Free cholesterol (mmol/L)	0.013	(-0.008, 0.034)	0.211	0.284	0.184
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	0.101	(0.056, 0.145)	<0.001	<0.001	0.390
Triglycerides in VLDL (mmol/L)*	0.125	(0.062, 0.187)	<0.001	<0.001	0.551
Triglycerides in LDL (mmol/L)	0.009	(0.006, 0.012)	<0.001	<0.001	0.026
Triglycerides in HDL (mmol/L)	0.007	(0.004, 0.011)	<0.001	<0.001	0.029
Total phosphoglycerides (mmol/L)	-0.018	(-0.047, 0.010)	0.206	0.282	0.249
Ratio of triglycerides to phosphoglycerides	0.051	(0.020, 0.083)	0.001	0.003	0.274
Phosphatidylcholine & other cholines (mmol/L)	-0.011	(-0.040, 0.018)	0.447	0.534	0.251
Sphingomyelins (mmol/L)	-0.001	(-0.009, 0.006)	0.706	0.757	0.063
Total cholines (mmol/L)	-0.017	(-0.046, 0.013)	0.268	0.354	0.257
Apolipoproteins					
Apolipoprotein A1 (g/L)	-0.030	(-0.048, -0.013)	0.001	0.002	0.155
Apolipoprotein B (g/L)	0.027	(0.012, 0.042)	0.001	0.002	0.133
Ratio of apolipoprotein B to apolipoprotein A	0.027	(0.016, 0.038)	<0.001	<0.001	0.098
Fatty acids					
Total fatty acids (mmol/L)	0.200	(0.011, 0.389)	0.038	0.065	1.650
Estimated degree of unsaturation	-0.016	(-0.022, -0.009)	<0.001	<0.001	0.060
22:6, docosahexaenoic acid (mmol/L)	-0.004	(-0.007, -0.000)	0.033	0.059	0.028
18:2, linoleic acid (mmol/L)	0.068	(0.015, 0.120)	0.011	0.024	0.458
Omega3 fatty acids (mmol/L)	-0.008	(-0.018, 0.001)	0.083	0.128	0.084
Omega6 fatty acids (mmol/L)	0.031	(-0.024, 0.087)	0.271	0.352	0.485
Polyunsat. fatty acids (mmol/L)	0.023	(-0.041, 0.086)	0.483	0.567	0.553
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.105	(0.034, 0.176)	0.004	0.009	0.623
Saturated fatty acids (mmol/L)	0.073	(-0.000, 0.146)	0.052	0.085	0.638
Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	-0.049	(-0.078, -0.019)	0.001	0.003	0.255
Ratio of 18:2 linoleic acid to total fatty acids (%)	0.197	(-0.173, 0.567)	0.297	0.379	3.233
Ratio of omega3 fatty acids to total fatty acids (%)	-0.145	(-0.213, -0.078)	<0.001	0.000	0.593
Ratio of omega6 fatty acids to total fatty acids (%)	-0.302	(-0.658, 0.054)	0.096	0.142	3.109
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.447	(-0.830, -0.065)	0.022	0.042	3.346
Ratio of monounsat. fatty acids to total fatty acids (%)	0.499	(0.202, 0.796)	0.001	0.003	2.606
Ratio of saturated fatty acids to total fatty acids (%)	-0.051	(-0.251, 0.148)	0.614	0.689	1.743
Glycolysis related					
Glucose (mmol/L)*	-0.013	(-0.026, 0.001)	0.061	0.098	0.118
Lactate (mmol/L)	-0.045	(-0.097, 0.007)	0.088	0.133	0.456
Pyruvate (mmol/L)	-0.001	(-0.004, 0.002)	0.524	0.606	0.024
Citrate (mmol/L)	0.007	(0.005, 0.009)	<0.001	<0.001	0.018
Glycerol (mmol/L)	0.006	(0.002, 0.010)	0.004	0.009	0.023
Amino acids					
Alanine (mmol/L)	0.011	(0.004, 0.017)	0.002	0.004	0.058
Glutamine (mmol/L)	0.023	(0.018, 0.029)	<0.001	<0.001	0.051
Glycine (mmol/L)	0.010	(0.006, 0.014)	<0.001	<0.001	0.032
Histidine (mmol/L)	0.001	(-0.000, 0.002)	0.075	0.118	0.008
Isoleucine (mmol/L)	0.000	(-0.003, 0.002)	0.637	0.693	0.018

Leucine (mmol/L)	-0.002	(-0.005, -0.000)	0.022	0.041	0.018
Valine (mmol/L)	-0.007	(-0.011, -0.003)	0.001	0.003	0.036
Phenylalanine (mmol/L)	-0.002	(-0.003, -0.001)	0.003	0.007	0.009
Tyrosine (mmol/L)	0.000	(-0.001, 0.002)	0.582	0.663	0.014
Ketone bodies					
Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.005	0.166
Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.393	0.992
3hydroxybutyrate (mmol/L)*	0.041	(-0.019, 0.101)	0.178	0.248	0.513
Fluid balance					
Albumin (signal area)	-0.001	(-0.001, -0.000)	0.037	0.064	0.005
Creatinine (mmol/L)	0.000	(-0.001, 0.001)	0.624	0.690	0.007
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.017	(-0.004, 0.038)	0.104	0.151	0.183

* Metabolite has been log transformed
^ Benjamini-Hochberg adjusted p-value
# Associations for children in Figure 3 are presented in SD-units. The conversion factor provided (unweighted standard deviation of each metabolite measure in children) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.
Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 4: Differences in mean metabolite levels in female compared to male adults in absolute concentration units.

Metabolic subgroup	Differences for adults (Female - Male)				
	Estimate	95% CI	pvalue	Adj_p-value^	Conversion factor (SD) #
Lipoprotein subclass lipids					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	-0.930	(-1.271, -0.589)	<0.001	<0.001	2.173
Total lipids in very large VLDL (mmol/L)*	-1.217	(-1.617, -0.818)	<0.001	<0.001	2.555
Total lipids in large VLDL (mmol/L)*	-0.900	(-1.107, -0.693)	<0.001	<0.001	1.343
Total lipids in medium VLDL (mmol/L)	-0.325	(-0.385, -0.264)	<0.001	<0.001	0.398
Total lipids in small VLDL (mmol/L)	-0.167	(-0.201, -0.133)	<0.001	<0.001	0.223
Total lipids in very small VLDL (mmol/L)	-0.022	(-0.039, -0.005)	0.013	0.018	0.107
Total lipids in IDL (mmol/L)	0.014	(-0.023, 0.051)	0.465	0.530	0.236
Total lipids in large LDL (mmol/L)	-0.011	(-0.058, 0.035)	0.634	0.671	0.293
Total lipids in medium LDL (mmol/L)	-0.028	(-0.057, 0.001)	0.058	0.076	0.182
Total lipids in small LDL (mmol/L)	-0.017	(-0.035, 0.001)	0.061	0.079	0.113
Total lipids in very large HDL (mmol/L)	0.195	(0.158, 0.231)	<0.001	<0.001	0.239
Total lipids in large HDL (mmol/L)	0.395	(0.334, 0.455)	<0.001	<0.001	0.405
Total lipids in medium HDL (mmol/L)	0.129	(0.101, 0.158)	<0.001	<0.001	0.184
Total lipids in small HDL (mmol/L)	0.002	(-0.021, 0.025)	0.850	0.874	0.145
Lipoprotein particle size					
Mean diameter for VLDL particles (nm)	-1.414	(-1.669, -1.159)	<0.001	<0.001	1.678
Mean diameter for LDL particles (nm)	0.081	(0.066, 0.096)	<0.001	<0.001	0.100
Mean diameter for HDL particles (nm)	0.278	(0.236, 0.320)	<0.001	<0.001	0.279
Cholesterol					
Serum total cholesterol (mmol/L)	0.112	(-0.018, 0.241)	0.091	0.116	0.817
Total cholesterol in VLDL (mmol/L)	-0.187	(-0.230, -0.145)	<0.001	<0.001	0.275
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	-0.184	(-0.244, -0.124)	<0.001	<0.001	0.383
Total cholesterol in LDL (mmol/L)	-0.048	(-0.117, 0.021)	0.175	0.213	0.437
Total cholesterol in HDL (mmol/L)	0.344	(0.286, 0.401)	<0.001	<0.001	0.382
Total cholesterol in HDL2 (mmol/L)	0.324	(0.271, 0.378)	<0.001	<0.001	0.354
Total cholesterol in HDL3 (mmol/L)	0.019	(0.014, 0.025)	<0.001	<0.001	0.034
Esterified cholesterol (mmol/L)	0.070	(-0.023, 0.163)	0.139	0.172	0.586
Free cholesterol (mmol/L)	0.046	(0.009, 0.084)	0.016	0.023	0.238
Glycerides and phospholipids					
Serum total triglycerides (mmol/L)*	-0.344	(-0.416, -0.273)	<0.001	<0.001	0.468
Triglycerides in VLDL (mmol/L)*	-0.530	(-0.630, -0.429)	<0.001	<0.001	0.659
Triglycerides in LDL (mmol/L)	0.008	(0.001, 0.014)	0.033	0.044	0.044
Triglycerides in HDL (mmol/L)	-0.004	(-0.010, 0.002)	0.228	0.272	0.040
Total phosphoglycerides (mmol/L)	0.106	(0.052, 0.159)	<0.001	<0.001	0.340
Ratio of triglycerides to phosphoglycerides	-0.289	(-0.337, -0.241)	<0.001	<0.001	0.318
Phosphatidylcholine & other cholines (mmol/L)	0.138	(0.086, 0.190)	<0.001	<0.001	0.331
Sphingomyelins (mmol/L)	0.032	(0.020, 0.045)	<0.001	<0.001	0.078
Total cholines (mmol/L)	0.170	(0.115, 0.224)	<0.001	<0.001	0.349
Apolipoproteins					
Apolipoprotein A1 (g/L)	0.146	(0.114, 0.178)	<0.001	<0.001	0.209
Apolipoprotein B (g/L)	-0.115	(-0.146, -0.084)	<0.001	<0.001	0.198
Ratio of apolipoprotein B to apolipoprotein AI	-0.126	(-0.148, -0.105)	<0.001	<0.001	0.144
Fatty acids					
Total fatty acids (mmol/L)	-0.711	(-1.091, -0.330)	<0.001	<0.001	2.388
Estimated degree of unsaturation	0.031	(0.021, 0.042)	<0.001	<0.001	0.066
22:6, docosahexaenoic acid (mmol/L)	-0.002	(-0.008, 0.005)	0.622	0.667	0.041
18:2, linoleic acid (mmol/L)	0.004	(-0.087, 0.094)	0.934	0.947	0.566
Omega3 fatty acids (mmol/L)	-0.035	(-0.054, -0.016)	<0.001	0.001	0.122
Omega6 fatty acids (mmol/L)	0.004	(-0.094, 0.102)	0.936	0.936	0.612
Polyunsat. fatty acids (mmol/L)	-0.031	(-0.144, 0.082)	0.592	0.644	0.708
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	-0.372	(-0.520, -0.225)	<0.001	<0.001	0.930
Saturated fatty acids (mmol/L)	-0.307	(-0.455, -0.159)	<0.001	<0.001	0.932
Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.064	(0.018, 0.110)	0.006	0.009	0.288
Ratio of 18:2 linoleic acid to total fatty acids (%)	1.527	(0.984, 2.070)	<0.001	<0.001	3.430
Ratio of omega3 fatty acids to total fatty acids (%)	-0.038	(-0.152, 0.075)	0.508	0.570	0.710
Ratio of omega6 fatty acids to total fatty acids (%)	1.882	(1.351, 2.412)	<0.001	<0.001	3.376
Ratio of polyunsat. fatty acids to total fatty acids (%)	1.843	(1.272, 2.414)	<0.001	<0.001	3.621
Ratio of monounsat. fatty acids to total fatty acids (%)	-1.456	(-1.914, -0.998)	<0.001	<0.001	2.904
Ratio of saturated fatty acids to total fatty acids (%)	-0.387	(-0.700, -0.074)	0.015	0.022	1.959
Glycolysis related					
Glucose (mmol/L)*	-0.071	(-0.097, -0.044)	<0.001	<0.001	0.168
Lactate (mmol/L)	-0.178	(-0.252, -0.105)	<0.001	<0.001	0.469
Pyruvate (mmol/L)	-0.007	(-0.013, -0.002)	0.004	0.006	0.032

Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.387	0.016
Glycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.328	0.022
Amino acids					
Alanine (mmol/L)	-0.020	(-0.030, -0.011)	<0.001	<0.001	0.060
Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	<0.001	<0.001	0.065
Glycine (mmol/L)	0.029	(0.020, 0.038)	<0.001	<0.001	0.059
Histidine (mmol/L)	-0.001	(-0.003, 0.000)	0.116	0.146	0.009
Isoleucine (mmol/L)	-0.016	(-0.019, -0.013)	<0.001	<0.001	0.021
Leucine (mmol/L)	-0.019	(-0.022, -0.016)	<0.001	<0.001	0.022
Valine (mmol/L)	-0.029	(-0.035, -0.022)	<0.001	<0.001	0.042
Phenylalanine (mmol/L)	0.000	(-0.002, 0.001)	0.576	0.637	0.010
Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	<0.001	<0.001	0.014
Ketone bodies					
Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.001	0.273
Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	0.863	1.048
3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.035	0.472
Fluid balance					
Albumin (signal area)	-0.002	(-0.002, -0.001)	<0.001	<0.001	0.005
Creatinine (mmol/L)	-0.013	(-0.015, -0.012)	<0.001	<0.001	0.010
Inflammation					
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	<0.001	0.239

* Metabolite has been log transformed

^ Benjamini-Hochberg adjusted p-value

Associations for parents in Figure 3 are presented in SD-units. The conversion factor provided (unweighted standard deviaton of each metabolite measure in adults/parents) can be used to convert the association in absolute concentration to SD units by dividing by the conversion factor.

Where metabolite has been log transformed conversion factor is standard deviation of log transformed metabolite

Supplementary table 5: Mother-child concordance; correlations and partial correlations between mothers and their sons, daughters and all children.

Metabolic subgroup	Mother																	
	Boys						Girls						All Children					
	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	433	0.23	0.14 - 0.31	518	0.24	0.16 - 0.32	476	0.21	0.12 - 0.29	987	0.23	0.17 - 0.29	909	0.22	0.16 - 0.28
Total lipids in very large VLDL (mmol/L)*	469	0.25	0.16 - 0.33	433	0.25	0.16 - 0.34	518	0.22	0.14 - 0.30	476	0.20	0.12 - 0.29	987	0.24	0.18 - 0.29	909	0.23	0.17 - 0.29
Total lipids in large VLDL (mmol/L)*	469	0.22	0.13 - 0.30	433	0.24	0.15 - 0.33	518	0.23	0.14 - 0.31	476	0.22	0.14 - 0.31	987	0.22	0.16 - 0.28	909	0.23	0.17 - 0.29
Total lipids in medium VLDL (mmol/L)	469	0.26	0.17 - 0.34	433	0.28	0.19 - 0.36	518	0.29	0.21 - 0.37	476	0.30	0.21 - 0.38	987	0.28	0.22 - 0.34	909	0.29	0.23 - 0.35
Total lipids in small VLDL (mmol/L)	469	0.26	0.18 - 0.35	433	0.28	0.19 - 0.36	518	0.30	0.21 - 0.37	476	0.30	0.21 - 0.38	987	0.29	0.23 - 0.34	909	0.29	0.23 - 0.35
Total lipids in very small VLDL (mmol/L)	469	0.22	0.14 - 0.31	433	0.21	0.12 - 0.30	518	0.26	0.17 - 0.34	476	0.26	0.18 - 0.35	987	0.25	0.19 - 0.30	909	0.25	0.18 - 0.31
Total lipids in IDL (mmol/L)	469	0.27	0.18 - 0.35	433	0.23	0.14 - 0.32	518	0.29	0.21 - 0.37	476	0.31	0.23 - 0.39	987	0.29	0.23 - 0.34	909	0.28	0.22 - 0.34
Total lipids in large LDL (mmol/L)	469	0.28	0.19 - 0.36	433	0.24	0.15 - 0.33	518	0.30	0.22 - 0.37	476	0.32	0.24 - 0.40	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Total lipids in medium LDL (mmol/L)	469	0.28	0.20 - 0.36	433	0.24	0.15 - 0.33	518	0.29	0.21 - 0.37	476	0.32	0.24 - 0.40	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Total lipids in small LDL (mmol/L)	469	0.28	0.19 - 0.36	433	0.24	0.15 - 0.32	518	0.29	0.21 - 0.37	476	0.32	0.23 - 0.40	987	0.28	0.23 - 0.34	909	0.28	0.22 - 0.34
Total lipids in very large HDL (mmol/L)	469	0.30	0.22 - 0.38	433	0.30	0.21 - 0.38	518	0.32	0.24 - 0.39	476	0.30	0.21 - 0.38	987	0.31	0.25 - 0.36	909	0.30	0.24 - 0.36
Total lipids in large HDL (mmol/L)	469	0.31	0.23 - 0.39	433	0.31	0.23 - 0.40	518	0.28	0.20 - 0.36	476	0.26	0.18 - 0.34	987	0.30	0.24 - 0.35	909	0.29	0.23 - 0.35
Total lipids in medium HDL (mmol/L)	469	0.22	0.13 - 0.30	433	0.20	0.11 - 0.29	518	0.12	0.03 - 0.20	476	0.13	0.04 - 0.22	987	0.17	0.11 - 0.23	909	0.17	0.10 - 0.23
Total lipids in small HDL (mmol/L)	469	0.23	0.14 - 0.31	433	0.22	0.13 - 0.31	518	0.20	0.12 - 0.29	476	0.20	0.11 - 0.28	987	0.21	0.15 - 0.27	909	0.21	0.15 - 0.27
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	469	0.30	0.22 - 0.38	433	0.32	0.23 - 0.40	518	0.27	0.19 - 0.35	476	0.25	0.16 - 0.33	987	0.29	0.23 - 0.35	909	0.28	0.22 - 0.34
Mean diameter for LDL particles (nm)	469	0.22	0.13 - 0.31	433	0.20	0.11 - 0.29	518	0.30	0.22 - 0.38	476	0.32	0.24 - 0.40	987	0.26	0.20 - 0.31	909	0.26	0.20 - 0.32
Mean diameter for HDL particles (nm)	469	0.32	0.23 - 0.40	433	0.32	0.23 - 0.40	518	0.33	0.26 - 0.41	476	0.31	0.23 - 0.39	987	0.33	0.27 - 0.38	909	0.31	0.25 - 0.37
Cholesterol																		
Serum total cholesterol (mmol/L)	469	0.27	0.19 - 0.35	433	0.23	0.14 - 0.32	518	0.32	0.24 - 0.39	476	0.34	0.26 - 0.42	987	0.30	0.24 - 0.35	909	0.30	0.24 - 0.35
Total cholesterol in VLDL (mmol/L)	469	0.25	0.17 - 0.34	433	0.27	0.18 - 0.36	518	0.29	0.21 - 0.36	476	0.29	0.21 - 0.37	987	0.28	0.22 - 0.33	909	0.29	0.23 - 0.35
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	469	0.25	0.17 - 0.34	433	0.26	0.17 - 0.34	518	0.29	0.21 - 0.37	476	0.31	0.22 - 0.39	987	0.28	0.22 - 0.34	909	0.29	0.23 - 0.35
Total cholesterol in LDL (mmol/L)	469	0.28	0.20 - 0.36	433	0.24	0.15 - 0.33	518	0.29	0.21 - 0.37	476	0.32	0.23 - 0.40	987	0.29	0.23 - 0.34	909	0.29	0.23 - 0.35
Total cholesterol in HDL (mmol/L)	469	0.30	0.22 - 0.38	433	0.30	0.21 - 0.39	518	0.25	0.16 - 0.33	476	0.23	0.15 - 0.32	987	0.28	0.22 - 0.33	909	0.27	0.20 - 0.33
Total cholesterol in HDL2 (mmol/L)	469	0.30	0.22 - 0.38	433	0.31	0.22 - 0.39	518	0.25	0.16 - 0.32	476	0.23	0.15 - 0.32	987	0.28	0.22 - 0.33	909	0.27	0.21 - 0.33
Total cholesterol in HDL3 (mmol/L)	469	0.25	0.16 - 0.33	433	0.23	0.14 - 0.32	518	0.25	0.16 - 0.33	476	0.24	0.16 - 0.33	987	0.25	0.19 - 0.31	909	0.24	0.17 - 0.30
Esterified cholesterol (mmol/L)	465	0.28	0.19 - 0.36	430	0.23	0.14 - 0.32	518	0.32	0.24 - 0.39	476	0.34	0.26 - 0.42	983	0.30	0.24 - 0.35	906	0.29	0.23 - 0.35
Free cholesterol (mmol/L)	465	0.26	0.18 - 0.34	430	0.22	0.13 - 0.31	518	0.32	0.24 - 0.40	476	0.35	0.27 - 0.43	983	0.30	0.24 - 0.35	906	0.30	0.24 - 0.35
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	469	0.28	0.20 - 0.36	433	0.30	0.21 - 0.38	518	0.29	0.21 - 0.37	476	0.29	0.20 - 0.37	987	0.29	0.23 - 0.35	909	0.30	0.24 - 0.36
Triglycerides in VLDL (mmol/L)*	469	0.30	0.22 - 0.38	433	0.31	0.23 - 0.40	518	0.28	0.20 - 0.36	476	0.27	0.18 - 0.35	987	0.29	0.23 - 0.35	909	0.29	0.23 - 0.35
Triglycerides in LDL (mmol/L)	469	0.19	0.10 - 0.28	433	0.18	0.09 - 0.27	518	0.26	0.18 - 0.34	476	0.27	0.18 - 0.35	987	0.23	0.17 - 0.29	909	0.23	0.17 - 0.29
Triglycerides in HDL (mmol/L)	469	0.21	0.12 - 0.30	433	0.23	0.14 - 0.32	518	0.26	0.18 - 0.34	476	0.26	0.18 - 0.34	987	0.24	0.18 - 0.30	909	0.25	0.19 - 0.31
Total phosphoglycerides (mmol/L)	465	0.26	0.17 - 0.34	430	0.23	0.14 - 0.32	518	0.26	0.17 - 0.34	476	0.27	0.18 - 0.35	983	0.26	0.20 - 0.32	906	0.25	0.18 - 0.31
Ratio of triglycerides to phosphoglycerides	465	0.23	0.15 - 0.32	430	0.26	0.16 - 0.34	518	0.26	0.18 - 0.34	476	0.27	0.19 - 0.35	983	0.25	0.20 - 0.31	906	0.27	0.20 - 0.33
Phosphatidylcholine & other cholines (mmol/L)	465	0.27	0.18 - 0.35	430	0.24	0.14 - 0.32	518	0.25	0.17 - 0.33	476	0.27	0.18 - 0.35	983	0.26	0.20 - 0.32	906	0.25	0.19 - 0.31
Sphingomyelins (mmol/L)	465	0.23	0.15 - 0.32	430	0.22	0.12 - 0.30	518	0.29	0.21 - 0.37	476	0.31	0.23 - 0.39	983	0.27	0.21 - 0.32	906	0.27	0.21 - 0.33
Total cholines (mmol/L)	465	0.27	0.18 - 0.35	430	0.23	0.14 - 0.32	518	0.28	0.20 - 0.35	476	0.28	0.20 - 0.37	983	0.27	0.21 - 0.33	906	0.26	0.20 - 0.32
Apolipoproteins																		
Apolipoprotein A1 (g/L)	469	0.28	0.20 - 0.36	433	0.26	0.17 - 0.35	518	0.26	0.18 - 0.34	476	0.26	0.17 - 0.34	987	0.27	0.21 - 0.33	909	0.26	0.20 - 0.32
Apolipoprotein B (g/L)	469	0.26	0.18 - 0.35	433	0.27	0.18 - 0.35	517	0.30	0.22 - 0.38	475	0.32	0.24 - 0.40	986	0.29	0.23 - 0.35	908	0.31	0.25 - 0.36
Ratio of apolipoprotein B to apolipoprotein AI	469	0.28	0.20 - 0.36	433	0.30	0.21 - 0.38	517	0.25	0.17 - 0.33	475	0.25	0.16 - 0.33	986	0.27	0.21 - 0.33	908	0.28	0.22 - 0.34
Fatty acids																		
Total fatty acids (mmol/L)	462	0.22	0.13 - 0.30	427	0.22	0.13 - 0.31	517	0.31	0.23 - 0.39	475	0.33	0.25 - 0.41	979	0.27	0.22 - 0.33	902	0.29	0.22 - 0.34
Estimated degree of unsaturation	462	0.30	0.22 - 0.38	427	0.32	0.23 - 0.41	517	0.27	0.19 - 0.35	475	0.24	0.16 - 0.33	979	0.29	0.23 - 0.34	902	0.28	0.22 - 0.34
22:6, docosahexaenoic acid (mmol/L)	462	0.20	0.11 - 0.29	427	0.18	0.09 - 0.27	517	0.34	0.26 - 0.41	475	0.32	0.24 - 0.40	979	0.27	0.21 - 0.32	902	0.25	0.19 - 0.31
18:2, linoleic acid (mmol/L)	462	0.22	0.13 - 0.31	427	0.22	0.13 - 0.31	517	0.27	0.19 - 0.35	475	0.30	0.21 - 0.38	979	0.25	0.19 - 0.31	902	0.27	0.21 - 0.33
Omega3 fatty acids (mmol/L)	462	0.20	0.11 - 0.29	427	0.19	0.09 - 0.28	517	0.34	0.27 - 0.42	475	0.34	0.26 - 0.42	979	0.27	0.21 - 0.33	902	0.26	0.20 - 0.32
Omega6 fatty acids (mmol/L)	462	0.23	0.14 - 0.32	427	0.22	0.13 - 0.31	517	0.31	0.23 - 0.38	475	0.33	0.25 - 0.41	979	0.27	0.21 - 0.33	902	0.28	0.22 - 0.34
Polyunsat. fatty acids (mmol/L)	462	0.23	0.14 - 0.31	427	0.22	0.12 - 0.30	517	0.32	0.24 - 0.39	475	0.34	0.26 - 0.42	979	0.28	0.22 - 0.33	902	0.28	0.22 - 0.34
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	462	0.24	0.15 - 0.33	427	0.25	0.16 - 0.34	517	0.33	0.25 - 0.40	475	0.33	0.25 - 0.41	979	0.29	0.24 - 0.35	902	0.30	0.24 - 0.36
Saturated fatty acids (mmol/L)	462	0.21	0.12 - 0.29	427	0.21	0.12 - 0.30	517	0.29	0.21 - 0.37	475	0.30	0.21 - 0.38	979	0.26	0.20 - 0.32	902	0.26	0.20 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	462	0.31	0.22 - 0.39	427	0.29	0.20 - 0.38	517	0.35	0.27 - 0.42	475	0.32	0.24 - 0.40	979	0.33	0.27 - 0.39	902	0.31	0.25 - 0.36
Ratio of 18:2 linoleic acid to total fatty acids (%)	462	0.13	0.04 - 0.22	427	0.15	0.06 - 0.24	517	0.20	0.11 - 0.28	475	0.16	0.08 - 0.25	979	0.17	0.11 - 0.23	902	0.16	0.09 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	462	0.32	0.24 - 0.40	427	0.32	0.23 - 0.40	517	0.40	0.33 - 0.47	475	0.37	0.29 - 0.44	979	0.36	0.31 - 0.41	902	0.34	0.28 - 0.39
Ratio of omega6 fatty acids to total fatty acids (%)	462	0.23	0.15 - 0.32	427	0.26	0.17 - 0.35	517	0.25	0.17 - 0.33	475	0.22	0.13 - 0.30	979	0.24	0.18 - 0.30	902	0.24	0.17 - 0.30
Ratio of polyunsat. fatty acids to total fatty acids (%)	462	0.27	0.19 - 0.36	427	0.30	0.21 - 0.38	517	0.28	0.20 - 0.36	475	0.24	0.16 - 0.33	979	0.28	0.22 - 0.33	902	0.27	0.20 - 0.33
Ratio of monounsat. fatty acids to total fatty acids (%)	462	0.29	0.21 - 0.38	427	0.30	0.22 - 0.39	517	0.33	0.25 - 0.40	475	0.32	0.23 - 0.40	979	0.32	0.26 - 0.37	902	0.31	0.25 - 0.37
Ratio of saturated fatty acids to total fatty acids (%)	462	0.20	0.11 - 0.29	427	0.21	0.12 - 0.30	517	0.26	0.18 - 0.34	475	0.24	0.16 - 0.33	979	0.23	0.17 - 0.29	902	0.23	0.17 - 0.29
Glycolysis related																		
Glucose (mmol/L)*	468	0.06	-0.03 - 0.15	432	0.05	-0.04 - 0.14	518	0.14	0.06 - 0.23	476	0.14	0.05 - 0.23	986	0.11	0.05 - 0.17	908	0.10	0.04 - 0.17
Lactate (mmol/L)	469	0.04	-0.05 - 0.13	433	0.06	-0.04 - 0.15	518	0.11	0.02 - 0.19	476	0.12	0.03 - 0.20	987	0.07	0.00 - 0.13	909	0.08	0.01 - 0.14
Pyruvate (mmol/L)	467	0.12	0.03 - 0.21	431	0.13	0.04 - 0.23	518	0.23	0.14 - 0.31	476	0.23	0.14 - 0.31	985	0.17	0.11 - 0.23	907	0.17	0.11 - 0.24
Citrate (mmol/L)	469	0.24	0.15 - 0.32	433	0.25	0.16 - 0.34	517	0.16	0.07 - 0.24	475	0.16	0.07 - 0.25	986	0.18	0.12 - 0.24	908	0.19	0.13 - 0.26
Glycerol (mmol/L)	124	0.25	0.07 - 0.41	113	0.24	0.06 - 0.41	155	0.10	-0.05 - 0.26	137	0.08	-0.09 - 0.24	279	0.16	0.05 - 0.28	250	0.16	0.04 - 0.28
Amino acids																		
Alanine (mmol/L)	469	0.22	0.13 - 0.31	433	0.21	0.12 - 0.30	517	0.32	0.24 - 0.40	475	0.30	0.21 - 0.38	986	0.28	0.22 - 0.33	908	0.26	0.20 - 0.32
Glutamine (mmol/L)	469	0.28	0.20 - 0.36	433	0.27	0.18 - 0.35	518	0.19	0.10 - 0.27	476	0.18	0.09 - 0.27	987	0.23	0.17 - 0.29	909	0.22	0.16 - 0.28
Glycine (mmol/L)	468	0.22	0.13 - 0.30	432	0.25	0.15 - 0.33	517	0.19	0.10 - 0.27	475	0.18	0.09 - 0.27	985	0.20	0.14 - 0.26	907	0.21	0.15 - 0.27
Histidine (mmol/L)	468	0.20	0.11 - 0.29	432	0.20	0.11 - 0.29	518	0.21	0.13 - 0.29	476	0.20	0.11 - 0.28	986	0.21	0.15 - 0.27	908	0.20	0.13 - 0.26
Isoleucine (mmol/L)	466	0.39	0.31 - 0.47	431	0.40	0.32 - 0.47	518	0.33	0.25 - 0.40	476	0.30	0.22 - 0.38	984	0.36	0.30 - 0.41	907	0.34	0.28 - 0.40
Leucine (mmol/L)	469	0.34	0.25 - 0.42	433	0.35	0.26 - 0.43	518	0.28	0.20 - 0.35	476	0.25	0.16 - 0.33	987	0.30	0.25 - 0.36	909	0.29	0.23 - 0.35
Valine (mmol/L)	469	0.39	0.31 - 0.46	433	0.38	0.30 - 0.46	516	0.27	0.19 - 0.35	474	0.26	0.17 - 0.34	985	0.33	0.27 - 0.38	907	0.32	0.26 - 0.38
Phenylalanine (mmol/L)	469	0.32	0.23 - 0.39	433	0.31	0.22 - 0.39	518	0.29	0.21 - 0.37	476	0.27	0.18 - 0.35	987	0.30	0.24 - 0.36	909	0.29	0.23 - 0.35
Tyrosine (mmol/L)	468	0.33	0.25 - 0.41	432	0.32	0.24 - 0.41	518	0.26	0.17 - 0.34	476	0.21	0.13 - 0.30	986	0.29	0.23 - 0.35	908	0.27	0.21 - 0.33
Ketone bodies																		
Acetate (mmol/L)*	468	0.11	0.02 - 0.20	433	0.08	-0.02 - 0.17	517	0.14	0.06 - 0.22	475	0.14	0.06 - 0.23	985	0.13	0.07 - 0.19	908	0.12	0.05 - 0.18
Acetoacetate (mmol/L)*	469	0.13	0.04 - 0.22	433	0.14	0.04 - 0.23	517	0.03	-0.05 - 0.12	475	0.01	-0.08 - 0.10	986	0.08	0.01 - 0.14	908	0.07	0.00 - 0.13
3hydroxybutyrate (mmol/L)*	437	0.23	0.14 - 0.32	403	0.18	0.08 - 0.27	486	0.34	0.26 - 0.42	445	0.27	0.19 - 0.36	923	0.28	0.22 - 0.34	848	0.22	0.16 - 0.29
Fluid balance																		
Albumin (signal area)	468	0.29	0.20 - 0.37	432	0.26	0.17 - 0.34	518	0.28	0.20 - 0.36	476	0.28	0.19 - 0.36	986	0.28	0.22 - 0.34	908	0.27	0.21 - 0.33
Creatinine (mmol/L)	458	0.31	0.23 - 0.39	423	0.30	0.21 - 0.39	514	0.25	0.17 - 0.33	473	0.26	0.17 - 0.34	972	0.28	0.22 - 0.34	896	0.28	0.22 - 0.34
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	469	0.23	0.15 - 0.32	433	0.23	0.14 - 0.32	518	0.28	0.19 - 0.35	476	0.28	0.19 - 0.36	987	0.26	0.20 - 0.32	909	0.26	0.19 - 0.32

* log transformation has been applied to metabolite
# adjusted for child and parent age, disadvantage index, fasting time, and processing lag time (and for child sex where appropriate).

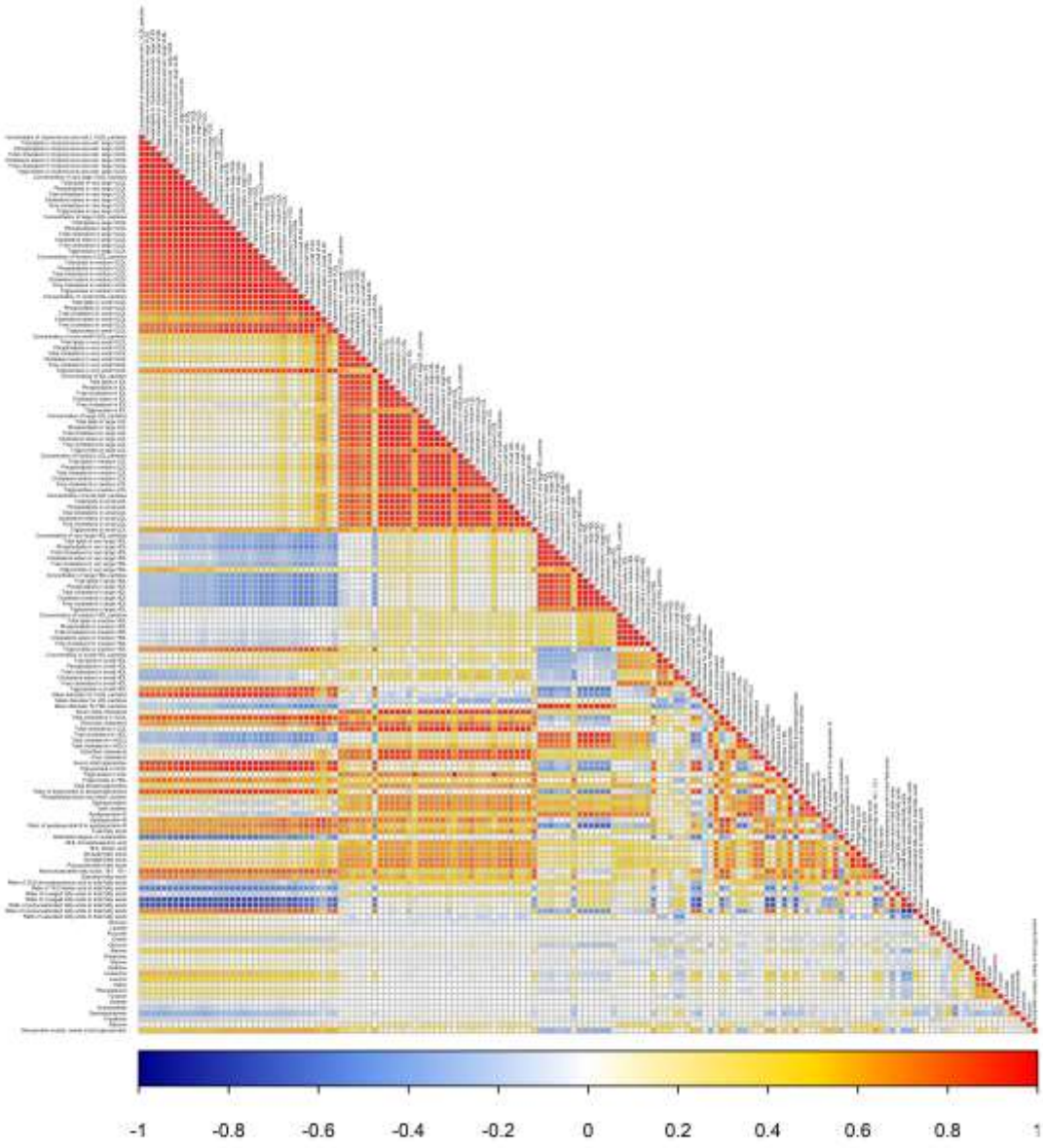
Supplementary table 6: Parent-child concordance; correlation and partial correlations between all parents and their sons, daughters and all children.

Metabolic subgroup	All Parents																	
	Male Child						Female Child							All Children				
	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI	n	CC	95% CI	n	PCC*	95% CI
Lipoprotein subclass lipids																		
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	547	0.22	0.14 - 0.30	505	0.23	0.14 - 0.31	586	0.20	0.12 - 0.28	544	0.17	0.08 - 0.25	1133	0.22	0.16 - 0.27	1049	0.20	0.15 - 0.26
Total lipids in very large VLDL (mmol/L)*	547	0.23	0.15 - 0.31	505	0.23	0.15 - 0.31	586	0.17	0.10 - 0.25	544	0.16	0.08 - 0.24	1133	0.21	0.15 - 0.26	1049	0.20	0.14 - 0.26
Total lipids in large VLDL (mmol/L)*	547	0.20	0.12 - 0.28	505	0.22	0.13 - 0.30	586	0.20	0.12 - 0.28	544	0.19	0.11 - 0.27	1133	0.20	0.15 - 0.26	1049	0.21	0.15 - 0.26
Total lipids in medium VLDL (mmol/L)	547	0.27	0.19 - 0.35	505	0.28	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.24	0.16 - 0.32	1133	0.26	0.21 - 0.31	1049	0.26	0.20 - 0.32
Total lipids in small VLDL (mmol/L)	547	0.27	0.19 - 0.34	505	0.27	0.19 - 0.35	586	0.26	0.18 - 0.33	544	0.26	0.17 - 0.33	1133	0.26	0.21 - 0.32	1049	0.26	0.21 - 0.32
Total lipids in very small VLDL (mmol/L)	547	0.23	0.15 - 0.31	505	0.22	0.13 - 0.30	586	0.25	0.17 - 0.33	544	0.26	0.18 - 0.34	1133	0.25	0.19 - 0.30	1049	0.24	0.19 - 0.30
Total lipids in IDL (mmol/L)	547	0.28	0.20 - 0.35	505	0.25	0.16 - 0.33	586	0.29	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.28	0.23 - 0.34
Total lipids in large LDL (mmol/L)	547	0.28	0.20 - 0.36	505	0.25	0.17 - 0.33	586	0.29	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.29	0.23 - 0.34
Total lipids in medium LDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.25	0.17 - 0.33	586	0.28	0.21 - 0.36	544	0.31	0.23 - 0.38	1133	0.28	0.23 - 0.34	1049	0.29	0.23 - 0.34
Total lipids in small LDL (mmol/L)	547	0.28	0.20 - 0.35	505	0.24	0.16 - 0.32	586	0.28	0.20 - 0.35	544	0.31	0.23 - 0.38	1133	0.28	0.22 - 0.33	1049	0.28	0.22 - 0.33
Total lipids in very large HDL (mmol/L)	547	0.29	0.21 - 0.37	505	0.28	0.20 - 0.36	586	0.29	0.21 - 0.36	544	0.27	0.19 - 0.35	1133	0.29	0.24 - 0.34	1049	0.28	0.22 - 0.33
Total lipids in large HDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.28	0.19 - 0.36	586	0.24	0.17 - 0.32	544	0.22	0.14 - 0.30	1133	0.27	0.21 - 0.32	1049	0.25	0.19 - 0.30
Total lipids in medium HDL (mmol/L)	547	0.15	0.07 - 0.24	505	0.13	0.04 - 0.21	586	0.10	0.02 - 0.18	544	0.10	0.02 - 0.19	1133	0.12	0.07 - 0.18	1049	0.11	0.05 - 0.17
Total lipids in small HDL (mmol/L)	547	0.19	0.10 - 0.27	505	0.16	0.08 - 0.25	586	0.18	0.10 - 0.26	544	0.17	0.09 - 0.25	1133	0.18	0.12 - 0.23	1049	0.17	0.11 - 0.22
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	547	0.29	0.21 - 0.36	505	0.30	0.22 - 0.38	586	0.22	0.14 - 0.29	544	0.19	0.11 - 0.27	1133	0.25	0.20 - 0.31	1049	0.24	0.19 - 0.30
Mean diameter for LDL particles (nm)	547	0.19	0.11 - 0.27	505	0.17	0.08 - 0.25	586	0.27	0.19 - 0.34	544	0.29	0.21 - 0.36	1133	0.23	0.17 - 0.28	1049	0.23	0.17 - 0.28
Mean diameter for HDL particles (nm)	547	0.31	0.23 - 0.38	505	0.30	0.22 - 0.38	586	0.30	0.22 - 0.37	544	0.27	0.19 - 0.35	1133	0.30	0.25 - 0.35	1049	0.29	0.23 - 0.34
Cholesterol																		
Serum total cholesterol (mmol/L)	547	0.28	0.20 - 0.36	505	0.24	0.16 - 0.32	586	0.31	0.24 - 0.38	544	0.33	0.26 - 0.41	1133	0.30	0.24 - 0.35	1049	0.29	0.24 - 0.35
Total cholesterol in VLDL (mmol/L)	547	0.26	0.18 - 0.34	505	0.27	0.19 - 0.35	586	0.26	0.18 - 0.33	544	0.26	0.18 - 0.34	1133	0.26	0.21 - 0.32	1049	0.27	0.21 - 0.32
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	547	0.27	0.19 - 0.34	505	0.26	0.18 - 0.34	586	0.28	0.20 - 0.35	544	0.29	0.21 - 0.37	1133	0.27	0.22 - 0.33	1049	0.28	0.22 - 0.34
Total cholesterol in LDL (mmol/L)	547	0.29	0.21 - 0.36	505	0.26	0.17 - 0.34	586	0.28	0.21 - 0.35	544	0.30	0.23 - 0.38	1133	0.29	0.23 - 0.34	1049	0.28	0.23 - 0.34
Total cholesterol in HDL (mmol/L)	547	0.27	0.19 - 0.35	505	0.25	0.17 - 0.33	586	0.22	0.14 - 0.29	544	0.20	0.12 - 0.28	1133	0.24	0.19 - 0.30	1049	0.22	0.17 - 0.28
Total cholesterol in HDL2 (mmol/L)	547	0.27	0.19 - 0.34	505	0.25	0.17 - 0.33	586	0.21	0.14 - 0.29	544	0.20	0.11 - 0.28	1133	0.24	0.18 - 0.29	1049	0.22	0.16 - 0.28
Total cholesterol in HDL3 (mmol/L)	547	0.25	0.17 - 0.33	505	0.22	0.14 - 0.30	586	0.23	0.15 - 0.31	544	0.23	0.14 - 0.30	1133	0.24	0.19 - 0.30	1049	0.22	0.16 - 0.28
Esterified cholesterol (mmol/L)	543	0.28	0.20 - 0.36	502	0.24	0.15 - 0.32	584	0.31	0.24 - 0.38	542	0.33	0.25 - 0.40	1127	0.30	0.24 - 0.35	1044	0.29	0.23 - 0.34
Free cholesterol (mmol/L)	543	0.27	0.19 - 0.34	502	0.23	0.14 - 0.31	584	0.32	0.24 - 0.39	542	0.34	0.26 - 0.41	1127	0.29	0.24 - 0.35	1044	0.29	0.23 - 0.34
Glycerides and phospholipids																		
Serum total triglycerides (mmol/L)*	547	0.28	0.20 - 0.35	505	0.29	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.24	0.16 - 0.32	1133	0.26	0.21 - 0.32	1049	0.26	0.21 - 0.32
Triglycerides in VLDL (mmol/L)*	547	0.28	0.20 - 0.36	505	0.29	0.21 - 0.37	586	0.23	0.15 - 0.30	544	0.21	0.13 - 0.29	1133	0.26	0.20 - 0.31	1049	0.25	0.20 - 0.31
Triglycerides in LDL (mmol/L)	547	0.20	0.12 - 0.28	505	0.19	0.10 - 0.27	586	0.27	0.19 - 0.34	544	0.28	0.20 - 0.35	1133	0.24	0.18 - 0.29	1049	0.24	0.18 - 0.29
Triglycerides in HDL (mmol/L)	547	0.25	0.17 - 0.33	505	0.26	0.18 - 0.34	586	0.27	0.20 - 0.35	544	0.28	0.20 - 0.35	1133	0.26	0.21 - 0.32	1049	0.27	0.21 - 0.32
Total phosphoglycerides (mmol/L)	543	0.28	0.20 - 0.36	502	0.24	0.16 - 0.32	584	0.27	0.19 - 0.34	542	0.29	0.21 - 0.36	1127	0.27	0.22 - 0.33	1044	0.26	0.20 - 0.32
Ratio of triglycerides to phosphoglycerides	543	0.23	0.15 - 0.31	502	0.24	0.16 - 0.32	584	0.21	0.13 - 0.28	542	0.20	0.12 - 0.28	1127	0.22	0.16 - 0.27	1044	0.22	0.16 - 0.28
Phosphatidylcholine & other cholines (mmol/L)	542	0.28	0.20 - 0.36	501	0.23	0.15 - 0.32	584	0.26	0.19 - 0.34	542	0.28	0.20 - 0.36	1126	0.27	0.21 - 0.32	1043	0.26	0.20 - 0.31
Sphingomyelins (mmol/L)	542	0.23	0.15 - 0.31	501	0.20	0.12 - 0.29	584	0.28	0.21 - 0.36	542	0.30	0.22 - 0.37	1126	0.26	0.20 - 0.31	1043	0.26	0.20 - 0.31
Total cholines (mmol/L)	542	0.27	0.19 - 0.35	501	0.23	0.14 - 0.31	584	0.29	0.21 - 0.36	542	0.30	0.22 - 0.37	1126	0.28	0.22 - 0.33	1043	0.26	0.20 - 0.32
Apolipoproteins																		
Apolipoprotein A1 (g/L)	547	0.26	0.18 - 0.34	505	0.23	0.14 - 0.31	586	0.25	0.17 - 0.33	544	0.25	0.17 - 0.32	1133	0.25	0.20 - 0.31	1049	0.23	0.18 - 0.29
Apolipoprotein B (g/L)	547	0.27	0.19 - 0.35	505	0.27	0.19 - 0.35	585	0.28	0.20 - 0.35	543	0.30	0.22 - 0.37	1132	0.28	0.23 - 0.33	1048	0.29	0.23 - 0.34
Ratio of apolipoprotein B to apolipoprotein AI	547	0.26	0.18 - 0.33	505	0.27	0.18 - 0.34	585	0.21	0.13 - 0.29	543	0.20	0.12 - 0.28	1132	0.23	0.18 - 0.29	1048	0.24	0.18 - 0.29
Fatty acids																		
Total fatty acids (mmol/L)	537	0.26	0.18 - 0.33	496	0.25	0.17 - 0.33	583	0.30	0.22 - 0.37	541	0.31	0.23 - 0.39	1120	0.28	0.23 - 0.33	1037	0.29	0.23 - 0.34
Estimated degree of unsaturation	537	0.30	0.22 - 0.37	496	0.32	0.23 - 0.39	583	0.24	0.17 - 0.32	541	0.22	0.14 - 0.30	1120	0.27	0.21 - 0.32	1037	0.26	0.20 - 0.32
22:6, docosahexaenoic acid (mmol/L)	537	0.23	0.15 - 0.31	496	0.21	0.13 - 0.30	583	0.33	0.26 - 0.40	541	0.33	0.25 - 0.40	1120	0.28	0.23 - 0.34	1037	0.27	0.21 - 0.32
18:2, linoleic acid (mmol/L)	537	0.24	0.16 - 0.32	496	0.25	0.16 - 0.33	583	0.27	0.19 - 0.34	541	0.29	0.21 - 0.37	1120	0.26	0.21 - 0.32	1037	0.28	0.22 - 0.33
Omega3 fatty acids (mmol/L)	537	0.24	0.16 - 0.32	496	0.22	0.14 - 0.31	583	0.34	0.27 - 0.41	541	0.34	0.27 - 0.41	1120	0.29	0.23 - 0.34	1037	0.28	0.22 - 0.33
Omega6 fatty acids (mmol/L)	537	0.26	0.18 - 0.33	496	0.25	0.16 - 0.33	583	0.30	0.23 - 0.38	541	0.33	0.25 - 0.40	1120	0.28	0.23 - 0.34	1037	0.29	0.24 - 0.35
Polyunsat. fatty acids (mmol/L)	537	0.26	0.17 - 0.33	496	0.24	0.16 - 0.33	583	0.31	0.24 - 0.39	541	0.34	0.26 - 0.41	1120	0.29	0.23 - 0.34	1037	0.29	0.24 - 0.35
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	537	0.27	0.19 - 0.35	496	0.28	0.19 - 0.36	583	0.30	0.22 - 0.37	541	0.30	0.23 - 0.38	1120	0.29	0.24 - 0.34	1037	0.29	0.24 - 0.35
Saturated fatty acids (mmol/L)	537	0.25	0.17 - 0.33	496	0.25	0.16 - 0.33	583	0.28	0.20 - 0.35	541	0.28	0.20 - 0.36	1120	0.27	0.21 - 0.32	1037	0.27	0.21 - 0.32
Fatty acid ratios																		
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	537	0.31	0.23 - 0.38	496	0.30	0.21 - 0.37	583	0.33	0.26 - 0.40	541	0.31	0.23 - 0.38	1120	0.32	0.27 - 0.37	1037	0.30	0.24 - 0.36
Ratio of 18:2 linoleic acid to total fatty acids (%)	537	0.15	0.07 - 0.23	496	0.17	0.08 - 0.25	583	0.18	0.10 - 0.26	541	0.16	0.08 - 0.24	1120	0.17	0.11 - 0.23	1037	0.16	0.10 - 0.22
Ratio of omega3 fatty acids to total fatty acids (%)	537	0.32	0.24 - 0.39	496	0.32	0.23 - 0.39	583	0.40	0.33 - 0.47	541	0.38	0.31 - 0.45	1120	0.36	0.31 - 0.41	1037	0.35	0.29 - 0.40
Ratio of omega6 fatty acids to total fatty acids (%)	537	0.24	0.16 - 0.32	496	0.26	0.18 - 0.34	583	0.23	0.15 - 0.30	541	0.20	0.12 - 0.28	1120	0.24	0.18 - 0.29	1037	0.23	0.17 - 0.28
Ratio of polyunsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.35	496	0.29	0.21 - 0.37	583	0.26	0.18 - 0.33	541	0.23	0.15 - 0.31	1120	0.26	0.21 - 0.32	1037	0.25	0.20 - 0.31
Ratio of monounsat. fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.34	496	0.27	0.19 - 0.35	583	0.30	0.23 - 0.37	541	0.29	0.21 - 0.37	1120	0.29	0.23 - 0.34	1037	0.28	0.22 - 0.34
Ratio of saturated fatty acids to total fatty acids (%)	537	0.22	0.14 - 0.30	496	0.23	0.14 - 0.31	583	0.26	0.19 - 0.34	541	0.25	0.17 - 0.33	1120	0.24	0.19 - 0.30	1037	0.24	0.19 - 0.30
Glycolysis related																		
Glucose (mmol/L)*	545	0.05	-0.04 - 0.13	503	0.04	-0.05 - 0.12	586	0.14	0.06 - 0.21	544	0.14	0.05 - 0.22	1131	0.1	0.04 - 0.15	1047	0.09	0.03 - 0.15
Lactate (mmol/L)	547	0.02	-0.07 - 0.10	505	0.03	-0.06 - 0.12	586	0.13	0.05 - 0.21	544	0.14	0.05 - 0.22	1133	0.07	0.01 - 0.12	1049	0.08	0.01 - 0.13
Pyruvate (mmol/L)	545	0.11	0.03 - 0.20	503	0.13	0.04 - 0.21	586	0.22	0.15 - 0.30	544	0.22	0.14 - 0.30	1131	0.17	0.11 - 0.22	1047	0.17	0.11 - 0.23
Citrate (mmol/L)	547	0.23	0.15 - 0.31	505	0.25	0.16 - 0.33	585	0.14	0.06 - 0.22	543	0.14	0.05 - 0.22	1132	0.17	0.12 - 0.23	1048	0.18	0.12 - 0.24
Glycerol (mmol/L)	151	0.30	0.15 - 0.44	137	0.28	0.12 - 0.43	175	0.12	-0.03 - 0.26	157	0.08	-0.08 - 0.23	326	0.20	0.09 - 0.30	294	0.19	0.07 - 0.29
Amino acids																		
Alanine (mmol/L)	546	0.24	0.16 - 0.31	504	0.22	0.14 - 0.31	585	0.32	0.25 - 0.39	543	0.30	0.22 - 0.37	1131	0.28	0.23 - 0.34	1047	0.27	0.21 - 0.32
Glutamine (mmol/L)	547	0.30	0.22 - 0.37	505	0.29	0.21 - 0.37	586	0.20	0.12 - 0.27	544	0.19	0.11 - 0.27	1133	0.24	0.19 - 0.30	1049	0.24	0.18 - 0.29
Glycine (mmol/L)	545	0.21	0.13 - 0.29	503	0.24	0.15 - 0.32	585	0.19	0.11 - 0.27	543	0.19	0.11 - 0.27	1130	0.20	0.15 - 0.26	1046	0.21	0.15 - 0.27
Histidine (mmol/L)	545	0.21	0.13 - 0.29	503	0.21	0.12 - 0.29	586	0.22	0.14 - 0.29	544	0.20	0.12 - 0.28	1131	0.21	0.16 - 0.27	1047	0.21	0.15 - 0.26
Isoleucine (mmol/L)	541	0.36	0.29 - 0.44	500	0.37	0.29 - 0.44	586	0.29	0.22 - 0.36	544	0.26	0.18 - 0.34	1127	0.33	0.27 - 0.38	1044	0.31	0.25 - 0.36
Leucine (mmol/L)	547	0.32	0.24 - 0.39	505	0.33	0.25 - 0.40	586	0.24	0.16 - 0.32	544	0.21	0.13 - 0.29	1133	0.28	0.23 - 0.33	1049	0.27	0.21 - 0.32
Valine (mmol/L)	547	0.38	0.31 - 0.45	505	0.38	0.31 - 0.45	584	0.27	0.19 - 0.34	542	0.25	0.17 - 0.32	1131	0.33	0.27 - 0.38	1047	0.32	0.26 - 0.37
Phenylalanine (mmol/L)	547	0.29	0.22 - 0.37	505	0.29	0.21 - 0.37	586	0.30	0.22 - 0.37	544	0.28	0.20 - 0.36	1133	0.29	0.24 - 0.35	1049	0.28	0.23 - 0.34
Tyrosine (mmol/L)	545	0.31	0.24 - 0.39	503	0.31	0.22 - 0.38	586	0.27	0.19 - 0.34	544	0.23	0.15 - 0.31	1131	0.29	0.24 - 0.34	1047	0.27	0.21 - 0.32
Ketone bodies																		
Acetate (mmol/L)*	546	0.09	0.01 - 0.17	505	0.05	-0.03 - 0.14	585	0.15	0.06 - 0.22	543	0.15	0.07 - 0.23	1131	0.12	0.06 - 0.17	1048	0.11	0.05 - 0.17
Acetoacetate (mmol/L)*	547	0.11	0.03 - 0.20	505	0.11	0.03 - 0.20	585	0.04	-0.04 - 0.12	543	0.04	-0.05 - 0.12	1132	0.07	0.02 - 0.13	1048	0.07	0.01 - 0.13
3hydroxybutyrate (mmol/L)*	511	0.22	0.13 - 0.30	472	0.16	0.07 - 0.24	551	0.34	0.26 - 0.41	510	0.28	0.20 - 0.36	1062	0.27	0.21 - 0.33	982	0.21	0.15 - 0.27
Fluid balance																		
Albumin (signal area)	546	0.32	0.24 - 0.39	504	0.29	0.20 - 0.36	586	0.25	0.17 - 0.32	544	0.25	0.17 - 0.33	1132	0.28	0.23 - 0.33	1048	0.27	0.21 - 0.32
Creatinine (mmol/L)	534	0.29	0.21 - 0.37	493	0.27	0.19 - 0.35	580	0.27	0.19 - 0.34	539	0.27	0.19 - 0.35	1114	0.28	0.22 - 0.33	1032	0.27	0.22 - 0.33
Inflammation																		
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	547	0.24	0.16 - 0.32	505	0.24	0.16 - 0.32	586	0.24	0.17 - 0.32	544	0.25	0.16 - 0.32	1133	0.24	0.19 - 0.30	1049	0.24	0.18 - 0.30

* log transformation has been applied to metabolite

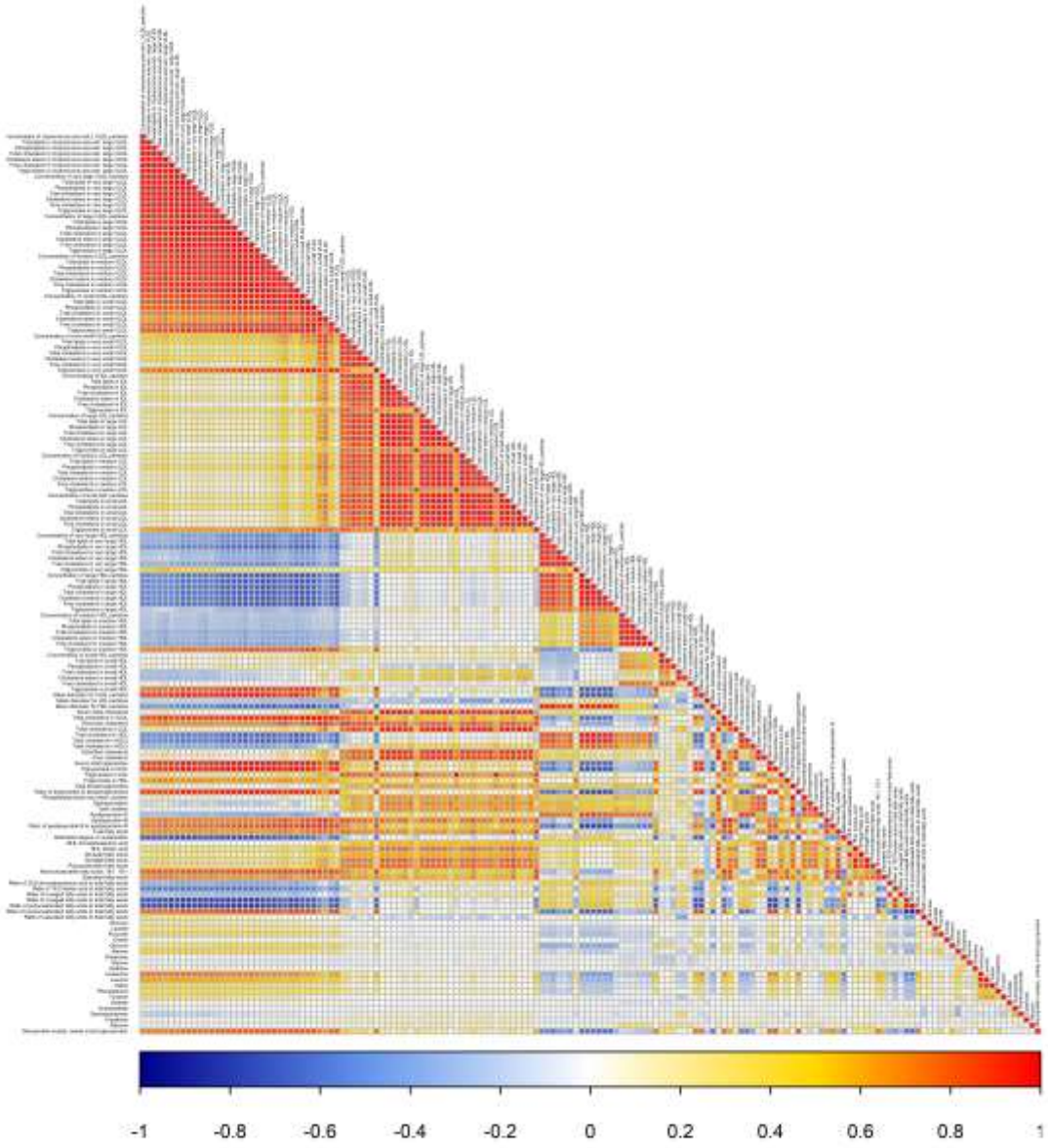
adjusted for child and parent age, disadvantage index, fasting time, and processing lag time (and for child and parent sex where appropriate).

Supplementary Figure 1: Correlations between Child Health CheckPoint metabolites - children



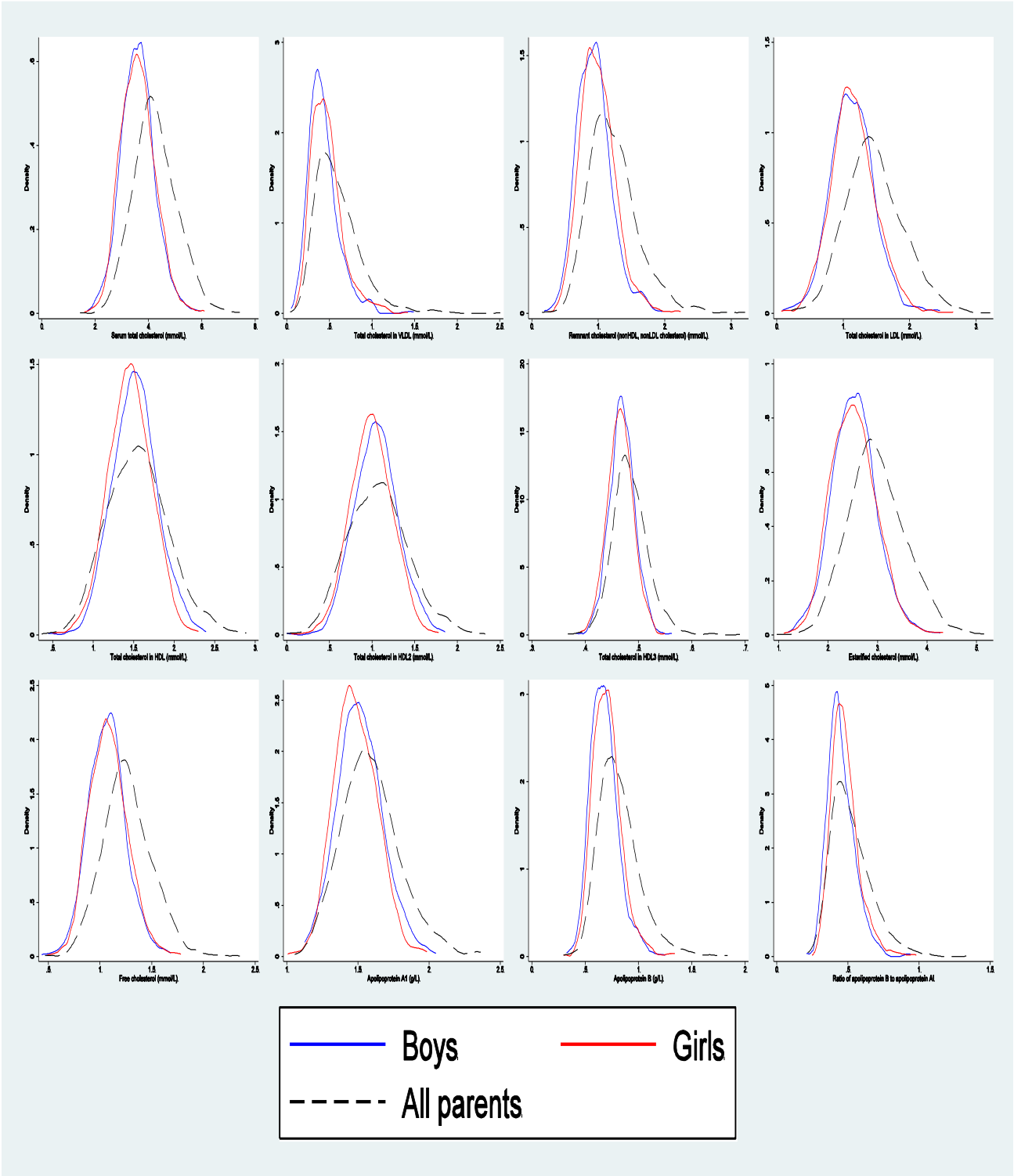
Note: Correlations (spearmans) between metabolites for the CheckPoint child metabolomics data

Supplementary Figure 2: Correlations between Child Health CheckPoint metabolites - parents

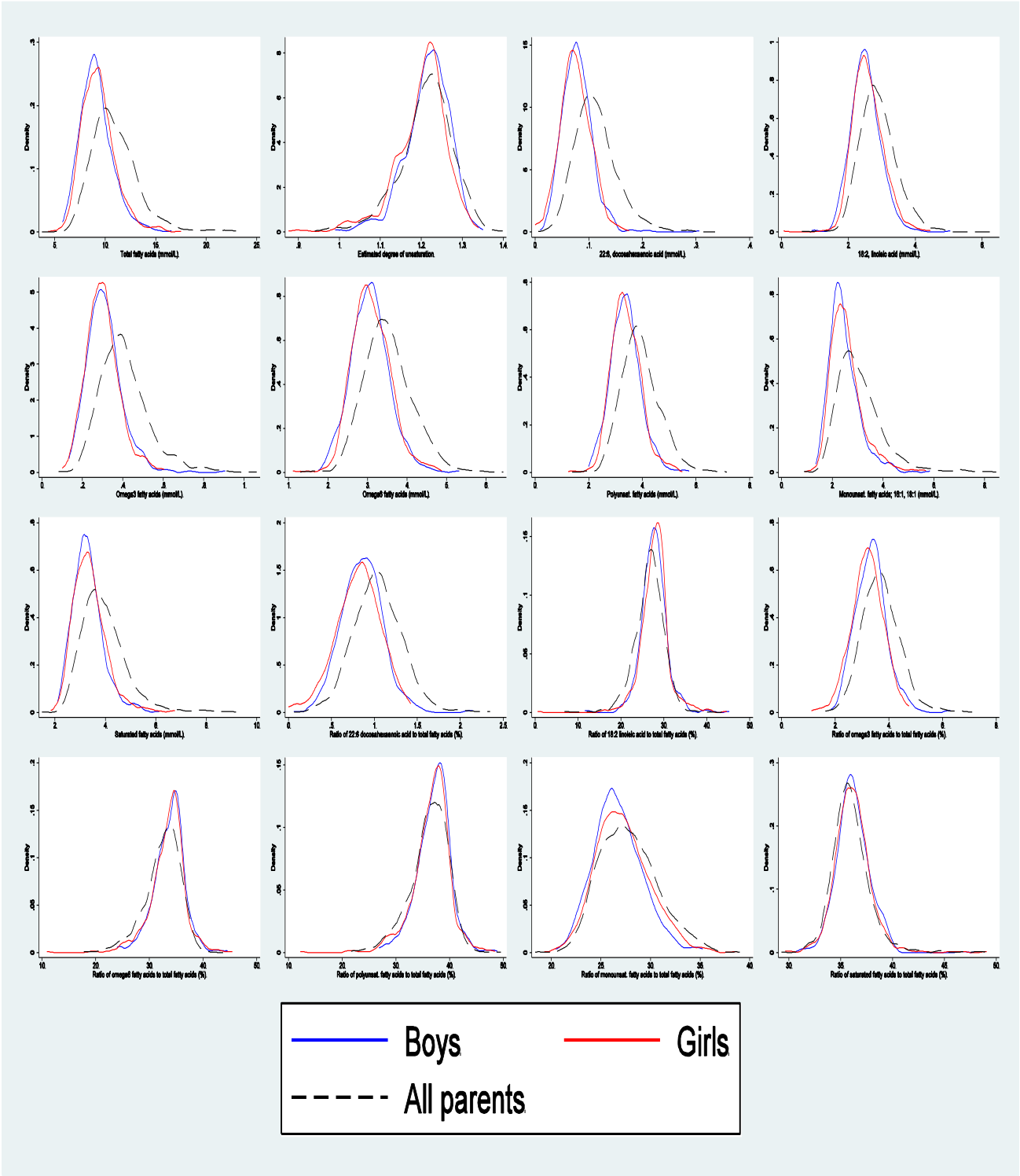


Note: Correlations (spearmans) between metabolites for the CheckPoint parent metabolomics data

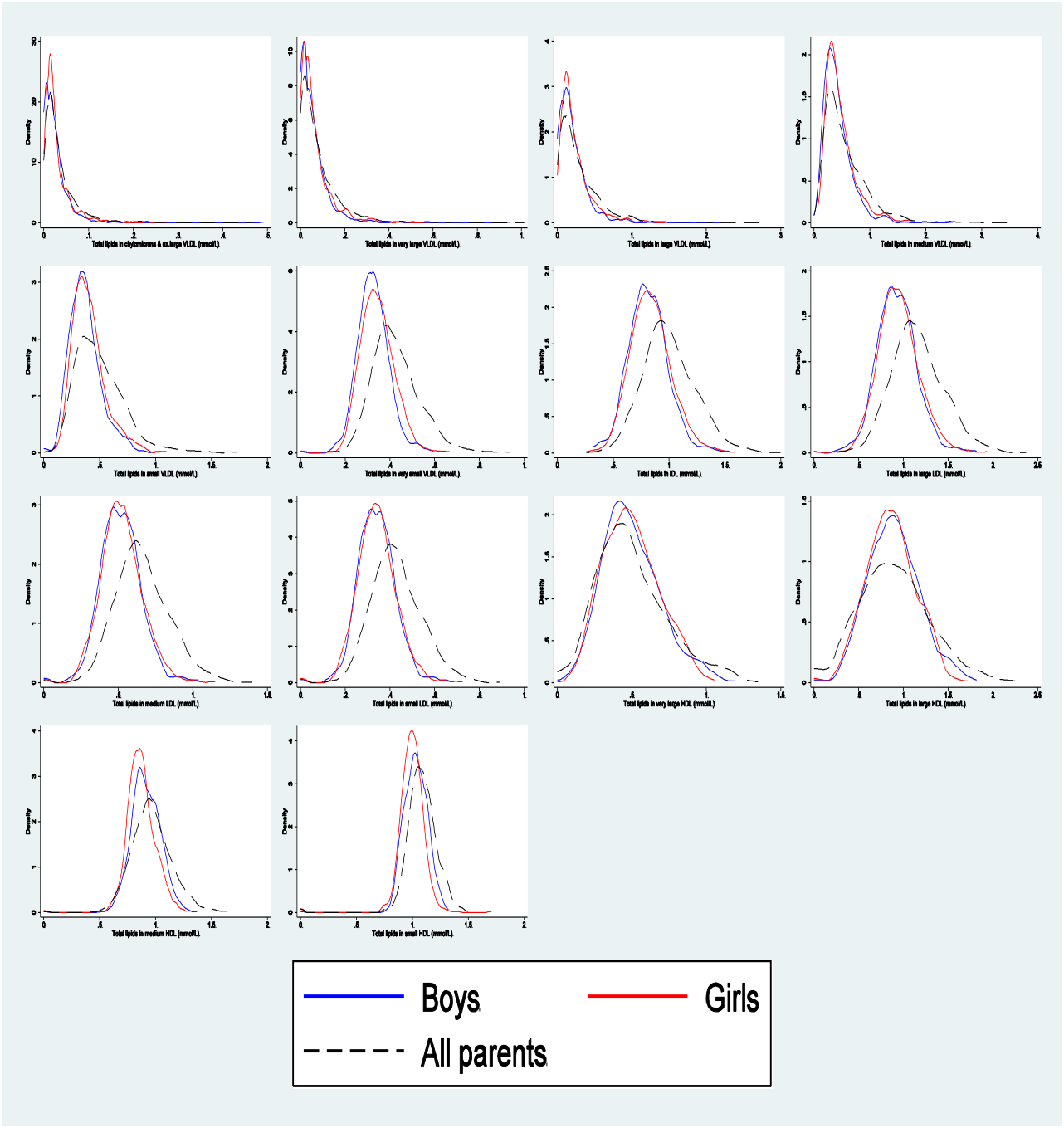
Supplementary Figure 3



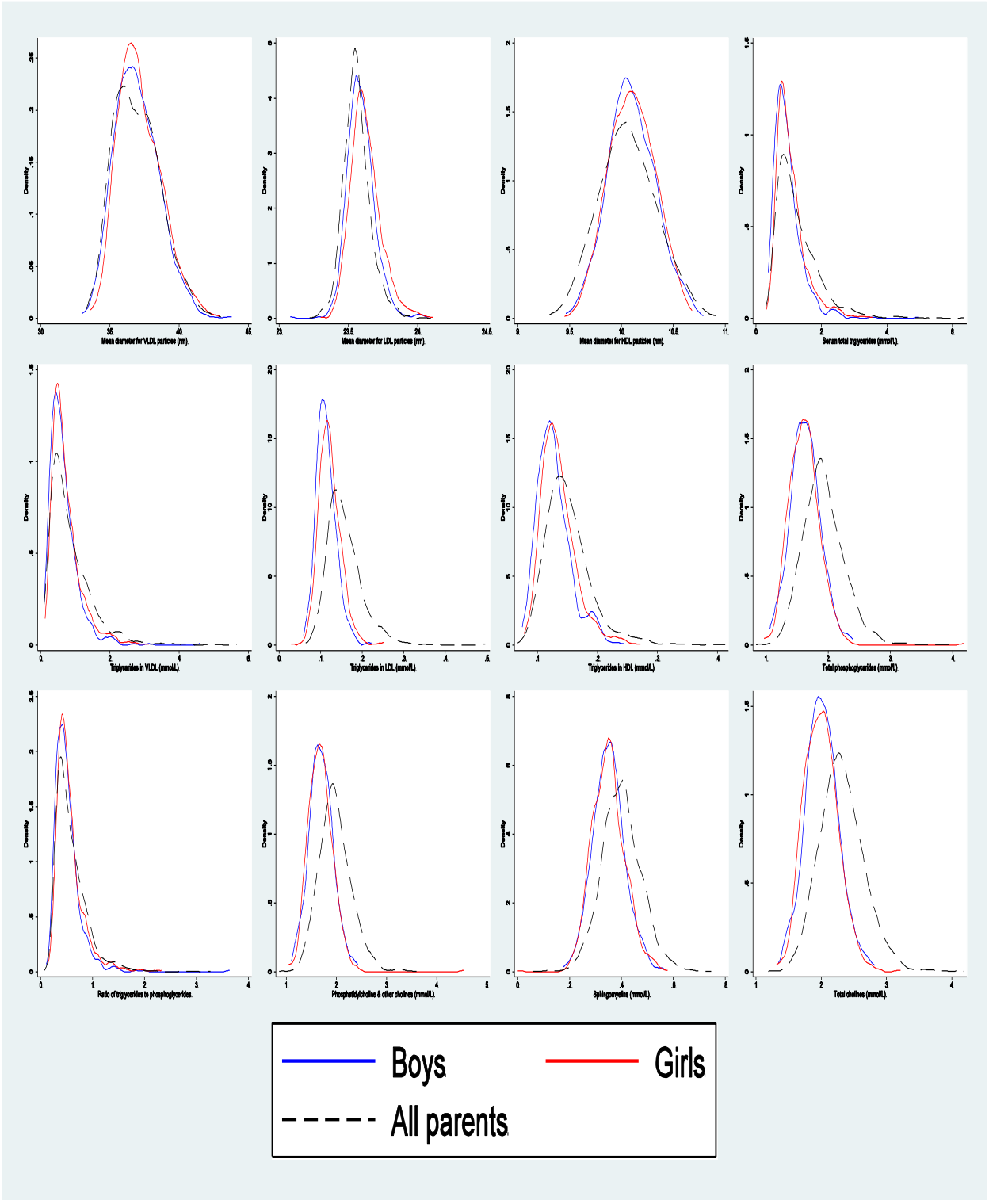
Supplementary Figure 4



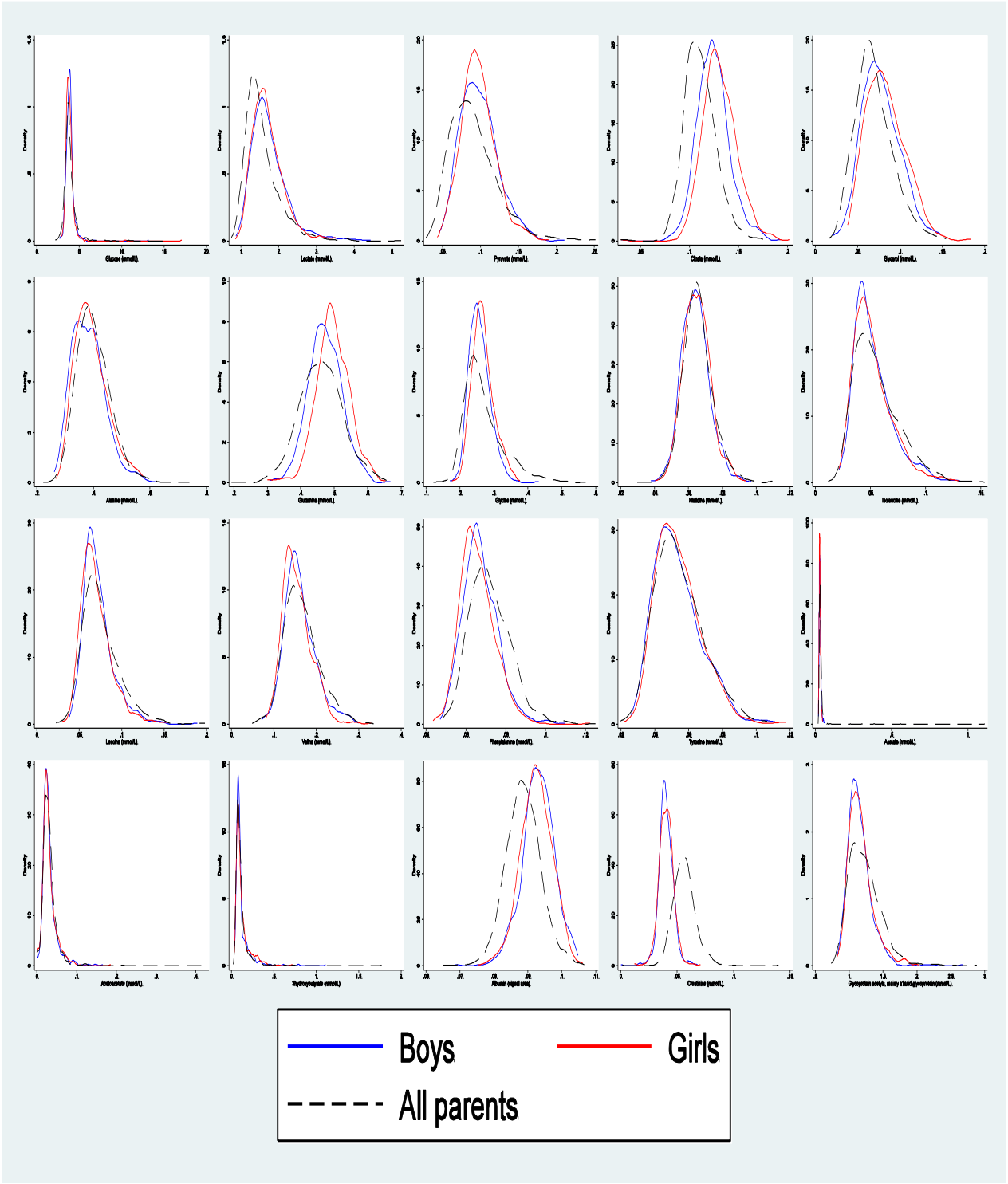
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



STROBE Statement—checklist of items that should be included in reports of observational studies

Paper title: Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents

Person completing checklist: Susan Ellul

	Item No	Recommendation	Page number	Line number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2	14-15
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	9-13 25-32
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5	Pg 4 (3-9 ,21-22, 31-32, 33-35) Pg 5 (5-7)
Objectives	3	State specific objectives, including any prespecified hypotheses	5	8-11
Methods				
Study design	4	Present key elements of study design early in the paper	5	14-22
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6	Pg 5 (17-20) Pg 6 (3-32)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ~~*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale~~ for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	23-31
		~~(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed~~ ~~*Case-control study*—For matched studies, give matching criteria and the number of controls per case~~		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9	Pg 7 (4-17) Pg 8 (table 1, 9-16) Pg 9 (1-24)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9	Pg 7 (4-17) Pg 8 (table 1, 9-16) Pg 9 (1-24)
Bias	9	Describe any efforts to address potential sources of bias	9 -10	Pg 9 (31-35) Pg 10 (16-18)

Study size	10	Explain how the study size was arrived at	11	5-14, figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-10	Pg 9 (28-35) Pg 10 (3-15)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10	Pg 9 (28-35) Pg 10 (3-15)
		(*b*) Describe any methods used to examine subgroups and interactions	9-10	Pg 9 (28-35) Pg 10 (3-15)
		(*c*) Explain how missing data were addressed	9 -10	Pg 9 (31-35) Pg 10 (16-18)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed ~~*Case-control study*—If applicable, explain how matching of cases and controls was addressed~~	9	31-35
		(*e*) Describe any sensitivity analyses	10	16-18

Results			**Page number**	**Line number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11 and figure 1	5-12
		(b) Give reasons for non-participation at each stage	11 6 figure 1	8-12 7-11
		(c) Consider use of a flow diagram	figure 1	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12 (table 2)	2
		(b) Indicate number of participants with missing data for each variable of interest	figure 1 table 2 sup Table 1	2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	sup table 1	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13 supp tables 2-6	21-24
		(*b*) Report category boundaries when continuous variables were categorized	NA	

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	supp tables 2-6	
Discussion				
Key results	18	Summarise key results with reference to study objectives	14	3-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14	18-28
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-17	Pg 14 (30-32) Pg 15 (all) Pg 16 (all) Pg 17 (1-2)
Generalisability	21	Discuss the generalisability (external validity) of the study results	14	18-28
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18-19	Pg 18 (21-33) Pg 19 (1-2)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.