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

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1	Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their
2	parents
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25	Keywords: Metabolomics; lipids; inflammation; reference values; parents; children;
26	inheritance patterns; correlation studies; epidemiologic studies; cross-sectional studies.
27	
28	Word count: 4181
29	
30	Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched
31	chain amino acid; CDC: Centers for Disease Prevention and Control; CVD: Cardiovascular
32	disease; CPS1: Carbamoyl-phosphate synthase 1; DHA: Docosahexaenoic acid; DOB: Date
33	of birth; EDTA: Ethylenediaminetetraacetic acid; GlycA: Glycoprotein acetyls; HbA1c:
34	Haemoglobin A1c; HDL: High-density lipoprotein; HOMA: Homeostatic model assessment;
35	IDL: Intermediate density lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein;
36	LiH: Lithium Heparin; LSAC: Longitudinal Study of Australian Children; MUFA:
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1 Monounsaturated fatty acid; NMR: Nuclear magnetic resonance; PCOS: Polycystic Ovary

2 Syndrome; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acids; SST: serum

3 separating tubes; T2D: Type 2 diabetes; T2DM: Type 2 diabetes mellitus; VLDL: Very low

4 density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS: Very
5 small.

ABSTRACT

9 Objectives: Nuclear Magnetic Resonance (NMR) metabolomics is high throughput and cost 10 effective, with the potential to improving the understanding of disease and risk. We examine 11 the circulating metabolic profile by quantitative NMR metabolomics of a sample of 12 Australian 11-12 year old children and their parents, describe differences by age and sex, and 13 explore correlation of metabolites in parent-child dyads.

14 Design: The population-based cross-sectional Child Health CheckPoint study nested within15 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.

19 Participants: 1180 children and 1325 parents provided a blood sample and had
20 metabolomics data available. This included 1133 parent-child dyads (518 mother-daughter,
21 469 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.

33 Conclusions: We report the serum metabolite profiles from mid-childhood and adulthood in
34 a population-based sample, together with parent-child concordance. Distinct age- and sex35 specific profiles were observed. These data will be informative for investigation of the

1	1	childhood origins of adult non-communicable diseases and for comparative studies in other
2	2	populations.
5 4	3	Strengths and limitations of this study:
5	4	• In a large population-based cohort, venous blood was collected for children and their
7	5	attending parent on the same day using the same methods
8 9	6	• Rapidly processed, high quality serum samples with standardised metabolomic data
10	7	generated as a single batch
11	8	• Cross-sectional design does not enable longitudinal analysis of specific metabolite
13 14	9	species over short term or longer periods of time
15	10	• Assessment of paternal influences on offspring metabolite measures is limited by a
10	11	relatively small sample size compared to mother-child pairs, reducing the precision of
18 10	12	estimates
20	13	• Factors known to influence metabolomic profile (such as body mass index) were not
21 22	14	considered
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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.⁶⁷

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.⁸⁹ 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

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(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 1112 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

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biological mother) at which both participated in a wide range of measures relevant to noncommunicable 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.

27 Measures

29 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

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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

Metabolic group	Species and derived measures	
Amino osida	Alanine, Glutamine, Glycine, Histidine	
Amino acids	Branched chain: Isoleucine, Leucine, Valine	
	Aromatic: Phenylalanine, Tyrosine	
Cholesterol	VLDL, LDL, HDL, HDL2, HDL3, Total, Free, Esterified, Remnant	
	Triglycerides (VLDL, LDL, HDL, total)	
	Phosphoglycerides	
Triglycerides and	Ratio of triglycerides to phosphoglycerides [*]	
phospholipids	Phosphatidylcholine	
	Sphingomyelins	
	Total cholines	
	Apolipoprotein A-1 (ApoA-1)	
Apolipoproteins	Apolipoprotein B (Apo B)	
	Ratio of Apolipoprotein B to Apolipoprotein A-1 (ApoB/Apo A-1)*	
	Total, Omega-3, Omega-6, Polyunsaturated (PUFA), Saturated (SFA)	
Fatty acids (FA)	Monounsaturated (MUFA), Docosahexaenoic acid (DHA), Linoleic (LA)	
	Estimated degree of unsaturation	
Fatty agid ratios	Omega-3/total FA [*] , Omega-6/total FA [*] , PUFA/total FA [*] (all %)	
ratty actu ratios	SFA/total FA [*] , MUFA/total FA [*] , DHA/total FA [*] , LA/total FA [*] (all %)	
Lipoprotein	12 lipids in each of 14 subclasses:	
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)	

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

9 Information obtained from <u>https://nightingalehealth.com/science/biomarkers</u>

* 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.

16 Many of the 228 metabolomics measures correlate substantially both in adults

17 (supplementary figure 1) and children (supplementary figure 2). For clarity, we therefore

18 focussed on an informative subset of 70 lipid and metabolites in analyses that capture the

19 majority of variation within the dataset. Most metabolites were reported as absolute

1 concentrations (ie not including ratios). We excluded glucose and lactate given the known

2 sensitivity of these measures to variable time of fasting and specific processing variables.

3 Some derived ratios, such as fatty acids and apolipoprotein B to apolipoprotein A-1, were

4 included because of previous data indicating clinical utility.¹⁷

5 <u>Other measures and sample characteristics</u>

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/m²) 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

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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

3 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.

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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	ood 14.16 (2.0) 14.12 (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.

3

4 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.

15 Sex-specific differences in metabolite levels in children and adults

- 16 Figure 3 shows differences in mean metabolite levels by sex for children and adults
- 17 separately in SD units, with estimates in absolute concentration units listed in supplementary
- 18 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

16 Figure 4 shows the correlations between metabolite measures for all children with all parents,

17 and for boys and girls with mothers (but not with the 177 fathers, given the small numbers).

18 The corresponding correlation coefficients and partial correlation coefficients are listed in

19 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

2 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.

11 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.

16 Replication studies exploring sex differences at earlier and later stages of childhood and

17 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.

30 Meaning and implications for clinicians and policymakers

31 Overall, we found a clear difference in metabolite profile between children and their parents.

32 This was apparent for specific metabolite measures (such as some amino acids) as well as the

1 distribution of metabolites (such as lipid composition of lipoproteins of different density).

2 Some measures were higher in adults, some similar, while a minority were lower. Previous

3 studies, largely in adults, have identified a range of specific metabolite changes with age,

4 particularly from mid to late adulthood.²³ This includes a general decrease in several amino

5 acid species, which contrasts with our findings from childhood to mid adulthood.⁸ Only the

6 amino acid glutamine showed this pattern in our dataset.

Sex-specific differences in children (± 0.2 SD) were generally much smaller than in adults $(\pm 0.8 \text{ SD})$. 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 value 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

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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 sexspecific 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 studies²⁵, we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of $\sim 10,000$ adults with cardiovascular disease (CVD), with replication in > 53,000subjects, 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 $(p=6.3 \times 10^{-5})$ 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 cholesterol³⁷ 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.

28 These are the first data on the mother-child or parent-child correlations of NMR metabolites.

29 Smaller studies have reported positive correlations between parents and children for a limited

30 range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL

- 31 cholesterol and triglycerides measured using conventional methods. We found positive
- 32 correlations between parents and children for the same lipid measures (although measured
- 33 using NMR) consistent with previously reported findings. One study reported a positive

1 association between the serum lipid levels of 4 year old children (n=127) and their parents

2 (122 mothers and 118 fathers) 40 while another study of children aged 6-18 (n=255) and their

3 parents (n=179) found that the age of the child influenced the degree of correlation of several

4 lipid measures, with older (10-18 years) children more similar to their parents in terms of

5 triglyceride levels than younger individuals (6-9 years).¹²

6 Unanswered questions and future research

7 The temporal and sex specific dynamism of the metabolomics data we describe here offer 8 considerable opportunities for identification of biomarkers of risk for a range of non-9 communicable diseases early in life, to inform targeted interventions and monitor their 10 efficacy. Combining metabolomics with other 'omics data (such as genetics), as is 11 increasingly reported from large adult studies, offers considerable promise in understanding 12 the causal pathways that link early life exposures, genetics and intermediate phenotypes with 13 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 sexspecific 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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3 Australian Children. The study is conducted in partnership between the Department of Social

4 Services (DSS), the Australian Institute of Family Studies (AIFS) and the Australian Bureau

of Statistics (ABS). The findings and views reported in this paper are those of the author and
should not be attributed to DSS, AIFS or the ABS.

7

8 REDCap (Research Electronic Data Capture) tools⁴¹ were used in this study. More
9 information about this software can be found at: www.project-redcap.org.

10 We thank the LSAC and CheckPoint study participants, staff and students for their11 contributions.

12 COMPETING INTERESTS 13

completed the 14 All authors ICMJE uniform have disclosure form at 15 www.icmje.org/coi disclosure.pdf and declare financial support for the submitted work from 16 the National Health and Medical Research Council of Australia, The Royal Children's 17 Hospital Foundation, the Murdoch Children's Research Institute, The University of 18 Melbourne, the National Heart Foundation of Australia, and the Financial Markets 19 Foundation for Children. Personal fees were received by MW from the Australian 20 Department of Social Services and by PW from Nightingale Health Ltd. MW and DB are 21 supported by the NHMRC; DB by the National Heart Foundation of Australia; and MW by 22 Cure Kids New Zealand. MW received grants from NZ Ministry of Business, Innovation & 23 Employment and A Better Start/Cure Kids New Zealand, and support from Sandoz to present 24 at a symposium outside the submitted work. PW is employee and shareholder of Nightingale 25 Health Ltd, a company offering NMR-based metabolic profiling.

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27

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12 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.

20 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

- 24 is available at <u>www.growingupinaustralia.gov.au</u>

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 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 concentration for females compared to males in children (A) and adults (B). Error bars represent 95% confidence intervals. 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 are Isted 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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 13 concentration units, 95% confidence intervals and associated p-values are listed in 14 supplementary table 2. HDL: High-density lipoprotein; IDL: Intermediate density 15 lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein. 16 17 Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood. 18 Association measures are SD difference in metabolite concentration for females compared to 19 males in children (A) and adults (B). Error bars represent 95% confidence intervals. 20 Association measures in absolute concentration units, 95% confidence intervals and 21 associated p-values are listed in supplementary table 3 and 4. HDL: High-density lipoprotein; 22 IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low 23 density lipoprotein.
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 IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.
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45 26 Pearson's correlation coefficients for all children with all parents (A); and for boys (blue)
47 27 with mothers and for girls (red) with mothers (B). Error bars represent 95% confidence
48 49 28 intervals. Correlation coefficients with associated 95% confidence intervals are listed in
50 29 supplementary table 5 and 6. HDL: High-density lipoprotein; IDL: Intermediate density
51 52 30 lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.
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3	1	SUPPLEMENTARY DOCUMENTS
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6	3	Supplementary figure 1: Correlation of NMR metabolite measures in children.
7	4	
8	5	Heatmap showing the correlation between metabolite measures in children. The correlations
9	6	shown are Spearman's correlation coefficients with blue cells representing negative
10	7	correlations and red cells representing positive correlations.
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12	9	Supplementary figure 2: Correlation of NMR metabolite measures in parents.
13	10	
14	11	Heatman showing the correlation between metabolite measures in parents. The correlations
15	12	shown are Snearman's correlation coefficients with blue cells representing negative
16	12	shown are spearman's correlation coefficients with blue cens representing negative
17	15	correlations and red cens representing positive correlations.
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19	15	Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.
20	16	
21	17	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
22	18	each cholesterol and apolipoprotein measure.
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25	21	Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.
26	22	
27	23	Boys (blue) girls (red) and all parents (thin dotted black line) plotted on the same graph for
28	20	each fatty acid and fatty acid ratio measure
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31	26	Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass
32	27	particles.
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34	29	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
35	30	total lipids within each of the 14 lipoprotein subclass particles.
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37	32	Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride
38	33	measures.
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40	35	Boys (blue) girls (red) and all parents (thin dotted black line) plotted on the same graph for
41	36	linoprotein particle sizes and triglyceride measures
42	27	ipoprotein particle sizes and trigificentae measures.
43	20	Supplementary figure 7: Density plats for glycolysis related aming agid katong body
44	20	supplementary lighter. Density plots for grycolysis related, annual acid, ketone body
45	39	and inflammation measures.
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47	41	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
48	42	glycolysis related, amino acid, ketone body and inflammation measures.
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50	44	Supplementary table 1: Weighted mean (SD) of metabolite measures in children and
51	45	parents.
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53	47	Supplementary table 2: Differences in mean metabolite levels in adults compared to
54	48	children in absolute concentration units.
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2	1	Supplementary table 3: Differences in mean metabolite levels in girls compared to boys
4	2	in absolute concentration units.
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6	4	Supplementary table 4: Differences in mean metabolite levels in female compared to
7	5	male adults in absolute concentration units.
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9	7	Supplementary table 5: Mother-child concordance; correlations and partial correlations
10 11	8	between mothers and their sons, daughters and all children.
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13	10	Supplementary table 6: Parent-child concordance; correlation and partial correlations
14	11	between all parents and their sons, daughters and all children.
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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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Page 2	27 of 5	51	Log Acetate (mmol/L) Log Acetoacetate (mmol/L)		BMJ Op	en _			Ketone bodies
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Page	31 of 51	Log Acetate (mmol/L) Log Acetoacetate (mmol/L) .og 3hydroxybutyrate (mmol/L)	_ _	BMJ Open				Ketone bodies
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Supplementary Figure 1: Correlations between Child Health CheckPoint metabolites - children

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



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










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

3						Children									Adults				
4	Metabolic subgroup		Boys			Girls			All			Male			Female			All	
5	Linenzatein subelees linide	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
5	Lipoprotein subclass lipids Total lipids in chylomicrons & ax large VI DL (mmol/L)*	575	0.005	0.60	605	0.011	0.54	1100	0.007	0 5 9	175	0.040	0 52	11/0	0.012	0.51	1275	0.015	0.52
6	Total lipids in environmentals & exhauge vebe (minor/e)	575	0.005	0.00	605	0.011	0.54	1100	0.007	0.56	177	0.040	0.55	1140	0.015	0.51	1325	0.015	0.52
7	Total lipids in large VLDL (mmol/L)*	575	0.000	0.75	605	0.014	0.74	1180	0.010	0.74	177	0.007	1 13	1140	0.022	0.07	1325	0.027	0.70
0	Total lipids in medium VLDL (mmol/L)	575	0.035	0.55	605	0.121	0.55	1180	0.005	0.55	177	0.425	0.63	1140	0.101	0.75	1325	0.102	0.70
0	Total lipids in small VI DI (mmol/I)	575	0.38	0.15	605	0.40	0.15	1180	0.40	0.15	177	0.50	0.05	1148	0.55	0.27	1325	0.52	0.45
9	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
10	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
11	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
11	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
12	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
13	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
14	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
14	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
15	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
16	Lipoprotein particle size																		
17	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
10	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
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
19	Cholesterol																		
20	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
21	Total cholesterol in VIDI (mmol/I)	575	0.44	0.19	605	0.47	0.19	1180	0.46	0.19	177	0.83	0.40	1148	0.59	0.05	1325	0.62	0.30
22	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
22	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
23	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
24	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
25	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
25	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
26	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
27	Chronides and phospholinids																		
28	Sorum total triglycaridas (mmal/L)*	575	0.019	4 77	605	1 005	74 69	1100	0.062	10.07	175	1 696	0.06	11/0	1 1 2 0	2 72	1225	1 10	2 75
20	Triglycerides in VLDL (mmol/L)*	575	0.518	4.77	605	0.648	1 21	1180	0.902	1 1 2	177	1.080	2.80	1140	0.694	1 75	1325	0.75	2.75
29	Triglycerides in LDL (mmol/L)	575	0.502	0.02	605	0.040	0.03	1180	0.013	0.03	177	0.15	0.04	1148	0.054	0.04	1325	0.16	0.04
30	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
31	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
22	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
52	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
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
34	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
35	Apolipoproteins																		
36	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
27	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
57	Ratio of apolipoprotein B to apolipoprotein Al	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
38	For the second se																		
39	raily dClOS Total fatty acids (mmol/L)	E70	0.21	1 70	604	0.27	1 7 2	1174	0.20	1 71	173	11 OF	רד ר	11/5	10.02	2 20	1210	11 02	0 <i>∧</i> ⊏
40	Estimated degree of unsaturation	570	5.21 1 21	1.70	604	5.37 1.20	1.75	1174	1 20	1.71	173	1 1 2	2.72	1145	1 21	2.39	1310	1 21	2.45
11	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
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18:2, linoleic acid (mmol/L)	570	2.54	0.46	604	2.59	0.46	11/4	2.57	0.46	1/3	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.

3			Differences by age (Parents - Ch	ild)	
4	Metabolic subgroup		Unadjusted	,	
5					Conversion
6	Linoprotein subclass linids	Estimate	95% Cl	p-value	factor (SD) #
7	Total linids in chylomicrons & ex large VI DI (mmol/I)*	0 704	(0.519, 0.890)	<0.001	2 53
8	Total lipids in very large VLDL (mmol/L)*	0.922	(0.515, 0.850)	<0.001	3.03
9	Total lipids in large VLDL (mmol/L)*	0.648	(0.502, 0.795)	<0.001	1.95
10	Total lipids in medium VLDL (mmol/L)	0.105	(0.080, 0.129)	< 0.001	0.35
11	Total lipids in small VLDL (mmol/L)	0.107	(0.094, 0.121)	< 0.001	0.20
10	Total lipids in very small VLDL (mmol/L)	0.093	(0.086, 0.099)	< 0.001	0.10
12	Total lipids in IDL (mmol/L)	0.181	(0.166, 0.196)	< 0.001	0.23
13	Total lipids in large LDL (mmol/L)	0.229	(0.211, 0.247)	< 0.001	0.29
14	Total lipids in medium LDL (mmol/L)	0.144	(0.132, 0.155)	< 0.001	0.18
15	Total lipids in small LDL (mmol/L)	0.089	(0.082, 0.096)	<0.001	0.11
16	Total lipids in very large HDL (mmol/L)	0.012	(-0.003, 0.027)	0.128	0.22
17	Total lipids in large HDL (mmol/L)	0.032	(0.007, 0.057)	0.011	0.35
18	Total lipids in medium HDL (mmol/L)	0.076	(0.064, 0.089)	< 0.001	0.17
10	Total lipids in small HDL (mmol/L)	0.068	(0.058, 0.078)	< 0.001	0.14
19					
20	Lipoprotein particle size				
21	Mean diameter for VLDL particles (nm)	-0.147	(-0.263, -0.031)	0.013	1.63
22	Mean diameter for LDL particles (nm)	-0.044	(-0.052, -0.037)	<0.001	0.11
23	Mean diameter for HDL particles (nm)	-0.027	(-0.045, -0.010)	0.002	0.26
24					
27	Cholesterol				
25	Serum total cholesterol (mmol/L)	0.670	(0.619, 0.721)	<0.001	0.80
26	Total cholesterol in VLDL (mmol/L)	0.146	(0.129, 0.163)	< 0.001	0.25
27	Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.261	(0.237, 0.284)	< 0.001	0.36
28	Total cholesterol in LDL (mmol/L)	0.327	(0.300, 0.354)	<0.001	0.42
29	Total cholesterol in HDL (mmol/L)	0.082	(0.058, 0.106)	< 0.001	0.34
30	Total cholesterol in HDL2 (mmol/L)	0.064	(0.042, 0.086)	<0.001	0.31
31	Fotorified chalasteral (mmol/L)	0.018	(0.016, 0.020)	<0.001	0.03
27	Esternied cholesterol (mmol/L)	0.474	(0.437, 0.511)	<0.001	0.38
32		0.195	(0.180, 0.210)	<0.001	0.25
33	Glycerides and phospholinids				
34	Serum total triglycerides (mmol/I)*	0 176	(0 145, 0 206)	<0.001	0 44
35	Triglycerides in VI DL (mmol/L)*	0 140	(0.096, 0.183)	<0.001	0.44
36	Triglycerides in LDL (mmol/L)	0.040	(0.038, 0.043)	<0.001	0.04
37	Triglycerides in HDL (mmol/L)	0.020	(0.017, 0.022)	< 0.001	0.04
20	Total phosphoglycerides (mmol/L)	0.311	(0.290, 0.332)	< 0.001	0.34
20	Ratio of triglycerides to phosphoglycerides	0.049	(0.027, 0.071)	< 0.001	0.30
39	Phosphatidylcholine & other cholines (mmol/L)	0.295	(0.274, 0.316)	< 0.001	0.33
40	Sphingomyelins (mmol/L)	0.052	(0.047, 0.057)	< 0.001	0.08
41	Total cholines (mmol/L)	0.323	(0.302, 0.345)	< 0.001	0.35
42					
43	Apolipoproteins				
44	Apolipoprotein A1 (g/L)	0.099	(0.086, 0.112)	<0.001	0.19
15	Apolipoprotein B (g/L)	0.125	(0.113, 0.137)	<0.001	0.18
40	Ratio of apolipoprotein B to apolipoprotein Al	0.055	(0.046, 0.064)	<0.001	0.13
46					
4/	Fatty acids				
48	Total fatty acids (mmol/L)	1.738	(1.592, 1.885)	<0.001	2.24
49	Estimated degree of unsaturation	0.005	(0.000, 0.009)	0.030	0.06
50	22:6, docosahexaenoic acid (mmol/L)	0.037	(0.035, 0.040)	< 0.001	0.04
51	18:2, linoleic acid (mmol/L)	0.347	(0.310, 0.384)	< 0.001	0.54
51	Omega3 fatty acids (mmol/L)	0.105	(0.098, 0.113)	< 0.001	0.12
52	Omega6 fatty acids (mmol/L)	0.453	(0.414, 0.492)	< 0.001	0.60
53	Polyunsat. ratty acids (mmol/L)	0.558	(0.513, 0.603)	<0.001	0.70
54	Monounsat. Tatty acids; 16:1, 18:1 (mmoi/L)	0.568	(0.512, 0.625)	<0.001	0.85
55	Saturated fatty acids (mmol/L)	0.012	(0.554, 0.669)	<0.001	0.86
56	Eatty acid ratios				
57	Ratio of 22:6 docosabevaenoic acid to total fatty acids (%)	0 209	(0 190 0 228)	<0.001	0 20
58	Ratio of 18:2 lingleic acid to total fatty acids (%)	-1 079	(0.130, 0.220) (_1 221 _0 827)	~0.001	2 20
50	Ratio of omegas fatty acids to total fatty acids (%)	0.446	() AN3 () AQN)	<0.001 <0.001	0.69
29	Ratio of omega6 fatty acids to total fatty acids (%)	-0.992	(-1,227 -0,757)	<0.001	3,30
60	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	
Glycolysis related				
Pyruvate (mmol/L)	-0.007	(-0.009, -0.005)	< 0.001	
Citrate (mmol/L)	-0.017	(-0.018, -0.016)	< 0.001	
Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	<0.001	
Amino acids				
Alanine (mmol/L)	0.013	(0.009, 0.017)	< 0.001	
Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	< 0.001	
Glycine (mmol/L)	0.007	(0.003, 0.010)	< 0.001	
Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	
Isoleucine (mmol/L)	0.003	(0.002, 0.004)	< 0.001	
Leucine (mmol/L)	0.004	(0.003, 0.006)	< 0.001	
Valine (mmol/L)	0.009	(0.006, 0.011)	< 0.001	
Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	< 0.001	
Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	
Ketone bodies				
Acetate (mmol/L)*	0.101	(0.084, 0.117)	< 0.001	
Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	
3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	
Inflammation				
Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.062	(0.047, 0.078)	<0.001	

*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 - Bo	ys)	
metabolic subgroup		Unadjusted		Conversio
	Estimate	95% CI	p-value	factor (SD
Lipoprotein subclass lipids	0 727	(0.414.1.050)	-0.001	2.04
Total lipids in unviornicions & ex.large VLDL (mmol/L)	0.737	(0.414, 1.059)	<0.001	2.64
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)*	0.005	(0.018, 0.080)	<0.001	2.41
Total lipids in medium VLDL (mmol/L)	0.049	(0.015, 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 (mmoi/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
	-0.033	(-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
A				
Apolipoproteins	0.020	(0.040, 0.012)	0.001	0.46
Apolipoprotein A1 (g/L)	-0.030	(-0.048, -0.013)	0.001	0.15
Apolipoprotein B (g/L) Batia of apolipoprotein B to apolipoprotein Al	0.027	(0.012, 0.042)	0.001	0.13
Ratio of apolipoprotein B to apolipoprotein A	0.027	(0.010, 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				
Byruyate (mmol/L)	_0.001	(-0.004.0.002)	0 524	0.02
r yi uvale (IIIIIIII/L) Citrata (mmal/L)	-0.001	(-0.004, 0.002)	0.524	0.02
Gucare (IIIII0i/L)	0.007	(0.005, 0.009)	<0.001	0.02
Giycerol (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	0.04
Glutamine (mmol/L)	0.022		0.002	0.00
Glucine (mmol/L)	0.025	(0.016, 0.029)	<0.001	0.05
Grycine (IIIII0)/L)	0.010	(0.000, 0.014)	<0.001	0.03
nisiume (mmol/L)	0.001	(-0.000, 0.002)	0.075	0.01
isoleucine (mmoi/L)	-0.000	(-0.003, 0.002)	0.637	0.02
Leucine (mmoi/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
i yrosine (mmol/L)	0.000	(-0.001, 0.002)	0.582	0.01
Makana kadha				
	0.022		0.000	o
Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.17
Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.9

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

*metabolite has been log transformed

<text> # 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

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

Differences for adults (Female - Male) Metabolic subgroup Unadjusted Conversion Estimate 95% CI p-value 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 10 Total lipids in large VLDL (mmol/L)* -0.900 (-1.107, -0.693) < 0.001 1.34 11 Total lipids in medium VLDL (mmol/L) -0.325 (-0.385, -0.264) < 0.001 0.40 12 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 13 Total lipids in IDL (mmol/L) 0.014 (-0.023, 0.051) 0.465 0.24 14 Total lipids in large LDL (mmol/L) -0.011 (-0.058, 0.035) 0.634 0.29 15 Total lipids in medium LDL (mmol/L) -0.028 (-0.057, 0.001)0.058 0.18 16 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 < 0.001 0.24 (0.158, 0.231)17 Total lipids in large HDL (mmol/L) 0.395 (0.334, 0.455) < 0.001 0.41 18 Total lipids in medium HDL (mmol/L) 0.129 (0.101, 0.158)< 0.001 0.18 19 Total lipids in small HDL (mmol/L) 0.002 (-0.021, 0.025) 0.850 0.14 20 21 Lipoprotein particle size Mean diameter for VLDL particles (nm) < 0.001 -1.414 (-1.669, -1.159)1.68 22 Mean diameter for LDL particles (nm) 0.081 (0.066, 0.096) < 0.001 0.10 23 Mean diameter for HDL particles (nm) 0.278 (0.236, 0.320)< 0.001 0.28 24 25 Cholesterol Serum total cholesterol (mmol/L) 0.112 (-0.018, 0.241) 0.091 0.82 26 Total cholesterol in VLDL (mmol/L) -0.187 (-0.230, -0.145)< 0.001 0.28 27 Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L) -0.184 (-0.244, -0.124) < 0.001 0.38 28 Total cholesterol in LDL (mmol/L) -0.048 (-0.117, 0.021)0.175 0.44 29 Total cholesterol in HDL (mmol/L) 0.344 (0.286, 0.401) < 0.001 0.38 30 Total cholesterol in HDL2 (mmol/L) 0.324 < 0.001 0.35 (0.271, 0.378)Total cholesterol in HDL3 (mmol/L) 0.019 (0.014, 0.025) < 0.001 0.03 31 Esterified cholesterol (mmol/L) 0.070 (-0.023, 0.163)0.139 0.59 32 Free cholesterol (mmol/L) 0.046 (0.009, 0.084) 0.016 0.24 33 34 Glycerides and phospholipids Serum total triglycerides (mmol/L)* -0.344 < 0.001 0.47 35 (-0.416, -0.273)Triglycerides in VLDL (mmol/L)* -0.530 (-0.630, -0.429) < 0.001 0.66 36 0.033 Triglycerides in LDL (mmol/L) 0.008 (0.001, 0.014)0.04 37 Triglycerides in HDL (mmol/L) -0.004 (-0.010, 0.002) 0.228 0.04 38 Total phosphoglycerides (mmol/L) 0.106 (0.052, 0.159)< 0.001 0.34 39 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 40 Sphingomyelins (mmol/L) 0.032 (0.020, 0.045)< 0.001 0.08 41 Total cholines (mmol/L) 0.170 < 0.001 0.35 (0.115, 0.224)42 43 Apolipoproteins 0.146 (0.114, 0.178)< 0.001 0.21 44 Apolipoprotein A1 (g/L) Apolipoprotein B (g/L) -0.115 (-0.146, -0.084)< 0.001 0.20 45 Ratio of apolipoprotein B to apolipoprotein Al (-0.148, -0.105) < 0.001 -0.126 0.14 46 47 Fatty acids 48 Total fatty acids (mmol/L) -0.711 (-1.091, -0.330)< 0.001 2.39 < 0.001 Estimated degree of unsaturation 0.031 (0.021, 0.042) 0.07 49 22:6. docosahexaenoic acid (mmol/L) -0.002 (-0.008, 0.005)0.622 0.04 50 18:2, linoleic acid (mmol/L) 0.004 (-0.087, 0.094) 0.934 0.57 51 Omega3 fatty acids (mmol/L) < 0.001 0.12 -0.035 (-0.054, -0.016)52 0.936 Omega6 fatty acids (mmol/L) 0.004 (-0.094, 0.102)0.61 0.592 Polyunsat. fatty acids (mmol/L) -0.031 (-0.144, 0.082)0.71 53 0.93 Monounsat. fatty acids; 16:1, 18:1 (mmol/L) -0.372 (-0.520, -0.225) < 0.001 54 Saturated fatty acids (mmol/L) 0.93 -0.307 (-0.455, -0.159) < 0.001 55 56 Fatty acid ratios Ratio of 22:6 docosahexaenoic acid to total fatty acids (%) 57 0.29 0.064 (0.018, 0.110) 0.006 Ratio of 18:2 linoleic acid to total fatty acids (%) 1.527 (0.984, 2.070)< 0.001 3.43 58 Ratio of omega3 fatty acids to total fatty acids (%) -0.038 (-0.152, 0.075) 0.508 0.71 59 Ratio of omega6 fatty acids to total fatty acids (%) 1.882 < 0.001 3.38 (1.351, 2.412)60 Ratio of polyunsat. fatty acids to total fatty acids (%) 1.843 (1.272, 2.414)< 0.001 3.62 2.90 Ratio of monounsat. fatty acids to total fatty acids (%) -1.456(-1.914, -0.998)< 0.001 Ratio of saturated fatty acids to total fatty acids (%) -0.387 (-0.700, -0.074) 0.015 1.96

2	Glycolysis related				
3	Pyruvate (mmol/L)	-0.007	(-0.013, -0.002)	0.004	0.03
4	Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.02
5	Glycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.02
6					
7	Amino acids				
<i>,</i>	Alanine (mmol/L)	-0.020	(-0.030, -0.011)	< 0.001	0.06
8	Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	< 0.001	0.07
9	Glycine (mmol/L)	0.029	(0.020, 0.038)	< 0.001	0.06
10	Histidine (mmol/L)	-0.001	(-0.003, 0.000)	0.116	0.01
11	Isoleucine (mmol/L)	-0.016	(-0.019, -0.013)	<0.001	0.02
11	Leucine (mmol/L)	-0.019	(-0.022, -0.016)	< 0.001	0.02
12	Valine (mmol/L)	-0.029	(-0.035, -0.022)	< 0.001	0.04
13	Phenylalanine (mmol/L)	-0.000	(-0.002, 0.001)	0.576	0.01
14	Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	<0.001	0.01
15					
16	Ketone bodies				
17	Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.27
17	Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	1.05
18	3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.47
19					
20	Inflammation				
21	Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	0.24
22					

*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.

2	Supplementary table 5: Mother-child concordance; correlati	ons and par	tial correl	ations betwee	n mother	s and the	ir sons, daugi	hters and	l all childr	en. Moth	er								
3	Metabolic subgroup			Boy:	s	DCC#	05% (1	-		Gir	ls	DCC#	05% (1	_		All Ch	ildren	DCC#	05% (1
4	Lipoprotein subclass lipids	n		95% CI	n	PCC#	95% CI	n	u	95% CI	n	PCC#	95% CI	n	u	95% CI	n	PLL#	95% CI
5	Total lipids in chylomicrons & ex.large VLDL (mmol/L)* Total lipids in very large VLDL (mmol/L)*	469 469	0.22	0.13 - 0.30 0.16 - 0.33	468 468	0.22	0.13 - 0.31 0.16 - 0.33	518 518	0.24	0.16 - 0.32 0.14 - 0.30	517 517	0.23	0.14 - 0.31 0.13 - 0.29	987 987	0.23	0.17 - 0.29 0.18 - 0.29	985 985	0.23	0.17 - 0.29 0.17 - 0.29
5	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
6	Total lipids in medium VLDL (mmol/L)	469	0.26	0.17 - 0.34 0.18 - 0.35	468	0.25	0.17 - 0.34	518	0.29	0.21 - 0.37	517	0.28	0.20 - 0.36	987 987	0.28	0.22 - 0.34 0.23 - 0.34	985	0.27	0.22 - 0.33
7	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 large LDL (mmol/L)	469	0.27	0.19 - 0.35	468	0.28	0.18 - 0.35	518	0.29	0.22 - 0.37	517	0.30	0.22 - 0.37	987	0.29	0.23 - 0.34	985	0.28	0.22 - 0.34
8	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
9	Total lipids in very large HDL (mmol/L)	469	0.28	0.19 - 0.38	468	0.27	0.21 - 0.38	518	0.32	0.21 - 0.37	517	0.32	0.21 - 0.37	987	0.28	0.25 - 0.34	985	0.28	0.22 - 0.34
10	Total lipids in large HDL (mmol/L) Total lipids in medium HDL (mmol/L)	469	0.31	0.23 - 0.39	468 468	0.31	0.22 - 0.39	518 518	0.28	0.20 - 0.36	517 517	0.28	0.20 - 0.36	987 987	0.30	0.24 - 0.35	985 985	0.29	0.23 - 0.35
10	Total lipids in small HDL (mmol/L)	469	0.22	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
11	Lipoprotein particle size																		
12	Mean diameter for VLDL particles (nm) Mean diameter for LDL particles (nm)	469 469	0.30	0.22 - 0.38	468 468	0.30	0.22 - 0.38	518 518	0.27	0.19 - 0.35	517 517	0.26	0.18 - 0.34	987 987	0.29	0.23 - 0.35	985 985	0.28	0.22 - 0.34
13	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
1.4	Cholesterol																		
14	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
15	Iotal cholesterol in VLDL (mmol/L) Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	469	0.25	0.17 - 0.34 0.17 - 0.34	468 468	0.25	0.16 - 0.33 0.16 - 0.33	518 518	0.29	0.21 - 0.36	517	0.28	0.20 - 0.36	987 987	0.28	0.22 - 0.33 0.22 - 0.34	985 985	0.27	0.21 - 0.33 0.22 - 0.33
16	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
10	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 - 0.33	517	0.24	0.16 - 0.32	987 987	0.28	0.22 - 0.33	985 985	0.27	0.21 - 0.32
17	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
18	Free cholesterol (mmol/L)	465	0.28	0.19 - 0.36	464	0.27	0.19 - 0.33	518	0.32	0.24 - 0.39	517	0.32	0.24 - 0.39	983	0.30	0.24 - 0.35	981	0.29	0.24 - 0.35
19	Glycerides and phospholipids																		
20	Serum total trigiycerides (mmol/L)* Triglycerides in VLDL (mmol/L)*	469	0.28	0.20 - 0.36 0.22 - 0.38	468 468	0.28	0.19 - 0.36 0.21 - 0.38	518 518	0.29	0.21 - 0.37	517	0.28	0.20 - 0.36 0.19 - 0.35	987 987	0.29	0.23 - 0.35 0.23 - 0.35	985 985	0.28	0.23 - 0.34 0.22 - 0.34
20	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
21	Total phosphoglycerides (mmol/L)	469	0.21	0.12 - 0.30	468	0.21	0.13 - 0.30	518	0.26	0.18 - 0.34 0.17 - 0.34	517	0.25	0.17 - 0.33 0.18 - 0.34	987	0.24	0.18 - 0.30	985 981	0.24	0.18 - 0.29 0.20 - 0.32
22	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
22	Sphingomyelins (mmol/L)	465	0.27	0.15 - 0.33	464	0.27	0.15 - 0.32	518	0.25	0.17 - 0.33	517	0.25	0.21 - 0.33	983	0.20	0.20 - 0.32	981	0.26	0.20 - 0.32
23	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
24	Apolipoproteins	469	0.28	0.20-0.26	469	0.28	0.10-0.26	519	0.26	0 18 - 0 24	517	0.26	0.18 - 0.24	097	0.27	0.21 - 0.22	095	0.27	0.21 - 0.22
25	Apolipoprotein B (g/L)	469	0.28	0.18 - 0.35	468	0.28	0.19 - 0.38	517	0.20	0.18 - 0.34	516	0.20	0.18 - 0.34	986	0.27	0.23 - 0.35	984	0.27	0.23 - 0.35
26	Ratio of apolipoprotein B to apolipoprotein Al	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
20	Fatty acids Total fatty acids (mmol/L)	467	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
27	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	0.22 - 0.33	977	0.28	0.22 - 0.33
28	22:6, docosahexaenoic acid (mmol/L) 18:2 linoleic acid (mmol/L)	462	0.20	0.11 - 0.29	461	0.19	0.10 - 0.28	517 517	0.34	0.26 - 0.41	516 516	0.33	0.25 - 0.40	979 979	0.27	0.21 - 0.32	977 977	0.26	0.20 - 0.31
20	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
29	Omega6 fatty acids (mmol/L) Polyunsat_fatty acids (mmol/L)	462	0.23	0.14 - 0.32	461 461	0.24	0.16 - 0.33	517 517	0.31	0.23 - 0.38	516 516	0.31	0.23 - 0.38	979 979	0.27	0.21 - 0.33	977 977	0.28	0.22 - 0.33
30	Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	462	0.23	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
31	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
32	Fatty acid ratios Batio of 22:6 docosabexaenoic 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
22	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
33	Ratio of omega3 fatty acids to total fatty acids (%) Ratio of omega6 fatty acids to total fatty acids (%)	462 462	0.32	0.24 - 0.40 0.15 - 0.32	461 461	0.33	0.24 - 0.40 0.14 - 0.32	517 517	0.40	0.33 - 0.47 0.17 - 0.33	516 516	0.38	0.30 - 0.45 0.15 - 0.32	979 979	0.36	0.31 - 0.41 0.18 - 0.30	977 977	0.35	0.29 - 0.40 0.18 - 0.30
34	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
35	Ratio of monounsat. fatty acids to total fatty acids (%) Ratio of saturated fatty acids to total fatty acids (%)	462 462	0.29	0.21 - 0.38 0.11 - 0.29	461 461	0.29	0.20 - 0.37 0.11 - 0.28	517 517	0.33	0.25 - 0.40	516 516	0.31 0.26	0.23 - 0.39 0.18 - 0.34	979 979	0.32	0.26 - 0.37 0.17 - 0.29	977 977	0.30	0.25 - 0.36 0.17 - 0.29
20	Glycolysis related																		
50	Pyruvate (mmol/L) Citrate (mmol/L)	467	0.12	0.03 - 0.21	466 468	0.12	0.02 - 0.20	518 517	0.23	0.14 - 0.31	517 516	0.23	0.14 - 0.31	985 986	0.17	0.11 - 0.23	983 984	0.17	0.11 - 0.23
3/	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
38	Amino acids	460	0.22	0.12 0.21	46.9	0.22	0.14 0.21	517	0.33	0.34 0.40	516	0.22	0.24 0.40	086	0.29	0.22 0.22	094	0.28	0.22 0.24
39	Glutamine (mmol/L)	469	0.22	0.20 - 0.36	468	0.23	0.20 - 0.37	518	0.19	0.10 - 0.27	517	0.19	0.10 - 0.27	987	0.28	0.17 - 0.29	985	0.23	0.17 - 0.29
40	Glycine (mmol/L) Histidine (mmol/L)	468 468	0.22	0.13 - 0.30	467 467	0.23	0.15 - 0.32	517 518	0.19	0.10 - 0.27	516 517	0.18	0.10 - 0.26	985 986	0.20	0.14 - 0.26	983 984	0.21	0.15 - 0.27
-0	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
41	Leucine (mmol/L) Valine (mmol/L)	469 469	0.34	0.25 - 0.42	468 468	0.34	0.25 - 0.42	518 516	0.28	0.20 - 0.35	517 515	0.27	0.19 - 0.35	987 985	0.30	0.25 - 0.36	985 983	0.30	0.24 - 0.36
42	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
43	iyrosine (mmol/L)	468	U.33	0.25 - 0.41	467	0.33	0.25 - 0.41	518	U.26	0.17 - 0.34	517	0.25	0.17 - 0.33	986	U.29	0.23 - 0.35	984	0.29	0.23 - 0.35
11	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
45	Acetoacetate (mmol/L)* 3hvdroxybutyrate (mmol/L)*	469 437	0.13	0.04 - 0.22	468 436	0.13	0.04 - 0.22	517 486	0.03	-0.05 - 0.12	516 485	0.02	-0.06 - 0.11	986	0.08	0.01 - 0.14	984 921	0.08	0.01 - 0.14
45	Inflammation																		
46	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
47																			

*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			Bo	ys					Gir	ls					All Chi	ildren	
	n	cc	95% CI	n	PCC#	95% CI	n	cc	95% CI	n	PCC#	95% CI	n	cc	95% CI	n	PCC#
Lipoprotein subclass lipids	547	0.22	0.4.4 . 0.20		0.22	0.4.4 . 0.20	500	0.20	0.42 0.20	505	0.40	0.44 0.07	4422	0.22	0.46 0.27	4420	0.24
Total lipids in very large VLDL (mmol/L)*	547	0.22	0.14 - 0.50	544	0.22	0.14 - 0.30	586	0.20	0.12 - 0.28	585	0.19	0.09 - 0.27	1133	0.22	0.16 - 0.27	1129	0.21
Total lipids in large VI DL (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
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
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
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
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
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
Total lipids in medium HDL (mmol/L) Total lipids in small HDL (mmol/L)	547	0.15	0.10 - 0.24	544 544	0.14	0.10 - 0.23	586 586	0.10	0.02 - 0.18 0.10 - 0.26	585 585	0.10	0.02 - 0.18 0.10 - 0.26	1133 1133	0.12	0.07 - 0.18 0.12 - 0.23	1129 1129	0.1
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	0.2
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	0.2
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	0.2
Chalasteral																	
Cholesterol		0.20	0.20 0.20		0.27	0.40 0.35	500	0.24	0.24 0.20	505	0.24	0.24 0.20	4422	0.20	0.24 0.25	44.20	
Total cholesterol in VIDL (mmol/L)	547	0.26	0.20 - 0.38	544	0.27	0.19 - 0.33	596	0.51	0.24 - 0.38	595	0.51	0.24 - 0.39	1122	0.50	0.24 - 0.33	1129	0.2
Remnant cholesterol (nonHDL nonLDL cholesterol) (mmol/L)	547	0.20	0.10 - 0.54	544	0.20	0.18 - 0.33	586	0.20	0.20 - 0.33	585	0.23	0.20 - 0.35	1133	0.20	0.22 - 0.32	1129	0.2
Total cholesterol in LDL (mmol/1)	547	0.29	0.21 - 0.34	544	0.20	0.20 - 0.34	586	0.28	0.21 - 0.35	585	0.28	0.21 - 0.35	1133	0.29	0.23 - 0.34	1129	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
Glycerides and phospholipids	547	0.20	0.20 - 0.25	544	0.27	0 19 - 0 24	596	0.25	0 17 - 0 22	595	0.24	0 16 - 0 21	1122	0.26	0 21 - 0 27	1170	0.7
Triglycerides in VLDL (mmol/L)*	547	0.20	0.20 - 0.35	544	0.27	0.20 - 0.34	586	0.25	0.15 - 0.32	585	0.24	0.14 - 0.20	1133	0.20	0.20 - 0.32	1129	0.2
Trighycerides in IDL (mmol/L)	547	0.20	0.12 - 0.30	544	0.20	0.11 - 0.27	586	0.25	0.19 - 0.30	585	0.21	0.19 - 0.25	1133	0.20	0.18 - 0.29	1129	0.2
Trighycerides in HDL (mmol/L)	547	0.20	0.12 - 0.28	544	0.15	0.17 - 0.27	586	0.27	0.19 - 0.34	585	0.27	0.19 - 0.34	1133	0.24	0.21 - 0.23	1129	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
Ratio of apolipoprotein B to apolipoprotein Al	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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
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	0.2
Fatty acid ratios Batio of 22:6 docosabexaenoic acid to total fatty acids (%)	537	0.31	0.23 - 0.39	524	0.30	0.22 - 0.27	5.83	0 32	0.26 - 0.40	587	0.32	0.24 - 0.39	1120	0 37	0.27 - 0.27	1116	0.3
Ratio of 18:2 linoleic acid to total fatty acids (%)	527	0.51	0.07 - 0.58	524	0.50	0.06 - 0.22	583	0.53	0.10 - 0.40	582	0.52	0.09 - 0.59	1120	0.52	0.11 - 0.22	1116	0.3
Ratio of omega3 fatty acids to total fatty acids (%)	527	0.13	0.24 - 0.25	524	0.13	0.24 - 0.25	583	0.40	0.33 - 0.20	582	0.17	0.32 - 0.25	1120	0.36	0.31 - 0.41	1116	0.1
Ratio of omega6 fatty acids to total fatty acids (%)	527	0.24	0.16 - 0.39	524	0.32	0.16 - 0.39	583	0.40	0.15 - 0.30	582	0.33	0.14 - 0.40	1120	0.30	0.18 - 0.91	1116	0.3
Ratio of polyunsat, fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.35	534	0.24	0.19 - 0.34	583	0.26	0.18 - 0.33	582	0.24	0.16 - 0.37	1120	0.24	0.21 - 0.37	1116	0.2
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	0.7
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	0.2
Glycolysis related				_			_										
Pyruvate (mmol/L) Citrate (mmol/L)	545 547	0.11	0.03 - 0.20	542 544	0.11	0.03 - 0.20	586 585	0.22	0.15 - 0.30	585	0.22	0.15 - 0.30	1131 1132	0.17	0.11 - 0.22	1127 1128	0.1
Glycerol (mmol/L)	151	0.25	0.15 - 0.44	149	0.23	0.13 - 0.42	175	0.14	-0.03 - 0.26	175	0.14	-0.02 - 0.22	326	0.20	0.09 - 0.30	324	0.1
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	0.2
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	0.2
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	0.2
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	0.2
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	0.3
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	0.2
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 <mark>- 0</mark> .33	1131	0.33	0.27 - 0.38	1127	0.3
Phenylalanine (mmol/L) Tyrosine (mmol/L)	547 545	0.29 0.31	0.22 - 0.37 0.24 - 0.39	544 542	0.29 0.32	0.22 - 0.37 0.24 - 0.39	586 586	0.30 0.27	0.22 - 0.37 0.19 - 0.34	585 585	0.30	0.22 - 0.37 0.19 - 0.34	1133 1131	0.29 0.29	0.24 - 0.35 0.24 - 0.34	1129 1127	0.2
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	0.1
Acetoacetate (mmol/L)* 3hydroxybutyrate (mmol/L)*	547	0.11	0.03 - 0.20	544	0.12	0.03 - 0.20	585 551	0.04	-0.04 - 0.12	584 550	0.04	-0.04 - 0.12	1132 1062	0.07	0.02 - 0.13	1128 1058	0.0
Shyoroxybutyrate (IIIIIO/L)	511	0.22	0.15 - 0.30	508	0.22	0.15 - 0.30	221	0.34	0.20-0.41	550	0.33	0.25 - 0.40	1002	0.27	0.21 - 0.33	1029	0.2
Inflammation Glycoprotein acetyls, mainly at acid discoprotein (mmol/L)	547	0.24	0 16 - 0 22	544	0.22	0 15 - 0 21	5.96	0.24	0 17 - 0 22	5.95	0.35	0 16 - 0 21	1122	0.24	0 19 - 0 20	1170	0.2
Grycoprotein acetyis, mainiy atacid giycoprotein (MMOI/L)	547	0.24	0.10 - 0.32	544	0.23	0.13 - 0.31	360	0.24	0.17 - 0.32	585	0.23	0.10 - 0.31	1133	0.24	0.19 - 0.30	1129	0.2:

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

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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	(<i>a</i>) Indicate the study's design with a commonly used	2	14-15
		term in the title or the abstract		
		(b) Provide in the abstract an informative and balanced	2	9-13
		summary of what was done and what was found		26-32
Introduction		0		
Background/rationale	2	Explain the scientific background and rationale for the	4-5	Pg 4 (3-9 ,21-22,
-		investigation being reported		31-32, 33-35)
				Pg 5 (3-4)
Objectives	3	State specific objectives, including any prespecified	5	5-7
2		hypotheses		
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,	5-6	Pg 5 (33-35)
C		including periods of recruitment, exposure, follow-up,		Pg 6 (1-27)
		and data collection		8 ()
Participants	6	(a) Cohort study—Give the eligibility criteria, and the	5	18-26
1		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		
		<i>Cross-sectional study</i> —Give the eligibility criteria, and		
		the sources and methods of selection of participants		
		(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,	6-8	Pg 6 (29-36)
		potential confounders, and effect modifiers. Give		Pg 7 (1-6, table 1
		diagnostic criteria, if applicable		15-19)
				Pg 8 (1-33)
Data sources/	8*	For each variable of interest, give sources of data and	6-8	Pg 6 (29-36)
measurement		details of methods of assessment (measurement).		Pg 7 (1-6, table 1
		Describe comparability of assessment methods if there is		15-19)
		more than one group		Pg 8 (1-33)
Bias	9	Describe any efforts to address potential sources of bias	9	6-10
	10		10	

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Statistical methods 12 (a) Describe all statistical methods, including those used 9 2-26 (b) Describe any methods used to examine subgroups 9 2-26 (c) Explain how missing data were addressed 9 6-10 (c) Explain how missing data were addressed 9 6-10 (c) Cohort study—If applicable, explain how loss to 9 6-10, 15-17, 20 (c) Explain how saddressed 23 23 (c) Describe any sensitivity analyses 9 15-17, 20-23 Results (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 5-12 (b) Give reasons for non-participantis of a cach stage 10 8-12 (c) Consider use of a flow diagram figure 1 2 (b) Indicate number of participants (eg demographic, the lights, escili) and information on exposures and potential (table 2) 2 (c) Cohort study—Summarise follow-up time (eg, average and total arount) NA (c) Cohort study—Summarise follow-up time (eg, average and total arount) NA (c) Cohort study—Report numbers of outcome events or summary sup table 1 12 (c) Cohort study—Report numbers of outcome events or summary cata	Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why)	2-26
(b) Describe any methods used to examine subgroups 9 2-26 and interactions	Statistical methods	12	2. (<i>a</i>) Describe all statistical methods, including those used 5. to control for confounding)	2-26
ic) Explain how missing data were addressed 9 6-10 (d) Cohort study—If applicable, explain how loss to follow-up was addressed 9 6-10, 15-17, 20 Case control study—If applicable, explain how matching of cases and controls was addressed 23 23 (e) Describe any sensitivity analyses 9 15-17, 20-23 Results Page number 10 and figure 1 5-12 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 8-12 (b) Give reasons for non-participation at each stage 10 8-12 6 (c) Consider use of a flow diagram figure 1 2 11 2 (b) Indicate number of participants (cg demographic, (b) Indicate number of participants with missing data for each variable of interest figure 1 1 1 (c) Cohort study—Supmarise follow-up time (eg, average and total amount) NA total amount) 1 Outcome data 15* Cohort study—Report numbers of outcome events or summary measures over time NA Case-control study—Report numbers of outcome events or summary measures <td></td> <td></td> <td>(<i>b</i>) Describe any methods used to examine subgroups and interactions</td> <td>)</td> <td>2-26</td>			(<i>b</i>) Describe any methods used to examine subgroups and interactions)	2-26
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(c) If relevant, consider translating estimates of relative risk into NA			(c) If relevant, consider translating estimates of relative risk into	NA	
absolute risk for a meaningful time period			absolute risk for a meaningful time period		
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Other analyses	17	Report other analyses done—eg analyses of subgroups and	supp tables	
		interactions, and sensitivity analyses	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	13	18-28
		potential bias or imprecision. Discuss both direction and		
		magnitude of any potential bias		
Interpretation	20	Give a cautious overall interpretation of results considering	13-16	Pg 13 (30-32)
		objectives, limitations, multiplicity of analyses, results from		Pg 14 (all)
		similar studies, and other relevant evidence		Pg 15 (all)
				Pg 16 (1-2)
Generalisability	21	Discuss the generalisability (external validity) of the study	13	19-27
		results		
Other information	on			
Funding	22	Give the source of funding and the role of the funders for the	17-18	Pg 17 (28-33)
		present study and, if applicable, for the original study on which		Pg 18 (1-3)
		the present article is based		

*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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Page 1 of 50

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1	Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their
2	parents
3	
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24	
25	Keywords: Metabolomics; lipids; inflammation; reference values; parents; children;
26	inheritance patterns; correlation studies; epidemiologic studies; cross-sectional studies.
27	
28	Word count: 4270
29	
30	Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched
31	chain amino acid; BD: Becton Dickinson; CDC: Centers for Disease Prevention and Control;
32	CVD: Cardiovascular disease; CPS1: Carbamoyl-phosphate synthase 1; DHA:
33	Docosahexaenoic acid; DOB: Date of birth; EDTA: Ethylenediaminetetraacetic acid; FDR:
34	False discovery rate; GlycA: Glycoprotein acetyls; HbA1c: Haemoglobin A1c; HDL: High-
35	density lipoprotein; HOMA: Homeostatic model assessment; IDL: Intermediate density
36	lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein; LiH: Lithium Heparin; LSAC:
	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml 1

1 Longitudinal Study of Australian Children; MUFA: Monounsaturated fatty acid; NMR:

2 Nuclear magnetic resonance; PCOS: Polycystic Ovary Syndrome; PUFA: Polyunsaturated

3 fatty acid; SFA: Saturated fatty acids; SST: serum separating tubes; T2D: Type 2 diabetes;

4 T2DM: Type 2 diabetes mellitus; VLDL: Very low density lipoprotein; XL: Very large;

5 XXL: Chylomicrons and extremely large; XS: Very small.

ABSTRACT

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

14 Design: The population-based cross-sectional Child Health CheckPoint study nested within15 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.

32 Conclusions: We describe the serum metabolite profiles from mid-childhood and adulthood 33 in a population-based sample, together with parent-child concordance. Distinct age- and sex-34 specific profiles were observed. These data will be informative for investigation of the 35 childhood origins of adult non-communicable diseases and for comparative studies in other 36 populations.

1	1	Strengths and limitations of this study:
2	2	• In a large population-based cohort, venous blood was collected for children and their
3 4	3	attending parent on the same day using the same methods
5	4	• Rapidly processed, high quality serum samples with standardised metabolomic data
0 7	5	generated as a single batch
8	5	Cross sectional design does not enable longitudinal analysis of specific metabolite
9 10	-	• cross-sectional design does not enable forgitudinal analysis of specific incluonite
11	/	species over short term or longer periods of time
12 13	8	• Assessment of paternal associations with offspring metabolite measures is limited by
14	9	a relatively small sample size compared to mother-child pairs, reducing the precision
15 16	10	of estimates
17	11	• Factors known to influence metabolomic profile (such as body mass index) were not
18 19	12	considered as the aim was to describe the distribution of metabolites in children and
20	13	their parents
21 22		and Fandrine.
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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.⁶⁷

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.⁸⁹ 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

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(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.

8 Here, we describe (1) the distribution of NMR-based metabolite measures in a population9 based cohort of 11-12 year old children and their parents, differences in metabolite
10 concentrations (2) by age (adults compared to children) and (3) by sex in children and adults;
11 and (4) report sex-specific parent-child concordance.

12 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

32 Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D)
34 and the Australian Institute of Family Studies Ethics Committee (14-26). The attending
35 parent/caregiver provided written informed consent for themselves and their child to

1 participate in the study, and asked to provide optional consent for the collection and use of

2 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.

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1 Measures

3 <u>Metabolomic profiling</u>

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

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

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

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

* 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. Page 9 of 50

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1 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

25 Statistical analysis

26 <u>Sample Characteristics</u>

28 Continuous descriptive variables and metabolite measures were summarised using means and 29 standard deviations (SD) for children and adults separately, by sex and overall. For skewed 30 metabolites, geometric means and relative SD were reported. To provide visual comparisons 31 of distributions of metabolites by age and sex, density plots were used. Population summary 32 statistics were estimated by applying survey weights and survey procedures that corrected for 33 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 34 weights.²³ More detail on the calculation of weights is provided elsewhere.²⁴ 35

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

2 (adults, children)

Skewed metabolites (skewness greater or equal to 2) were log-transformed. We used twosided 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.

9 Parent-Child concordance

10 Concordance between parents and children was assessed by 1) Pearson's correlation 11 coefficients (CC) with 95% confidence intervals, and 2) partial correlation coefficients 12 (PCC), adjusting for child and parent age, disadvantage index, fasting time, processing lag 13 time (and for child and parent sex where appropriate). Scatterplots of parent versus child 14 metabolites (log-transformed where needed as above) were examined to check for outliers 15 and to ensure assumptions were met.

16 The analyses were repeated using weighted multi-level survey analyses and compared to 17 unweighted analyses. As there appeared to be no major effect of response patterns on 18 results we reported results from unweighted analyses. Analyses were undertaken using 19 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.

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RESULTS

3 Sample characteristics 4

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. of metabourus ...

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)

I)

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

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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.

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

13 Parent-child concordance

14 Figure 4 shows the correlations between metabolite measures for all children with all parents,

15 and for boys and girls with mothers (but not with the 177 fathers, given the small numbers).

16 The corresponding correlation coefficients and partial correlation coefficients are listed in

17 supplementary tables 5 and 6.

18 Correlations for all parents and all children showed similar patterns to that observed for 19 mother and child by sex. While there was little suggestion of substantial correlation within 20 parent-child dyads for some metabolites (eg glucose, acetate) a positive correlation was found 21 for many metabolite measures irrespective of child sex. For example, positive correlations 22 were observed for isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total serum cholesterol (CC 23 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-24 child comparisons. Additional adjustment for factors that potentially influence metabolite 25 levels (age, socioeconomic status, fasting time and processing lag time) had little effect on 26 the degree of correlation in any comparison (supplementary tables 5 and 6).

1 DISCUSSION

2 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.

11 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.

14 Further strengths include the large number of parent-child dyads representing a wide range of

15 parent ages, the national population-based sample and the state-of-the-art measurements.

16 Replication studies exploring sex differences at earlier and later stages of childhood and

17 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

31 Overall, we found a clear difference in metabolite profile between children and their parents.

32 This was apparent for specific metabolite measures (such as some amino acids) as well as the

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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.

7 Sex-specific differences in children (± 0.2 SD) were generally much smaller than in adults 8 $(\pm 0.8 \text{ SD})$. Large metabolomic studies using alternative platforms have previously reported reproducible, sex-specific signatures in circulating metabolite profile in adults.²⁷ ²⁸ This 9 10 includes differences in amino acid and lipid serum concentrations, potentially influenced by 11 sex-specific effects of genetic polymorphisms on metabolite levels. 28 29 As in our study, most 12 amino acids have usually been reported to be higher in men than women.^{28 30} For example, in a 13 recent study of 507 metabolic markers in 1756 individuals (903 female and 853 male aged 14 ~ 60 years), one third of metabolites showed significant sexual dimorphism. These were 15 predominantly related to pathways of steroid metabolism, fatty acids, other lipids, and a large 16 proportion of amino acids.³⁰ Of particular note, branched chain amino acids (BCAAs) and 17 their related metabolic products were amongst the most differentially represented, with much 18 higher isoleucine, leucine and valine in males. A similar finding of higher leucine and valine 19 was also noted in the Cooperative Health Research in the Region of Augsburg (KORA) 20 follow-ups 3 (F3) and 4 (F4) analysis of >3000 adults²⁸ consistent with our observations in 21 adulthood.

22 In children, we found sex-specific differences for leucine and valine were smaller but in the 23 same direction as adults. Several lines of evidence implicate BCAA metabolism with 24 metabolic risk in humans. For example, three candidate genes for obesity and/or type 2 25 diabetes mellitus (T2DM) are involved in the BCAA metabolic pathway.³¹ In a recent large 26 meta-analysis of metabolomics in diabetes, a >30% higher risk of type 2 diabetes was found 27 per SD increase in isoleucine, leucine, valine or tyrosine, whereas glycine and glutamine 28 were inversely associated with risk.³¹ Several clinical studies have also reported that BCAAs 29 positively correlate with insulin resistance, homeostatic model assessment (HOMA) index 30 and levels of haemoglobin A1c (HbA1c), while longitudinal studies have reported that 31 increased blood BCAAs are predictive of future insulin resistance and type 2 diabetes 32 (T2D).³² It is intriguing to speculate that the higher BCAA in males from early life could 33 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 sexspecific 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 studies²⁸, we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of $\sim 10,000$ adults with cardiovascular disease (CVD), with replication in > 53,000subjects, 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 $(p=6.3 \times 10^{-5})$ 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 cholesterol⁴⁰ 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.

28 These are the first data on the mother-child or parent-child correlations of NMR metabolites.

29 Smaller studies have reported positive correlations between parents and children for a limited

30 range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL

- 31 cholesterol and triglycerides measured using conventional methods. We found positive
- 32 correlations between parents and children for the same lipid measures (although measured
- 33 using NMR) consistent with previously reported findings. One study reported a positive

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1	association between the serum lipid levels of 4 year old children (n=127) and their parents
2	(122 mothers and 118 fathers) 43 while another study of children aged 6-18 (n=255) and their
3	parents (n=179) found that the age of the child influenced the degree of correlation of several
4	lipid measures, with older (10-18 years) children more similar to their parents in terms of
5	triglyceride levels than younger individuals (6-9 years). ¹²
6	Unanswered questions and future research
7	The temporal and sex specific dynamism of the metabolomics data we describe here offer
8	considerable opportunities for identification of biomarkers of risk for a range of non-
9	communicable diseases early in life, to inform targeted interventions and monitor their
10	efficacy. Combining metabolomics with other 'omics data (such as genetics), as is
11	increasingly reported from large adult studies, offers considerable promise in understanding
12	the causal pathways that link early life exposures, genetics and intermediate phenotypes with
13	later onset chronic disease, and in identifying clinically relevant biomarkers.
14	In conclusion, we describe the metabolite profile from mid-childhood and adulthood in a
15	population-based sample together with parent-child concordance and sex-specific differences

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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2 This paper uses unit record data from *Growing Up in Australia*, the Longitudinal Study of

3 Australian Children. The study is conducted in partnership between the Department of Social

4 Services (DSS), the Australian Institute of Family Studies (AIFS) and the Australian Bureau

5 of Statistics (ABS). The findings and views reported in this paper are those of the author and

6 should not be attributed to DSS, AIFS or the ABS.

8 REDCap (Research Electronic Data Capture) tools <u>ENREF 43⁴⁴</u> were used in this study. More
9 information about this software can be found at: www.project-redcap.org.

10 We thank the LSAC and CheckPoint study participants, staff and students for their11 contributions.

12 COMPETING INTERESTS

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2	the Victorian Government's Operational Infrastructure Support Program.
3	
4 5	CONTRIBUTIONS
6	DB, RS and JC conceptualised and developed the Metabolomics Checkpoint study. SE and
7	JC undertook all aspects of data analysis. SAC coordinated the acquisition of metabolomics
8	data and provided critical review of this manuscript. MW, the Principal Investigator of the
9	Child Health CheckPoint, planned the analyses and provided critical review of this
10	manuscript. SE and RS drafted the manuscript. PW, MJ, TD, KL, JC, DB provided critical
11	expert advice and critical review of this manuscript.
12 13	DATA SHARING STATEMENT
14	Dataset and technical documents available from Growing Up in Australia: The Longitudinal
15	Study of Australian Children via low-cost license for bone fide researchers. More information
16	is available at www.growingupinaustralia.gov.au
17	
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1	FIGURE CAPTIONS AND FOOTNOTES
3	Figure 1: Participant flow chart.
4	n=number of families, c=number of children, p=number of attending adults,
5	MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,
6	LSAC=Longitudinal Study of Australian Children
7	*Unable to analyse due to insufficient volume or poor quality sample
8	^Data from 6 non-biological child-parent pairs excluded from concordance analyses
9	
10	Figure 2: Differences in metabolite levels between children and adults.
11	Association measures are SD difference in metabolite concentration for adults compared to
12	children. Error bars represent 95% confidence intervals. Significant associations after p-
13	values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold
14	(FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals
15	and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein;
16	IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low
17	density lipoprotein.
18	
19	Figure 3: Sex-specific differences in metabolite levels in childhood and adulthood.
20	Association measures are SD difference in metabolite concentration for females compared to
21	males in children (A) and adults (B). Error bars represent 95% confidence intervals.
22	Significant associations after p-values adjusted for multiple testing using Benjamini-
23	Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute
24	concentration units, 95% confidence intervals and associated p-values are listed in
25	supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density
26	lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.
27	
28	Figure 4: Parent: child correlation for metabolite measures.
29	Pearson's correlation coefficients for all children with all parents (A); and for boys (blue)

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

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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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1 2	SUPPLEMENTARY DOCUMENTS
3	Supplementary figure 1: Correlation of NMR measures in children.
4	
5	Heatmap showing the correlation between metabolite measures in children. The correlations
6	shown are Spearman's correlation coefficients with blue cells representing negative
7	correlations and red cells representing positive correlations.
8	
9	Supplementary figure 2: Correlation of NMR metabolite measures in parents.
10	
11	Heatmap showing the correlation between metabolite measures in parents. The correlations
12	shown are Spearman's correlation coefficients with blue cells representing negative
13	correlations and red cells representing positive correlations.
14	
15	Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.
16	
17	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
18	each cholesterol and apolipoprotein measure.
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21	Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.
22	
23	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
24	each fatty acid and fatty acid ratio measure.
25	
26	Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass
27	particles.
28	
29	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
30	total lipids within each of the 14 lipoprotein subclass particles.
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32	Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride
33	measures.
34	$\mathbf{D} = (11)^{-1} (11)^{-$
35	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
30	inpoprotein particle sizes and trigryceride measures.
37	Supplementary figure 7. Density plate for glycolysis veloted amine acid betwee hady
30	Supplementary figure 7: Density plots for glycolysis related, animo acid, ketone body,
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44 15	Supplementary table 1: weighted mean (SD) of metabolite measures in children and
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3	1	Supplementary table 3: Differences in mean metabolite levels in girls compared to boys
4	2	in absolute concentration units.
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6	л Л	Supplementary table 1. Differences in mean metabolite levels in female compared to
7	4	Supplementary table 4. Differences in mean metabolite levels in female compared to
, 8	5	male adults in absolute concentration units.
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10	7	Supplementary table 5: Mother-child concordance; correlations and partial correlations
10	8	between mothers and their sons, daughters and all children.
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12	10	Supplementary table 6: Parent-child concordance; correlation and partial correlations
13	11	between all parents and their sons, daughters and all children.
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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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6	Differences in metabolite levels of adults and children	
7	Log Total lipids in chylomicrons and extr. large VLDL (mmol/L) -	
8	Log Total lipids in medium VLD (mmol/L) -	
9	Total lipids in small VLD. (mmo/L) - Total lipids in very small VLD. (mmo/L) -	
10	Total lipids in large LDL (mmol/L) - Lipioprotein Subclass	
11	Total lipids in very large HDL (mmo/L) - Total lipids in large HDL (mmo/L) - Total lipids in large HDL (mmo/L) -	
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∠∠ 23	Apolipoprotein B (g/L)	
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30	Ratio of saturated fatty acids to total fatty acids (%)	
31	Lactate (minol/L) = Glycolysis related	
32	Giycerol (mmo/L)	
32	Alanine (mmol/L) = Gklanine (mmol/L) =	
34	Histidine (mmo/L) = Severine (mmo/L) = Amino acids	
35	Valine (mmol/L) - Phenytalania (mmol/L) - Turcine (mmol/L) -	
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37	Log Aceľoacetate (mmol/L) – Log 3hydroxybutyrate (mmol/L) – Ketone bodies	
38	Albumin (signal area) - Creatinine (mmol/L) - Fluid balance	
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41	sub-binerence in metabolite concentration (95% Cr) for adults compared to children	
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45	Figure 2: Differences in metabolite levels between children and adults.	
46	Association measures are SD difference in metabolite concentration for adults compared to children. E	rror
47	bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple te	esting
48	using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolu	ite
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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 pvalues 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.

Motabolio subgroup	Children All								Adults Formale									
Metabolic subgroup	n	Mean	SD	n	Female	SD	n	All Mean	SD		Mean	SD		Female	SD	n	All Mean	SD
Lipoprotein subclass lipids		WEall	30		Weatt	30		Weatt	30		Ivicali	30		Ivicali	30		Weatt	
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	575	0.005	4.826	605	0.011	3.260	1180	0.007	4.054	17	0.040	2.101	1148	0.013	2.822	1325	0.015	2.77
Total lipids in very large VLDL (mmol/L)*	575	0.006	6.246	605	0.014	4.800	1180	0.010	5.521	17	0.087	2.419	1148	0.022	3.458	1325	0.027	3.39
Total lipids in large VIDI (mmol/L)*	575	0.059	3 945	605	0.121	2 488	1180	0.085	3 234	17	0.423	1 287	1148	0.022	1 666	1325	0.182	1.66
Total lipids in medium VI DI (mmol/I)	575	0.441	0.270	605	0.478	0.272	1180	0.460	0.271	17	0.959	0.630	1148	0.548	0.366	1325	0.602	0.43
Total lipids in small VI DL (mmol/L)	575	0.381	0.153	605	0.405	0.1/6	1180	0.393	0.1/9	17	0.555	0.000	11/18	0.340	0.216	1325	0.520	0.432
Total linids in very small VI DL (mmol/L)	575	0.301	0.133	605	0.342	0.076	1180	0.334	0.145	17	0.050	0.275	1148	0.426	0.110	1325	0.320	0.23-
Total lipids in IDL (mmol/L)	575	0.923	0.176	605	0.834	0.070	1180	0.934	0.075	17	0.451	0.111	11/18	0.420	0.240	1325	0.425	0.110
Total lipids in large LDL (mmol/L)	575	0.004	0.170	605	0.034	0.105	1100	0.010	0.100	17	1 162	0.201	1140	1 1 5 5	0.240	1225	1 156	0.24
Total lipids in mage LDL (mmol/L)	575	0.517	0.220	605	0.541	0.220	1100	0.525	0.224	17	0.676	0.327	1140	0.655	0.256	1225	0.659	0.30
Total lipids in medium EDE (mmol/L)	575	0.311	0.150	005	0.319	0.140	1100	0.330	0.156	17	0.676	0.214	1140	0.055	0.165	1525	0.056	0.10
Total lipids in small LDL (mmol/L)	575	0.338	0.083	605	0.340	0.087	1180	0.339	0.085	17	0.439	0.135	1148	0.425	0.114	1325	0.427	0.11
Total lipids in very large HDL (mmol/L)	575	0.482	0.196	605	0.495	0.184	1180	0.488	0.189	1/	0.320	0.189	1148	0.497	0.229	1325	0.474	0.23
Total lipids in large HDL (mmol/L)	575	0.874	0.291	605	0.859	0.275	1180	0.866	0.282	1/	0.509	0.335	1148	0.900	0.382	1325	0.849	0.39
Total lipids in medium HDL (mmol/L)	575	0.917	0.127	605	0.871	0.126	1180	0.894	0.128	17	0.828	0.241	1148	0.9/1	0.175	1325	0.952	0.19
Total lipids in small HDL (mmol/L)	575	1.039	0.103	605	0.997	0.115	1180	1.018	0.111	17	1.055	0.254	1148	1.085	0.138	1325	1.081	0.15
Lipoprotein particle size																		
Mean diameter for VLDL particles (nm)	575	37.063	1.633	605	37.238	1.557	1180	37.152	1.591	17	38.527	1.737	1148	36.943	1.599	1325	37.152	1.70
Mean diameter for LDL particles (nm)	575	23.587	0.103	605	23.628	0.109	1180	23.608	0.107	17	23.487	0.093	1148	23.573	0.100	1325	23.562	0.10
Mean diameter for HDL particles (nm)	575	10.081	0.233	605	10.102	0.221	1180	10.092	0.226	17	9.798	0.244	1148	10.068	0.262	1325	10.032	0.27
Cholesterol																		
Serum total cholesterol (mmol/L)	575	3.576	0.620	605	3.596	0.643	1180	3.586	0.629	17	4.161	0.885	1148	4.234	0.828	1325	4.225	0.83
Total cholesterol in VLDL (mmol/L)	575	0.438	0.188	605	0.472	0.189	1180	0.455	0.189	17	0.826	0.395	1148	0.592	0.265	1325	0.623	0.29
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	575	0.944	0.269	605	0.994	0.271	1180	0.970	0.270	17	1.452	0.472	1148	1.219	0.383	1325	1.250	0.40
Total cholesterol in LDL (mmol/L)	575	1.130	0.330	605	1.149	0.341	1180	1.139	0.334	17	1.492	0.498	1148	1.460	0.442	1325	1.464	0.44
Total cholesterol in HDL (mmol/L)	575	1.503	0.274	605	1.453	0.266	1180	1.477	0.270	17	1.217	0.350	1148	1.556	0.363	1325	1.511	0.37
Total cholesterol in HDL2 (mmol/L)	575	1.035	0.254	605	0.988	0.246	1180	1.011	0.250	17	0.751	0.327	1148	1.072	0.335	1325	1.030	0.35
Total cholesterol in HDL3 (mmol/L)	575	0.468	0.024	605	0.466	0.024	1180	0.467	0.024	17	0.466	0.035	1148	0.483	0.033	1325	0.481	0.03
Esterified cholesterol (mmol/L)	572	2.516	0.447	604	2.517	0.460	1176	2.517	0.452	17	5 2.941	0.636	1147	2.975	0.593	1323	2.971	0.59
Free cholesterol (mmol/L)	572	1.062	0.179	604	1.079	0.186	1176	1.070	0.182	17	5 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	17	1 686	0.809	1148	1 1 2 9	0 755	1325	1 190	0.78
Triglycerides in VI DL (mmol/L)*	575	0.582	0.885	605	0.648	0.830	1180	0.615	0.858	17	1 249	0.927	1148	0.694	0.945	1325	0 750	0.70
Triglycerides in LDL (mmol/L)	575	0.113	0.02/	605	0.123	0.027	1180	0.118	0.026	17	0.153	0.040	11/18	0.054	0.044	1325	0.157	0.04
Triglycerides in HDL (mmol/L)	575	0.129	0.021	605	0.126	0.020	1180	0.133	0.020	17	0.161	0.047	1148	0.150	0.040	1325	0.153	0.04
Total phosphoglycoridos (mmol/L)	575	1 622	0.030	604	1,620	0.030	1176	1 6 2 6	0.050	17	1 965	0.047	1140	1 026	0.040	1222	1 010	0.04
Patia of trighteerides to phosphoghysorides	572	0.526	0.240	604	1.020	0.201	1170	0.549	0.250	17	1.805	0.554	1147	1.520	0.340	1222	1.510	0.34
Ratio of trigiycendes to phosphogrycendes	572	0.520	0.255	604	0.569	0.277	1170	0.546	0.205	17	0.946	0.567	1147	1.070	0.279	1323	1.005	0.55
Phosphatidylcholine & other cholines (mmol/L)	572	1.691	0.240	604	1.687	0.267	11/6	1.689	0.253	1/	1.8//	0.320	1147	1.978	0.336	1322	1.965	0.33
Sphingomyelins (mmol/L)	572	0.348	0.061	604	0.349	0.064	1176	0.348	0.062	1/	0.370	0.070	1147	0.397	0.078	1322	0.394	0.07
Total cholines (mmol/L)	572	2.005	0.256	604	1.997	0.264	1176	2.001	0.259	1/	2.185	0.334	1147	2.317	0.351	1322	2.299	0.35
Apolipoproteins																		
Apolipoprotein A1 (g/L)	575	1.509	0.159	605	1.484	0.151	1180	1.497	0.155	17	1.461	0.178	1148	1.589	0.205	1325	1.572	0.20
Apolipoprotein B (g/L)	575	0.682	0.135	604	0.706	0.136	1179	0.694	0.135	17	0.955	0.245	1148	0.812	0.196	1325	0.831	0.20
Ratio of apolipoprotein B to apolipoprotein A	575	0.455	0.097	604	0.479	0.098	1179	0.467	0.098	17	0.660	0.178	1148	0.518	0.136	1325	0.537	0.15
Fatty acids																		
Total fatty acids (mmol/L)	570	9.215	1.697	604	9.370	1.730	1174	9.294	1.709	17	11.850	2.723	1145	10.917	2.392	1318	11.034	2.44
Estimated degree of unsaturation	570	1.212	0.056	604	1.196	0.065	1174	1.204	0.061	17	1.179	0.070	1145	1.212	0.066	1318	1.208	0.06
	570	0.078	0.028	604	0.074	0.029	1174	0.076	0.028	17	0.118	0.051	1145	0.111	0.039	1318	0.112	0.04
18:2. linoleic acid (mmol/L)	570	2,539	0,456	604	2,592	0.464	1174	2,566	0,459	17	2.919	0,567	1145	2,880	0.584	1318	2,885	0.5
Omega3 fatty acids (mmol/L)	570	0.309	0.086	604	0.296	0.083	1174	0.302	0.085	17	0.451	0.160	1145	0.400	0.117	1318	0.406	0.12
Omega6 fatty acids (mmol/L)	570	3 077	0.493	604	3 094	0.492	1174	3 086	0.491	17	2 5 5 2	0.648	1145	3,507	0.627	1318	3,513	0.6
Polyunsat fatty acids (mmol/L)	570	3 386	0.565	604	3 300	0.561	1174	3 388	0.560	17	1 004	0 775	11/15	3 907	0 721	1212	3 910	0.02
Monounsat fatty acide: 16:1-18:1 (mmol/L)	570	2 500	0.505	604	2.550	0.501	1174	2 544	0.500	17	2 5 20	1.059	11/15	3.907	0.021	1210	3 125	0.7
Saturated fatty acids (mmol/L)	570	2.300	0.052	604	2.307	0.001	1174	2.344	0.040	17	5 5.52U	1.000	1145	2,000	0.917	1210	2.122	0.94
	570	3.3∠8	U.042	004	3.393	0.082	11/4	3.302	0.001	1/	9 4.3Z5	1.088	1145	3.930	0.932	1319	3.979	0.95

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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	0.3
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
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
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
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
Ratio of polyunsut, fatty acids to total fatty acids (%)	570	26 911	2 569	604	27 375	2 678	1174	27 1/18	2 625	173	29 366	2 968	1145	27 889	2 842	1318	28 075	2
Ratio of saturated fatty acids to total fatty acids (%)	570	36.110	1.675	604 604	36.167	1.802	1174	36.139	1.734	173	36.402	2.027	1145	35.950	2.032	1318	36.007	2.
Success (mmol/L)*	574	1 250	0.115	COF	1 226	0.105	1170	1 2 4 2	0.11	176	1 415	0.205	11/0	1 224	0 176	1224	1 244	0
actate (mmol/L)	574	1.550	0.115	005	1.550	0.105	11/9	1.542	0.11	178	1.415	0.205	1140	1.554	0.170	1224	1.544	0
actate (mmoi/L)	575	1.770	0.459	605	1./18	0.434	1180	1.743	0.446	177	1.696	0.472	1148	1.562	0.480	1325	1.580	0
vyruvate (mmol/L)	574	0.100	0.024	605	0.098	0.023	1179	0.099	0.023	1//	0.101	0.031	1147	0.093	0.034	1324	0.094	0
Jitrate (mmoi/L)	575	0.125	0.017	604	0.131	0.018	11/9	0.128	0.018	1//	0.110	0.016	1148	0.111	0.016	1325	0.111	0
Jiycerol (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.
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.
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
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
listidine (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.
soleucine (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
.eucine (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
√aline (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
henylalanine (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
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.
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.
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
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.
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
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.
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.
* accurate in a contract the CDT when allowed variable																		
* geometric mean [relative SD] when skewed variable # Note: The presence of ethanol in a sample can affect quantification	ion of glycerol	and on som	e occasions	3hvdroxyl	outvrate													
Ethanol can be introduced in to a sample from disinfectants used of	during blood co	ollection/pro	cessing of s	ample.	July fulle.													

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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 <i>,</i> 0.890)	< 0.001	<0.001	2.534					
Total lipids in very large VLDL (mmol/L)*	0.922	(0.700 <i>,</i> 1.145)	< 0.001	<0.001	3.031					
Total lipids in large VLDL (mmol/L)*	0.648	(0.502 <i>,</i> 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 <i>,</i> 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 <i>,</i> 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 <i>,</i> 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					
	0.027	(0.043, 0.010)	0.002	0.000	0.250					
Cholesterol										
Serum total cholesterol (mmol/L)	0.670	(0.619 <i>,</i> 0.721)	< 0.001	<0.001	0.805					
Total cholesterol in VLDL (mmol/L)	0.146	(0.129 <i>,</i> 0.163)	<0.001	<0.001	0.249					
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	0.261	(0.237 <i>,</i> 0.284)	< 0.001	< 0.001	0.357					
Total cholesterol in LDL (mmol/L)	0.327	(0.300 <i>,</i> 0.354)	<0.001	<0.001	0.424					
Total cholesterol in HDL (mmol/L)	0.082	(0.058 <i>,</i> 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 Al	0.055	(0.046. 0.064)	<0.001	< 0.001	0.127					
······································	2.200									
Fatty acids	1 700		10 001	-0.001	2.245					
i otal fatty acids (mmol/L)	1./38	(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					

59

2	Fatty acid ratios					
3	Patio of 22:6 decosphereneis asid to total fatty asids (%)	0 200	(0 100 0 228)	<0.001	<0.001	0 202
4	Ratio of 22.0 docosalies acid to total fatty acids (%) Patio of 18:2 lipolois acid to total fatty acids (%)	1.079	(0.190, 0.220)	<0.001	<0.001	0.292
5	Ratio of 18.2 infoleic actual to total ratio actual $(\%)$	-1.079	(-1.551, -0.627)	<0.001	<0.001	5.567
6	Ratio of omegas fatty acids to total fatty acids (%)	0.446	(0.405, 0.490)	<0.001	<0.001	0.095
7	Patio of polyupsat, fatty acids to total fatty acids (%)	-0.552	(-1.227, -0.737)	<0.001	<0.001	3.290
/	Patio of monouncat, fatty acids to total fatty acids (%)	-0.340	(-0.794, -0.298)	<0.001	<0.001	3.307
8	Patio of saturated fatty acids to total fatty acids (%)	0.741	(0.347, 0.334)	<0.001	<0.001	1 962
9	Natio of saturated fatty acids to total fatty acids (76,	-0.195	(-0.328, -0.002)	0.004	0.005	1.805
10	Glycolysis related					
11	Glucose (mmol/L)*	-0.002	(-0.014.0.009)	0 700	0 709	0 147
12	Lactate (mmol/L)	-0.180	(-0.215, -0.144)	<0.001	<0.001	0.177
13	Pyruvate (mmol/l)	-0.007	(-0.009, -0.005)	<0.001	<0.001	0.029
14	Citrate (mmol/L)	-0.017	(-0.018, -0.016)	< 0.001	< 0.001	0.019
15	Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	< 0.001	< 0.001	0.023
16			(,			
10	Amino acids					
17	Alanine (mmol/L)	0.013	(0.009, 0.017)	< 0.001	<0.001	0.060
18	Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	< 0.001	< 0.001	0.060
19	Glycine (mmol/L)	0.007	(0.003, 0.010)	<0.001	<0.001	0.049
20	Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	0.006	0.009
21	Isoleucine (mmol/L)	0.003	(0.002, 0.004)	< 0.001	<0.001	0.019
22	Leucine (mmol/L)	0.004	(0.003, 0.006)	<0.001	<0.001	0.021
23	Valine (mmol/L)	0.009	(0.006, 0.011)	< 0.001	< 0.001	0.039
20	Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	< 0.001	<0.001	0.010
24	Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	0.105	0.014
25						
26	Ketone bodies					
27	Acetate (mmol/L)*	0.101	(0.084, 0.117)	< 0.001	<0.001	0.235
28	Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	0.922	1.022
29	3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	0.001	0.493
30						
31	Fluid balance					
32	Albumin (signal area)	-0.004	(-0.004, -0.004)	<0.001	<0.001	0.005
32	Creatinine (mmol/L)	0.016	(0.015, 0.017)	<0.001	0.001	0.012
24						
54 25	Inflammation					
35	Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.062	(0.047, 0.078)	<0.001	<0.001	0.217
36						
37	4 NA . I P. I I I . C I					
38	* Metabolite has been log transformed					
39	* Benjamini-Hochberg adjusted p-value	for other ways wild and (او اورو او موقع او مغطوة ورورو			
40	# Associations in Figure 2 are presented in SD-units. The conversion	ractor provided (u	a conversion factor W	eviation of each	o has been log t	ransformed
41	conversion factor is standard doviation of log transformed metaboli	to		lere metabolit	e has been log t	lansionneu
42	conversion factor is standard deviation of log transformed metaboli	te				
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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^	Conversi factor (SI					
Lipoprotein subclass lipids					140001 (01					
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	0.737	(0.414, 1.059)	<0.001	<0.001	2.845					
l otal 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 VEDE (mmol/L)	0.052	(0.015, 0.048)	<0.001	<0.001	0.146					
Total lipids in IDL (mmol/L)	0.018	(0.010, 0.027)	0.001	0.035	0.074					
Total linids in large I DL (mmol/L)	0.020	(-0.004, 0.046)	0.132	0.055	0.102					
Total lipids in medium I DI (mmol/L)	0.020	(-0.008, 0.040)	0.338	0.416	0.227					
Total linids in small I DI (mmol/I)	0.001	(-0.008, 0.024)	0.338	0.822	0.155					
Total linids in very large HDI (mmol/L)	-0.002	(-0.023, 0.020)	0.882	0.882	0.000					
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					
Linearctein particle cite										
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	0.22€					
		,								
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					
Giveerides and phospholinids										
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	0.55					
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	0.24					
Ratio of triglycerides to phosphoglycerides	0.051	(0.020, 0.083)	0.001	0.003	0.27					
Phosphatidylcholine & other cholines (mmol/L)	-0.011	(-0.040, 0.018)	0.447	0.534	0.25					
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	0.25					
Anglingproteins										
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					
F-44										
Fatty acids Total fatty acids (mmol/L)	0.200	(0.011.0.389)	0.038	0.065	1.65					
Estimated degree of unsaturation	-0.016	(-0.022 -0.009)	<0.000	<0.001	0.06					
22:6. docosabexaenoic acid (mmol/L)	-0.004	(-0.007 -0.000)	0.033	0.059	0.025					
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	0.450					
Omega6 fatty acids (mmol/L)	0.031	(-0.024. 0.087)	0.271	0.352	0.48					
Polyunsat. fatty acids (mmol/L)	0.023	(-0.041, 0.086)	0.483	0.567	0.55					
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.105	(0.034, 0.176)	0.004	0.009	0.62					
Saturated fatty acids (mmol/L)	0.073	(-0.000, 0.146)	0.052	0.085	0.638					
Fatty and ratios										
Ratio of 22:6 docosabexaenoic acid to total fatty acids (%)	-0.049	(-0.078, -0.019)	0.001	0.003	0.25					
Ratio of 18:2 linoleic acid to total fatty acids (%)	0.197	(-0.173, 0.567)	0.297	0.379	3 23					
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	3.10					
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.447	(-0.830, -0.065)	0.022	0.042	3.34f					
Ratio of monounsat. fatty acids to total fatty acids (%)	0.499	(0.202, 0.796)	0.001	0.003	2.600					
Ratio of saturated fatty acids to total fatty acids (%)	-0.051	(-0.251, 0.148)	0.614	0.689	1.74					
Glycorysis related	_0.012	(-0.026.0.001)	0.061	0.000	0.110					
Lactate (mmol/L)	-0.013	(-0.020, 0.001)	0.002	0.098	0.118					
Laccare (IIIIII0/L)	-0.045	(-0.097, 0.007)	0.088	0.133	0.456					
ryiuvale (mmol/L) Citrate (mmol/L)	-U.UUI	(-0.004, 0.002)	0.524	0.000 -0.001	0.024					
Giveenol (mmol/L)	0.007	(0.005, 0.009)	0.001	0.001	0.018					
	0.000	(0.002, 0.010)	0.004	0.003	0.02					
Amino acids										
Alanine (mmol/L)	0.011	(0.004, 0.017)	0.002	0.004	0.05					
Glutamine (mmol/L)	0.023	(0.018, 0.029)	<0.001	<0.001	0.05					
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					

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2	Leucine (mmol/L)	-0.002	(-0.005, -0.000)	0.022	0.041	0.018
2	Valine (mmol/L)	-0.007	(-0.011, -0.003)	0.001	0.003	0.036
3	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
4						
5	Ketone bodies					
5	Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.005	0.166
6	Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.393	0.992
7	3hydroxybutyrate (mmol/L)*	0.041	(-0.019, 0.101)	0.178	0.248	0.513
8	Fluid balance					
0	Albumin (signal area)	-0.001	(-0.001, -0.000)	0.037	0.064	0.005
2	Creatinine (mmol/L)	0.000	(-0.001, 0.001)	0.624	0.690	0.007
10						
11	Inflammation					
11	Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.017	(-0.004, 0.038)	0.104	0.151	0.183
12						

* 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 or beer teries only

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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^	Conversio				
ī	ipoprotein subclass lipids									
Т	Fotal lipids in chylomicrons & ex.large VLDL (mmol/L)*	-0.930	(-1.271, -0.589)	<0.001	<0.001	2.173				
Т	Fotal lipids in very large VLDL (mmol/L)*	-1.217	(-1.617, -0.818)	<0.001	<0.001	2.555				
Т	Fotal lipids in large VLDL (mmol/L)*	-0.900	(-1.107, -0.693)	<0.001	<0.001	1.343				
Т	Fotal lipids in medium VLDL (mmol/L)	-0.325	(-0.385, -0.264)	<0.001	<0.001	0.398				
Т	Fotal lipids in small VLDL (mmol/L)	-0.167	(-0.201, -0.133)	<0.001	<0.001	0.223				
Т	Fotal lipids in very small VLDL (mmol/L)	-0.022	(-0.039, -0.005)	0.013	0.018	0.107				
Т	Fotal lipids in IDL (mmol/L)	0.014	(-0.023, 0.051)	0.465	0.530	0.236				
Т	Fotal lipids in large LDL (mmol/L)	-0.011	(-0.058, 0.035)	0.634	0.671	0.293				
Т	Fotal lipids in medium LDL (mmol/L)	-0.028	(-0.057, 0.001)	0.058	0.076	0.182				
Т	Fotal lipids in small LDL (mmol/L)	-0.017	(-0.035, 0.001)	0.061	0.079	0.113				
Т	Fotal lipids in very large HDL (mmol/L)	0.195	(0.158, 0.231)	<0.001	<0.001	0.239				
Т	Fotal lipids in large HDL (mmol/L)	0.395	(0.334, 0.455)	<0.001	<0.001	0.405				
Т	Fotal lipids in medium HDL (mmol/L)	0.129	(0.101, 0.158)	<0.001	<0.001	0.184				
Т	Fotal lipids in small HDL (mmol/L)	0.002	(-0.021, 0.025)	0.850	0.874	0.145				
L	ipoprotein particle size									
٨	Mean diameter for VLDL particles (nm)	-1.414	(-1.669, -1.159)	< 0.001	<0.001	1.678				
Ν	Vlean diameter for LDL particles (nm)	0.081	(0.066, 0.096)	< 0.001	<0.001	0.100				
٨	Vean diameter for HDL particles (nm)	0.278	(0.236, 0.320)	<0.001	<0.001	0.279				
,										
S	Serum total cholesterol (mmol/L)	0 112	(-0.018, 0.241)	0 091	0.116	0.817				
т	Fotal cholesterol in VIDL (mmol/L)	-0.187	(-0.230,-0.145)	<0.001	<0.001	0.275				
R	Remnant cholesterol (nonHDL, nonIDL cholesterol) (mmol/L)	-0 184	(-0.244 -0.124)	<0.001	<0.001	0.273				
т	Fotal cholesterol in LDL (mmol/L)	-0.104	(-0.117_0.021)	0.175	0.213	0.303				
T	Fotal cholesterol in HDL (mmol/L)	0.344	(0.286, 0.401)	<0.001	<0.01	0.382				
Ť	Fotal cholesterol in HDL2 (mmol/L)	0.344	(0.230, 0.401)	<0.001	<0.001	0.35/				
т Т	Fotal cholesterol in HDL3 (mmol/L)	0.019	(0.271, 0.378)	<0.001	<0.001	0.03/				
F	Esterified cholesterol (mmol/L)	0.015	(-0.023, 0.163)	0.139	0 172	0.00-				
F	Free cholesterol (mmol/L)	0.046	(0.009, 0.084)	0.135	0.023	0.238				
			(,							
G	Slycerides and phospholipids									
S	Serum total triglycerides (mmol/L)*	-0.344	(-0.416, -0.273)	<0.001	<0.001	0.468				
Т	Friglycerides in VLDL (mmol/L)*	-0.530	(-0.630, -0.429)	<0.001	<0.001	0.659				
Т	Triglycerides in LDL (mmol/L)	0.008	(0.001, 0.014)	0.033	0.044	0.044				
Т	Triglycerides in HDL (mmol/L)	-0.004	(-0.010, 0.002)	0.228	0.272	0.040				
Т	Fotal phosphoglycerides (mmol/L)	0.106	(0.052, 0.159)	< 0.001	<0.001	0.340				
R	Ratio of triglycerides to phosphoglycerides	-0.289	(-0.337, -0.241)	< 0.001	<0.001	0.318				
Ρ	Phosphatidylcholine & other cholines (mmol/L)	0.138	(0.086, 0.190)	<0.001	<0.001	0.333				
S	Sphingomyelins (mmol/L)	0.032	(0.020, 0.045)	< 0.001	<0.001	0.078				
Т	Fotal cholines (mmol/L)	0.170	(0.115, 0.224)	<0.001	<0.001	0.349				
_										
A ^	Apolipoproteins	0.146	(0.114.0.179)	<0.001	<0.001	0.200				
A	Apolipoprotein AI (g/L)	0.146	(0.114, 0.178)	<0.001	<0.001	0.20				
μ 	Apolipoprotein B (g/L)	-0.115	(-0.146, -0.084)	<0.001	<0.001	0.198				
к	Ratio of apolipoprotein B to apolipoprotein Al	-0.126	(-0.148, -0.105)	<0.001	<0.001	0.144				
F	Fatty acids									
Т	Fotal fatty acids (mmol/L)	-0.711	(-1.091, -0.330)	<0.001	< 0.001	2.38				
E	Estimated degree of unsaturation	0.031	(0.021, 0.042)	< 0.001	<0.001	0.066				
2	22:6, docosahexaenoic acid (mmol/L)	-0.002	(-0.008, 0.005)	0.622	0.667	0.043				
1	L8:2, linoleic acid (mmol/L)	0.004	(-0.087, 0.094)	0.934	0.947	0.566				
С	Dmega3 fatty acids (mmol/L)	-0.035	(-0.054, -0.016)	< 0.001	0.001	0.122				
С	Dmega6 fatty acids (mmol/L)	0.004	(-0.094, 0.102)	0.936	0.936	0.612				
Р	Polyunsat, fatty acids (mmol/L)	-0.031	(-0.144, 0.082)	0.592	0.644	0.70				
N	Vionounsat, fatty acids: 16:1. 18:1 (mmol/L)	-0.372	(-0.520, -0.225)	< 0.001	< 0.001	0.93				
S	Saturated fatty acids (mmol/L)	-0.307	(-0.455, -0.159)	< 0.001	< 0.001	0.93				
	· · · ·		,							
F	atty acid ratios									
R	Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	0.064	(0.018, 0.110)	0.006	0.009	0.28				
R	Ratio of 18:2 linoleic acid to total fatty acids (%)	1.527	(0.984, 2.070)	<0.001	<0.001	3.430				
R	Ratio of omega3 fatty acids to total fatty acids (%)	-0.038	(-0.152, 0.075)	0.508	0.570	0.710				
R	Ratio of omega6 fatty acids to total fatty acids (%)	1.882	(1.351, 2.412)	<0.001	<0.001	3.370				
R	Ratio of polyunsat. fatty acids to total fatty acids (%)	1.843	(1.272, 2.414)	<0.001	<0.001	3.62				
R	Ratio of monounsat. fatty acids to total fatty acids (%)	-1.456	(-1.914, -0.998)	<0.001	<0.001	2.904				
R	Ratio of saturated fatty acids to total fatty acids (%)	-0.387	(-0.700, -0.074)	0.015	0.022	1.959				
	Shuchlysis related									
6	Slucose (mmol/L)*	-0 071	(-0.097 -0.044)	<0.001	<0.001	0 1 6				
G	Slucose (mmol/L)*	-0.071	(-0.097, -0.044) (-0.252, -0.105)	<0.001	<0.001	0.16				

1						
2	Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.387	0.016
2	Giycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.328	0.022
ر ۱	Amino acids					
4	Alanine (mmol/L)	-0.020	(-0.030, -0.011)	<0.001	<0.001	0.060
5	Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	< 0.001	< 0.001	0.065
6	Glycine (mmol/L)	0.029	(0.020, 0.038)	< 0.001	< 0.001	0.059
7	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
8	Leucine (mmol/L)	-0.019	(-0.022, -0.016)	< 0.001	< 0.001	0.022
9	Valine (mmol/L)	-0.029	(-0.035, -0.022)	< 0.001	< 0.001	0.042
10	Phenylalanine (mmol/L)	0.000	(-0.002, 0.001)	0.576	0.637	0.010
10	Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	< 0.001	<0.001	0.014
11						
12	Ketone bodies					
12	Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.001	0.273
13	Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	0.863	1.048
14	3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.035	0.472
15						
16	Fluid balance		(0.000, 0.004)	0.004	0.004	0.005
10	Albumin (signal area)	-0.002	(-0.002, -0.001)	<0.001	<0.001	0.005
17	Creatinine (mmol/L)	-0.013	(-0.015, -0.012)	<0.001	<0.001	0.010
18	Inflammation					
19	Glycoprotein acetyls, mainly alacid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	<0.001	0.239
20			(,,			
 21						
21	* Matabalita has been log transformed					

* 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			Ro	ws					Mot	her Is					All Ch	ildren	
	n	сс	95% CI	,,, n	PCC*	95% CI	n	сс	95% CI	n	PCC*	95% CI	n	сс	95% CI	n	PCC
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
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
otal lipids in large VLDL (mmol/L)* Fotal lipids in medium 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
Total lipids in small 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	0.29	0.22 - 0.34	909	0.29
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
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
Total lipids in large LDL (mmol/L) Total lipids in medium 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
Total lipids in small LDL (mmol/L)	469	0.28	0.19 - 0.36	433	0.24	0.15 - 0.33	518	0.29	0.21 - 0.37	476	0.32	0.24 - 0.40	987	0.25	0.23 - 0.33	909	0.29
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
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
Total lipids in medium HDL (mmol/L) Total lipids in small HDL (mmol/L)	469 469	0.22 0.23	0.13 - 0.30 0.14 - 0.31	433 433	0.20	0.11 - 0.29 0.13 - 0.31	518 518	0.12	0.03 - 0.20 0.12 - 0.29	476 476	0.13 0.20	0.04 - 0.22 0.11 - 0.28	987 987	0.17 0.21	0.11 - 0.23 0.15 - 0.27	909 909	0.17
Lipoprotein particle size																	
Mean diameter for VLDL particles (nm) Mean diameter for LDL particles (nm)	469 469	0.30	0.22 - 0.38 0.13 - 0.31	433 433	0.32	0.23 - 0.40 0.11 - 0.29	518 518	0.27	0.19 - 0.35 0.22 - 0.38	476 476	0.25	0.16 - 0.33 0.24 - 0.40	987 987	0.29	0.23 - 0.35 0.20 - 0.31	909 909	0.28
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
Cholesterol	460	0.27	0.10 0.25	400	0.22	0.14 0.00	F 10	0.22	0.24 0.20	470	0.24	0.25 0.42	007	0.20	0.24 0.25	000	0.20
Total cholesterol in VLDL (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
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	0.25
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	0.25
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
Total cholesterol in HDL2 (mmol/L) Total cholesterol in HDL3 (mmol/L)	469	0.30	0.16 - 0.32	433	0.31	0.14 - 0.39	518 518	0.25	0.16 - 0.32	4/6 476	0.23	0.15-0.32	987 987	0.28	0.19 - 0.21	a0a 90a	0.27
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
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
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
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
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
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
Ratio of triglycerides to phosphoglycerides	465	0.28	0.15 - 0.32	430	0.25	0.14 - 0.32	518	0.26	0.17 - 0.34	476	0.27	0.18 - 0.35	983	0.25	0.20 - 0.32	906	0.2
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
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
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
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
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
Ratio of apolipoprotein B to apolipoprotein Al	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
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
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
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
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	0.2
Omega6 fatty acids (mmol/L)	462	0.20	0.11 - 0.29	42/	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	0.2
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	0.2
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	0.3
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
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	0.3
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	0.1/
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	0.3
Ratio of omega6 fatty acids to total fatty acids (%) Ratio of polyupsat, 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
Ratio of monounsat, fatty acids to total fatty acids (%)	462	0.29	0.21 - 0.38	427	0.30	0.22 - 0.39	517	0.28	0.25 - 0.56	475	0.32	0.23 - 0.40	979	0.28	0.22 - 0.33	902	0.2
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	0.2
Glycolysis related		0.00	0.02 0.1-	400	0.05	0.04 0.4			0.05 0.05	477		0.05 0.05	000		0.05 0.15	000	
Lactate (mmol/L)	468 469	0.06	-0.03 - 0.15 -0.05 - 0.13	432 433	0.05	-0.04 - 0.14 -0.04 - 0.15	518	0.14	0.00 - 0.23	476	0.14	0.03 - 0.23	985 987	0.11	0.05 - 0.17 0.00 - 0.13	908 909	0.10
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	0.1
Citrate (mmol/L) Glycerol (mmol/L)	469 124	0.24 0.25	0.15 - 0.32 0.07 - 0.41	433 113	0.25 0.24	0.16 - 0.34 0.06 - 0.41	517 155	0.16 0.10	0.07 - 0.24 -0.05 - 0.26	475 137	0.16	0.07 - 0.25	986 279	0.18 0.16	0.12 - 0.24 0.05 - 0.28	908 250	0.19
Amino acids						*								1.20			
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	0.2
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	0.2
Gycine (mmol/L) Histidine (mmol/L)	468	0.22	0.13 - 0.30	432	0.25	0.15 - 0.33	517	0.19	0.10 - 0.27	475 476	0.18	0.09 - 0.27	985	0.20	0.14 - 0.26	907	0.2
Isoleucine (mmol/L)	466	0.39	0.31 - 0.47	431	0.40	0.32 - 0.47	518	0.33	0.25 - 0.29	476	0.30	0.22 - 0.38	984	0.36	0.30 - 0.41	907	0.34
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
Valine (mmol/L) Phenvlalanine (mmol/L)	469 469	0.39	0.31 - 0.46	433 433	0.38	0.30 - 0.46	516 518	0.27	0.19 - 0.35	474 476	0.26	0.17 - 0.34	985 987	0.33	0.27 - 0.38	907 909	0.3
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	0.2
Ketone bodies																	
Acetate (mmol/L)* Acetoacetate (mmol/L)*	468 469	0.11 0.13	0.02 - 0.20 0.04 - 0.22	433 433	0.08 0.14	-0.02 - 0.17 0.04 - 0.23	517 517	0.14 0.03	0.06 - 0.22 -0.05 - 0.12	475 475	0.14 0.01	0.06 - 0.23 -0.08 - 0.10	985 986	0.13 0.08	0.07 - 0.19 0.01 - 0.14	908 908	0.12
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	0.2
Fluid balance	400	0.32	0.00 0.07	433	0.35	0.17 0.24	F 10	0.30	0.20 0.25	476	0.30	0.10 0.25	086	0.20	0.32 0.24	0.08	0.23
Aldumin (signal area) Creatinine (mmol/L)	468 458	0.29 0.31	0.20 - 0.37 0.23 - 0.39	432 423	0.26 0.30	0.17 - 0.34 0.21 - 0.39	518 514	0.28 0.25	0.20 - 0.36 0.17 - 0.33	476 473	0.28 0.26	0.19 - 0.36 0.17 - 0.34	986 972	0.28 0.28	0.22 - 0.34 0.22 - 0.34	908 896	0.27
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
* log transformation has been any to the second allow																	
 log transformation has been applied to metabolite 																	

* 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.

2										All Par	ents								
3	Metabolic subgroup	n	сс	Male 9 95% Cl	Child n	PCC*	95% CI	n	сс	Female 95% Cl	Child n	PCC*	95% CI	n	сс	All Chi 95% Cl	ldren n	PCC*	95% CI
4	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
5	Total lipids in very large VLDL (mmol/L)* Total lipids in large VLDL (mmol/L)*	547 547	0.23 0.20	0.15 - 0.31 0.12 - 0.28	505 505	0.23	0.15 - 0.31 0.13 - 0.30	586 586	0.17 0.20	0.10 - 0.25 0.12 - 0.28	544 544	0.16 0.19	0.08 - 0.24 0.11 - 0.27	1133 1133	0.21 0.20	0.15 - 0.26 0.15 - 0.26	1049 1049	0.20 0.21	0.14 - 0.26 0.15 - 0.26
6	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) Total lipids in very small VLDL (mmol/L)	547	0.27	0.19 - 0.34 0.15 - 0.31	505 505	0.27	0.19 - 0.35 0.13 - 0.30	586 586	0.26	0.18 - 0.33 0.17 - 0.33	544 544	0.26	0.17 - 0.33 0.18 - 0.34	1133 1133	0.25	0.21 - 0.32 0.19 - 0.30	1049 1049	0.26	0.21 - 0.32 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
8	Total lipids in large LDL (mmol/L) Total lipids in medium LDL (mmol/L)	547	0.28	0.20 - 0.36	505	0.25	0.17 - 0.33	586	0.29	0.21 - 0.36	544 544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34 0.23 - 0.34	1049	0.29	0.23 - 0.34 0.23 - 0.34
0	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
9	Total lipids in very large HDL (mmol/L) Total lipids in large HDL (mmol/L)	547	0.29	0.21 - 0.37	505	0.28	0.20 - 0.36 0.19 - 0.36	586	0.29	0.21 - 0.36	544 544	0.27	0.19 - 0.35 0.14 - 0.30	1133	0.29	0.24 - 0.34 0.21 - 0.32	1049	0.28	0.22 - 0.33 0.19 - 0.30
10	Total lipids in medium HDL (mmol/L) Total lipids in small HDL (mmol/L)	547 547	0.15 0.19	0.07 - 0.24 0.10 - 0.27	505 505	0.13 0.16	0.04 - 0.21 0.08 - 0.25	586 586	0.10 0.18	0.02 - 0.18 0.10 - 0.26	544 544	0.10 0.17	0.02 - 0.19 0.09 - 0.25	1133 1133	0.12 0.18	0.07 - 0.18 0.12 - 0.23	1049 1049	0.11 0.17	0.05 - 0.17 0.11 - 0.22
11	Lipoprotein particle size																		
12	Mean diameter for VLDL particles (nm) Mean diameter for LDL particles (nm)	547 547	0.29 0.19	0.21 - 0.36 0.11 - 0.27	505 505	0.30 0.17	0.22 - 0.38 0.08 - 0.25	586 586	0.22 0.27	0.14 - 0.29 0.19 - 0.34	544 544	0.19 0.29	0.11 - 0.27 0.21 - 0.36	1133 1133	0.25 0.23	0.20 - 0.31 0.17 - 0.28	1049 1049	0.24 0.23	0.19 - 0.30 0.17 - 0.28
13	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
14	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
15	Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	547	0.26	0.18 - 0.34 0.19 - 0.34	505	0.27	0.19 - 0.35 0.18 - 0.34	586	0.26	0.18 - 0.33 0.20 - 0.35	544 544	0.26	0.18 - 0.34 0.21 - 0.37	1133	0.26	0.22 - 0.32	1049	0.27	0.22 - 0.32
16	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
10	Total cholesterol in HDL2 (mmol/L)	547	0.27	0.19 - 0.33	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
17	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
18	Esternied cholesterol (mmol/L) Free cholesterol (mmol/L)	543	0.28	0.20 - 0.36	502	0.24	0.15 - 0.32	584 584	0.31	0.24 - 0.38	542	0.33	0.25 - 0.40	1127	0.30	0.24 - 0.35 0.24 - 0.35	1044	0.29	0.23 - 0.34 0.23 - 0.34
19	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
20	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
20	Triglycerides in LDL (mmol/L) Triglycerides in HDL (mmol/L)	547 547	0.20	0.12 - 0.28	505 505	0.19	0.10 - 0.27 0.18 - 0.34	586 586	0.27 0.27	0.19 - 0.34 0.20 - 0.35	544 544	0.28	0.20 - 0.35	1133 1133	0.24	0.18 - 0.29	1049 1049	0.24	0.18 - 0.29 0.21 - 0.32
21	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
22	Ratio of triglycerides to phosphoglycerides Phosphaticylcholine & other cholines (mmol/L)	543 542	0.23	0.15 - 0.31	502 501	0.24	0.16 - 0.32	584 584	0.21	0.13 - 0.28	542 542	0.20	0.12 - 0.28	1127 1126	0.22	0.16 - 0.27	1044 1043	0.22	0.16 - 0.28
	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
23	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
24	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
25	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
26	Ratio of apolipoprotein B to apolipoprotein Al	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
20	Fatty acids	527	0.26	0 19 - 0 22	406	0.25	0 17 - 0 22	593	0.20	0.22 - 0.27	541	0.21	0.22 - 0.20	1120	0.28	0.22 - 0.22	1027	0.20	0.22.0.24
27	Estimated degree of unsaturation	537	0.20	0.18 - 0.33	496	0.23	0.23 - 0.39	583	0.30	0.22 - 0.37	541	0.22	0.14 - 0.30	1120	0.28	0.23 - 0.33	1037	0.25	0.23 - 0.34
28	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
20	Omega3 fatty acids (mmol/L)	537	0.24	0.16 - 0.32	496	0.25	0.16 - 0.33	583	0.27	0.19 - 0.34 0.27 - 0.41	541	0.29	0.21 - 0.37	1120	0.28	0.21 - 0.32	1037	0.28	0.22 - 0.33
29	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
30	Polyunsat. fatty acids; (mmol/L) Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	537	0.26	0.17 - 0.33 0.19 - 0.35	496	0.24	0.16 - 0.33	583	0.31	0.24 - 0.39 0.22 - 0.37	541 541	0.34	0.26 - 0.41 0.23 - 0.38	1120	0.29	0.23 - 0.34 0.24 - 0.34	1037	0.29	0.24 - 0.35
31	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
20	Fatty acid ratios	627	0.21	0.22 0.28	406	0.20	0.21 0.27	592	0.22	0.26 0.40	E 4 1	0.21	0.22 0.29	1120	0.22	0.27 0.27	1027	0.20	0.24 0.26
52	Ratio of 18:2 linoleic acid to total fatty acids (%)	537	0.15	0.23 - 0.38	496	0.30	0.21 - 0.37	583	0.33	0.10 - 0.26	541 541	0.16	0.23 - 0.38	1120	0.32	0.27 - 0.37	1037	0.30	0.24 - 0.36
33	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
34	Ratio of polyunsat, fatty acids to total fatty acids (%)	537	0.24	0.19 - 0.35	496	0.20	0.21 - 0.37	583	0.25	0.13 - 0.30	541	0.20	0.12 - 0.28	1120	0.24	0.21 - 0.32	1037	0.25	0.20 - 0.31
35	Ratio of monounsat. fatty acids to total fatty acids (%) Ratio of saturated fatty acids to total fatty acids (%)	537 537	0.27 0.22	0.19 - 0.34 0.14 - 0.30	496 496	0.27 0.23	0.19 - 0.35 0.14 - 0.31	583 583	0.30 0.26	0.23 - 0.37 0.19 - 0.34	541 541	0.29 0.25	0.21 - 0.37 0.17 - 0.33	1120 1120	0.29 0.24	0.23 - 0.34 0.19 - 0.30	1037 1037	0.28 0.24	0.22 - 0.34 0.19 - 0.30
36	Glycolysis related																		
50	Glucose (mmol/L)*	545 547	0.05	-0.04 - 0.13 -0.07 - 0.10	503 505	0.04	-0.05 - 0.12 -0.06 - 0.12	586 586	0.14	0.06 - 0.21	544 544	0.14	0.05 - 0.22	1131 1133	0.1	0.04 - 0.15	1047 1049	0.09	0.03 - 0.15
37	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
38	Citrate (mmol/L) Glycerol (mmol/L)	547 151	0.23 0.30	0.15 - 0.31 0.15 - 0.44	505 137	0.25 0.28	0.16 - 0.33 0.12 - 0.43	585 175	0.14 0.12	0.06 - 0.22 -0.03 - 0.26	543 157	0.14	0.05 - 0.22 -0.08 - 0.23	1132 326	0.17 0.20	0.12 - 0.23 0.09 - 0.30	1048 294	0.18 0.19	0.12 - 0.24 0.07 - 0.29
39	Amino acids																		
40	Alanine (mmol/L) Glutamine (mmol/L)	546 547	U.24 0.30	0.16 - 0.31 0.22 - 0.37	504 505	0.22 0.29	0.14 - 0.31 0.21 - 0.37	585 586	0.32 0.20	0.25 - 0.39 0.12 - 0.27	543 544	0.30	0.22 - 0.37 0.11 - 0.27	1131 1133	0.28 0.24	0.23 - 0.34 0.19 - 0.30	1047 1049	0.27 0.24	0.21 - 0.32 0.18 - 0.29
41	Glycine (mmol/L) Histidine (mmol/L)	545 545	0.21 0.21	0.13 - 0.29 0.13 - 0.29	503 503	0.24	0.15 - 0.32 0.12 - 0.29	585 586	0.19 0.22	0.11 - 0.27 0.14 - 0.29	543 544	0.19	0.11 - 0.27	1130 1131	0.20 0.21	0.15 - 0.26 0.16 - 0.27	1046 1047	0.21 0.21	0.15 - 0.27 0.15 - 0.26
40	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
42	Valine (mmol/L)	547	0.32	0.24 - 0.39	505	0.35	0.23 - 0.40	584	0.24	0.18 - 0.32	544	0.21	0.13 - 0.29	1135	0.28	0.25 - 0.35	1049	0.27	0.21 - 0.32
43	Phenylalanine (mmol/L) Tyrosine (mmol/L)	547 545	0.29 0.31	0.22 - 0.37 0.24 - 0.39	505 503	0.29 0.31	0.21 - 0.37 0.22 - 0.38	586 586	0.30 0.27	0.22 - 0.37 0.19 - 0.34	544 544	0.28 0.23	0.20 - 0.36 0.15 - 0.31	1133 1131	0.29 0.29	0.24 - 0.35 0.24 - 0.34	1049 1047	0.28 0.27	0.23 - 0.34 0.21 - 0.32
44	Ketone bodies																		
45	Acetate (mmol/L)* Acetoacetate (mmol/L)*	546 547	0.09 0.11	0.01 - 0.17 0.03 - 0.20	505 505	0.05 0.11	-0.03 - 0.14 0.03 - 0.20	585 585	0.15 0.04	0.06 - 0.22 -0.04 - 0.12	543 543	0.15 0.04	0.07 - 0.23 -0.05 - 0.12	1131 1132	0.12 0.07	0.06 - 0.17 0.02 - 0.13	1048 1048	0.11 0.07	0.05 - 0.17 0.01 - 0.13
46	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
47	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
48	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
49	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
50	• L																		

* 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).

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

Supplementary figure 1: Correlation of NMR measures in children.

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



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 Spearmah 's correlation coefficients with blue conservations and red cells representing positive correlations.

46 47

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Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for each chelestory and another provide in mensure.





Boys (blue), girls (red), and all parents (thin dotted black ding) plotted on the same/graph for each fatty agid and fatty residenting assure





Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for lippprotein particlasizes and trial yearide measures.





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

All parents

Boys

.08 .09 Albumin (signal area)

Creatinine (mmol/L)

Girls

1.5

mmol/I

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	2	14-15
		term in the title or the abstract		
		(b) Provide in the abstract an informative and balanced	2	9-13
		summary of what was done and what was found		25-32
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the	4-5	Pg 4 (3-9 ,21-22,
		investigation being reported		31-32, 33-35)
				Pg 5 (5-7)
Objectives	3	State specific objectives, including any prespecified	5	8-11
		hypotheses		
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,	5-6	Pg 5 (17-20)
		including periods of recruitment, exposure, follow-up,		Pg 6 (3-32)
		and data collection		
Participants	6	(a) Cohort study—Give the eligibility criteria, and the	5	23-31
		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		
		(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,	7-9	Pg 7 (4-17)
		potential confounders, and effect modifiers. Give		Pg 8 (table 1, 9-
		diagnostic criteria, if applicable		16)
				Pg 9 (1-24)
Data sources/	8*	For each variable of interest, give sources of data and	7-9	Pg 7 (4-17)
measurement		details of methods of assessment (measurement).		Pg 8 (table 1, 9-
		Describe comparability of assessment methods if there is		16)
		more than one group		Pg 9 (1-24)
Bias	9	Describe any efforts to address potential sources of bias	9 -10	Pg 9 (31-35)
				D 10 (16 10)

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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	9-10	Pg 9 (28-35)
		analyses. If applicable, describe which groupings were chosen and why		Pg 10 (3-15)
Statistical methods	12	2 (<i>a</i>) Describe all statistical methods, including those used	9-10	Pg 9 (28-35)
		to control for confounding		Pg 10 (3-15)
		(b) Describe any methods used to examine subgroups	9-10	Pg 9 (28-35)
		and interactions		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	9	31-35
		follow-up was addressed		
		Case-control study If applicable, explain how matching		
		of cases and controls was addressed		
		(<u>e</u>) Describe any sensitivity analyses	10	16-18
Results			Page	Line numbe
Particinants	13*	(a) Report numbers of individuals at each stage of study—eg	11 and	5-12
i uniterpuillo	15	numbers potentially eligible examined for eligibility, confirmed	figure 1	0.12
		eligible, included in the study, completing follow-up, and	8	
		analysed		
		(b) Give reasons for non-participation at each stage	11	8-12
			6	7-11
			figure 1	
		(c) Consider use of a flow diagram	figure 1	
Descriptive	14*	(a) Give characteristics of study participants (eg demographic,	12	2
data		clinical, social) and information on exposures and potential	(table 2))
		confounders		
		(b) Indicate number of participants with missing data for each	figure 1	
		variable of interest	table 2	2
			sup Tab	le
			1	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and	NA	
	1.5.4	total amount)		
Outcome data	15*	Cohort study—Report numbers of outcome events or summary	sup tabl	e I
		Create control at the Depart numbers in each our course extension	NTA	
		case-control study—Report numbers in each exposure category,	, NA	
		Cross sectional study Papert numbers of outcome events or	NA	
		summary measures	IVA	
Main results	16	(a) Give unadjusted estimates and if applicable confounder-	13	21_24
ivianii results	10	adjusted estimates and their precision (eq. 95% confidence	sunn tak	21-2 4
		interval). Make clear which confounders were adjusted for and	2-6	
		why they were included	- ·	
		(b) Report category boundaries when continuous variables were	NA	
		categorized		
	For p	eer review only - http://bmjopen?bmj.com/site/about/guide	elines.xhtm	I

		(<i>c</i>) 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	on			
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.

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

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Page 1 of 50

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1	Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their
2	parents
3	
4	Authors: Susan Ellul ¹ , Melissa Wake ¹⁻³ , Susan A. Clifford ^{1,2} , Katherine Lange ¹ , Peter
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25	Keywords: Metabolomics; lipids; inflammation; reference values; parents; children;
26	inheritance patterns; correlation studies; epidemiologic studies; cross-sectional studies.
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28	Word count: 4328
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30	Abbreviations: ApoA-1: Apolipoprotein A-1; Apo B: Apolipoprotein B; BCAA: Branched
31	chain amino acid; BD: Becton Dickinson; CDC: Centers for Disease Prevention and Control;
32	CVD: Cardiovascular disease; CPS1: Carbamoyl-phosphate synthase 1; DHA:
33	Docosahexaenoic acid; DOB: Date of birth; EDTA: Ethylenediaminetetraacetic acid; FDR:
34	False discovery rate; GlycA: Glycoprotein acetyls; HbA1c: Haemoglobin A1c; HDL: High-
35	density lipoprotein; HOMA: Homeostatic model assessment; IDL: Intermediate density
36	lipoprotein; LA: Linoleic acid; LDL: Low-density lipoprotein; LiH: Lithium Heparin; LSAC:
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1 Longitudinal Study of Australian Children; MUFA: Monounsaturated fatty acid; NMR:

2 Nuclear magnetic resonance; PCOS: Polycystic Ovary Syndrome; PUFA: Polyunsaturated

3 fatty acid; REDCap: Research Electronic Data Capture; SFA: Saturated fatty acids; SST:

4 serum separating tubes; T2D: Type 2 diabetes; T2DM: Type 2 diabetes mellitus; VLDL:

5 Very low density lipoprotein; XL: Very large; XXL: Chylomicrons and extremely large; XS:
6 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.

15 Design: The population-based cross-sectional Child Health CheckPoint study nested within16 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.

19 Participants: 1180 children and 1325 parents provided a blood sample and had
20 metabolomics data available. This included 1133 parent-child dyads (518 mother-daughter,
21 469 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.

32 Conclusions: We describe the serum metabolite profiles from mid-childhood and adulthood 33 in a population-based sample, together with parent-child concordance. Differences in profiles 34 by age and sex were observed. These data will be informative for investigation of the 35 childhood origins of adult non-communicable diseases and for comparative studies in other 36 populations.

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2	2	Strengths and limitations of this study:
3 4	3	• In a large population-based cohort, venous blood was collected for children and their
5	4	attending parent on the same day using the same methods
0 7	5	• Rapidly processed, high quality serum samples with standardised metabolomic data
8	6	generated as a single batch
9 10	7	Cross sectional design does not enable longitudinal analysis of specific metabolite
11 12	/	• Cross-sectional design does not enable fongitudinal analysis of specific metabolite
12	8	species over short term of longer periods of time
14 15	9	• Assessment of paternal associations with offspring metabolite measures is limited by
16	10	a relatively small sample size compared to mother-child pairs, reducing the precision
17 18	11	of estimates
19	12	• Factors known to influence metabolomic profile (such as body mass index) were not
20 21	13	considered as the aim was to describe the distribution of metabolites in children and
22	14	their parents.
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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.⁶⁷

Previous research indicates that some blood metabolites change with age, particularly from mid to late adulthood.⁸⁹ 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

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(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.

8 Here, we describe (1) the distribution of NMR-based metabolite measures in a population9 based cohort of 11-12 year old children and their parents, differences in metabolite
10 concentrations (2) by age (adults compared to children) and (3) by sex in children and adults;
11 and (4) report sex-specific parent-child concordance.

12 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

32 Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D)
34 and the Australian Institute of Family Studies Ethics Committee (14-26). The attending
35 parent/caregiver provided written informed consent for themselves and their child to

1 participate in the study, and asked to provide optional consent for the collection and use of

2 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.

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1 Measures

3 <u>Metabolomic profiling</u>

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

Metabolic group	Species and derived measures					
A mino poid-	Alanine, Glutamine, Glycine, Histidine					
Amino acias	Branched chain: Isoleucine, Leucine, Valine					
	Aromatic: Phenylalanine, Tyrosine					
Cholesterol	VLDL, LDL, HDL, HDL2, HDL3, Total, Free, Esterified, Remnant					
	Triglycerides (VLDL, LDL, HDL, total)					
	Phosphoglycerides					
Triglycerides and	Ratio of triglycerides to phosphoglycerides [*]					
phospholipids	Phosphatidylcholine					
	Sphingomyelins					
	Total cholines					
	Apolipoprotein A-1 (ApoA-1)					
Apolipoproteins	Apolipoprotein B (Apo B)					
	Ratio of Apolipoprotein B to Apolipoprotein A-1 (ApoB/Apo A-1)*					
	Total, Omega-3, Omega-6, Polyunsaturated (PUFA), Saturated (SFA)					
Fatty acids (FA)	Monounsaturated (MUFA), Docosahexaenoic acid (DHA), Linoleic (LA					
	Estimated degree of unsaturation					
Fatter and mation	Omega-3/total FA [*] , Omega-6/total FA [*] , PUFA/total FA [*] (all %)					
Fatty acid ratios	SFA/total FA [*] , MUFA/total FA [*] , DHA/total FA [*] , LA/total FA [*] (all %)					
	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)					
Lipoprotein	Particle concentration, Total lipids, Esterified cholesterol, Total					
subclasses [*]	cholesterol, Phospholipids, Free cholesterol, Triglycerides and					
	Esterified cholesterol/I otal lipids (%), Free cholesterol/I otal lipids (%), Total h blogtom // Total lipids (%), Trickwaridag/Total lipids (%),					
	Phospholipids/Total lipids (%), Trigrycerides/Total lipids (%) and					
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					
	7					

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

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.

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1 <u>Other measures and sample characteristics</u>

2 Age and sex: For children, LSAC provided date of birth (DOB) and sex, which was originally

3 exported from the Medicare Australia database. In parents, DOB and sex was self-reported in

4 the CheckPoint questionnaire, which was administered on an iPad using the Research

5 Electronic Data Capture (REDCap) tool.²¹ Age in years was calculated as the difference

6 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

15 *Time of blood collection, processing and fasting time*: Time of blood collection and start of 16 laboratory processing were recorded. When missing, collection time was estimated using the 17 midpoint between the time the CheckPoint visit began and time that processing of the sample 18 commenced. Processing lag time was calculated as the minutes between blood collection and 19 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

26 Statistical analysis

27 <u>Sample Characteristics</u>

29 Continuous descriptive variables and metabolite measures were summarised using means and 30 standard deviations (SD) for children and adults separately, by sex and overall. For skewed 31 metabolites, geometric means and relative SD were reported. To provide visual comparisons 32 of distributions of metabolites by age and sex, density plots were used. Population summary 33 statistics were estimated by applying survey weights and survey procedures that corrected for 34 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 35 weights.²⁴ More detail on the calculation of weights is provided elsewhere.²⁵ 36

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

2 (adults, children)

Skewed metabolites (skewness greater or equal to 2) were log-transformed. We used twosided 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.

9 Parent-Child concordance

10 Concordance between parents and children was assessed by 1) Pearson's correlation 11 coefficients (CC) with 95% confidence intervals, and 2) partial correlation coefficients 12 (PCC), adjusting for child and parent age, disadvantage index, fasting time, processing lag 13 time (and for child and parent sex where appropriate). Scatterplots of parent versus child 14 metabolites (log-transformed where needed as above) were examined to check for outliers 15 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.

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RESULTS

3 Sample characteristics 4

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. of metabourus ...

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	5		
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)

11.2.0 (standard dariati • I)

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.

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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).

1 DISCUSSION

2 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.

13 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.

18 Replication studies exploring sex differences at earlier and later stages of childhood and

19 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.

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1 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 metaanalysis 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 studies²⁹, we found higher levels of glycine in mothers than fathers, and (less markedly) in girls than boys. Interestingly, recent metabolomics and genetic analyses of $\sim 10,000$ adults with cardiovascular disease (CVD), with replication in > 53,000subjects, 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 $(p=6.3 \times 10^{-5})$ 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 cholesterol⁴¹ 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

33 range of cardiometabolic risk factors including total cholesterol, LDL cholesterol, HDL

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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

9 Unanswered questions and future research

10 The temporal and sex specific dynamism of the metabolomics data we describe here offer 11 considerable opportunities for identification of biomarkers of risk for a range of non-12 communicable diseases early in life, to inform targeted interventions and monitor their 13 efficacy. Combining metabolomics with other 'omics data (such as genetics), as is 14 increasingly reported from large adult studies, offers considerable promise in understanding 15 the causal pathways that link early life exposures, genetics and intermediate phenotypes with 16 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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2 This paper uses unit record data from *Growing Up in Australia*, the Longitudinal Study of

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5 of Statistics (ABS). The findings and views reported in this paper are those of the author and

6 should not be attributed to DSS, AIFS or the ABS.

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

10 We thank the LSAC and CheckPoint study participants, staff and students for their 11 contributions.

12 COMPETING INTERESTS

All authors ICMJE uniform have completed the disclosure form at www.icmje.org/coi disclosure.pdf and declare financial support as described in the funding section. MW received support from Sandoz to present at a symposium outside the submitted work. PW is employee and shareholder of Nightingale Health Ltd, a company offering NMR-based metabolic profiling.

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3	
4 5	CONTRIBUTIONS
6	DB, RS and JC conceptualised and developed the Metabolomics Checkpoint study. SE and
7	JC undertook all aspects of data analysis. SAC coordinated the acquisition of metabolomics
8	data and provided critical review of this manuscript. MW, the Principal Investigator of the
9	Child Health CheckPoint, planned the analyses and provided critical review of this
10	manuscript. SE and RS drafted the manuscript. PW, MJ, TD, KL, JC, DB provided critical
11	expert advice and critical review of this manuscript.
12 13	DATA SHARING STATEMENT
14	Dataset and technical documents available from Growing Up in Australia: The Longitudinal
15	Study of Australian Children via low-cost license for bone fide researchers. More information
16	is available at www.growingupinaustralia.gov.au
17	
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1	FIGURE CAPTIONS AND FOOTNOTES
2	Figure 1: Participant flow chart.
4	n=number of families, c=number of children, p=number of attending adults,
5	MAC=Main assessment centre, mAC=Mini assessment centre, HV=Home visit assessment,
6	LSAC=Longitudinal Study of Australian Children
7	*Unable to analyse due to insufficient volume or poor quality sample
8	^Data from 6 non-biological child-parent pairs excluded from concordance analyses
9	
10	Figure 2: Differences in metabolite levels between children and adults.
11	Association measures are SD difference in metabolite concentration for adults compared to
12	children. Error bars represent 95% confidence intervals. Significant associations after p-
13	values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold
14	(FDR=0.10). Association measures in absolute concentration units, 95% confidence intervals
15	and associated p-values are listed in supplementary table 2. HDL: High-density lipoprotein;
16	IDL: Intermediate density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low
17	density lipoprotein.
18	
19	Figure 3: Sex differences in metabolite levels in childhood and adulthood.
20	Association measures are SD difference in metabolite concentration for females compared to
21	males in children (A) and adults (B). Error bars represent 95% confidence intervals.
22	Significant associations after p-values adjusted for multiple testing using Benjamini-
23	Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolute
24	concentration units, 95% confidence intervals and associated p-values are listed in
25	supplementary table 3 and 4. HDL: High-density lipoprotein; IDL: Intermediate density
26	lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein.
27	
28	Figure 4: Parent:child correlation for metabolite measures.
29	Pearson's correlation coefficients for all children with all parents (A); and for boys (blue)
30	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

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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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1 2	SUPPLEMENTARY DOCUMENTS
3	Supplementary figure 1: Correlation of NMR measures in children.
4	
5	Heatmap showing the correlation between metabolite measures in children. The correlations
6	shown are Spearman's correlation coefficients with blue cells representing negative
7	correlations and red cells representing positive correlations.
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9	Supplementary figure 2: Correlation of NMR metabolite measures in parents.
10	
11	Heatmap showing the correlation between metabolite measures in parents. The correlations
12	shown are Spearman's correlation coefficients with blue cells representing negative
13	correlations and red cells representing positive correlations.
14	
15	Supplementary figure 3: Density plots for cholesterol and apolipoprotein measures.
16	
17	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
18	each cholesterol and apolipoprotein measure.
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21	Supplementary figure 4: Density plots for fatty acid and fatty acid ratio measures.
22	
23	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
24	each fatty acid and fatty acid ratio measure.
25	
26	Supplementary figure 5: Density plots for total lipids in the 14 lipoprotein subclass
27	particles.
28	
29	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
30	total lipids within each of the 14 lipoprotein subclass particles.
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32	Supplementary figure 6: Density plots for lipoprotein particle size and triglyceride
33	measures.
34	
35	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
36	lipoprotein particle sizes and triglyceride measures.
37	
38	Supplementary figure 7: Density plots for glycolysis related, amino acid, ketone body,
39	fluid balance and inflammation measures.
40	
41	Boys (blue), girls (red), and all parents (thin dotted black line) plotted on the same graph for
42	glycolysis related, amino acid, ketone body, fluid balance and inflammation measures.
43	
44	Supplementary table 1: Weighted mean (SD) of metabolite measures in children and
45	parents.
46	
47	Supplementary table 2: Mean difference in metabolite levels in adults compared to
48	children in absolute concentration units.
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3	1	Supplementary table 3: Differences in mean metabolite levels in girls compared to boys
4	2	in absolute concentration units.
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6	л Л	Supplementary table 1. Differences in mean metabolite levels in female compared to
7	4	Supplementary table 4. Differences in mean metabolite levels in female compared to
, 8	5	male adults in absolute concentration units.
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10	7	Supplementary table 5: Mother-child concordance; correlations and partial correlations
10	8	between mothers and their sons, daughters and all children.
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12	10	Supplementary table 6: Parent-child concordance; correlation and partial correlations
13	11	between all parents and their sons, daughters and all children.
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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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6	Differences in metabolite levels of adults and children	
7	Log Total lipids in chylomicrons and extr. large VLDL (mmol/L) -	
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10	Total lipids in large LDL (mmol/L) - Lipioprotein Subclass	
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12	Total lipids in small HDL (mmo//L)	
13	Mean diameter for VLDL particles (nm) - Mean diameter for VLDL particles (nm) - Mean diameter for VLDL particles (nm) - Mean diameter for VLDL particles (nm) -	
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∠∠ 23	Apolipoprotein B (g/L)	
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24	22:6, docosañexaencia cad (mmo/l.) = 18.2, linoleic acid (mmo/l.) = Omega3 staty acidi (mmo/l.) = Fatty acids	
25	Omega6 fatty acids (mmo/L) Polyunsaturated fatty acids (mmo/L) Monounsaturated fatty acids; 16:1, 18:1 (mmo/L)	
20	Saturated fatty acids (mmol/L)	
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30	Ratio of saturated fatty acids to total fatty acids (%)	
31	Lactate (minol/L) = Glycolysis related	
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32	Alanine (mmol/L) = Gklanine (mmol/L) =	
34	Histidine (mmo/L) = Severine (mmo/L) = Amino acids	
35	Valine (mmol/L) - Phenytalania (mmol/L) - Turcine (mmol/L) -	
36		
37	Log Aceľoacetate (mmol/L) – Log 3hydroxybutyrate (mmol/L) – Ketone bodies	
38	Albumin (signal area) - Creatinine (mmol/L) - Fluid balance	
39	Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	
40	-1.0 -0.5 0.0 0.5 1.0 1.5	
41	sub-binerence in metabolite concentration (95% Cr) for adults compared to children	
42	∼ aduns iower (cinicier i nginer) aduns inginer (cinicier i ower) ∽	
43		
44		
45	Figure 2: Differences in metabolite levels between children and adults.	
46	Association measures are SD difference in metabolite concentration for adults compared to children. E	rror
47	bars represent 95% confidence intervals. Significant associations after p-values adjusted for multiple te	esting
48	using Benjamini-Hochberg procedure are shown in bold (FDR=0.10). Association measures in absolu	ite
49	HDL: High-density linoprotein: IDL: Intermediate density linoprotein: IDL: Low-density linoprotein: VI	DI:
50	Verv low density lipoprotein, 122. Intermediate density lipoprotein, 222. 2000 density lipoprotein.	
51		
52		
53	254x338mm (300 x 300 DPI)	
54		
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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 pvalues 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.

169x169mm (300 x 300 DPI)



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.

169x169mm (300 x 300 DPI)

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

Motabolio subgroup	 Male Female ΔI										Male Female									
Metabolic subgroup	n	Mean	SD	n	Female	SD	n	All Mean	SD		Mean	SD	n	Female	SD	n	All Mean	SD		
Lipoprotein subclass lipids		WEall	30		Weatt	30		Weatt	30		Ivicali	30		Ivicali	30		Weatt			
Total lipids in chylomicrons & ex.large VLDL (mmol/L)*	575	0.005	4.826	605	0.011	3.260	1180	0.007	4.054	17	7 0.040	2.101	1148	0.013	2.822	1325	0.015	2.77		
Total lipids in very large VLDL (mmol/L)*	575	0.006	6.246	605	0.014	4.800	1180	0.010	5.521	17	7 0.087	2.419	1148	0.022	3.458	1325	0.027	3.39		
Total linids in large VI DI (mmol/L)*	575	0.059	3 945	605	0 121	2 488	1180	0.085	3 234	17	7 0.423	1 287	1148	0 161	1 666	1325	0.182	1.66		
Total lipids in medium VI DI (mmol/I)	575	0.441	0.270	605	0.478	0.272	1180	0.460	0.271	17	7 0.959	0.630	1148	0.548	0.366	1325	0.602	0.43		
Total lipids in small VI DL (mmol/L)	575	0.381	0.153	605	0.405	0.1/6	1180	0.393	0.1/9	17	7 0.690	0.000	11/18	0.340	0.216	1325	0.520	0.432		
Total lipids in small VEDE (mmol/L)	575	0.301	0.133	605	0.342	0.076	1180	0.334	0.145	17	7 0.050	0.275	11/18	0.426	0.110	1325	0.320	0.23-		
Total lipids in IDL (mmol/L)	575	0.923	0.176	605	0.834	0.070	1180	0.934	0.075	17	7 0.985	0.111	11/18	0.420	0.240	1325	0.425	0.110		
Total lipids in large LDL (mmol/L)	575	0.004	0.170	605	0.034	0.105	1100	0.010	0.100	17	7 0.585	0.201	1140	1 1 5 5	0.240	1225	1 156	0.24		
Total lipids in mage LDE (minol/L)	575	0.517	0.220	605	0.541	0.220	1100	0.525	0.224	17	7 1.102	0.327	1140	0.655	0.256	1225	0.659	0.30		
Total lipids in medium EDE (mmol/L)	575	0.311	0.150	005	0.319	0.140	1100	0.330	0.156	17	7 0.676	0.214	1140	0.055	0.165	1325	0.056	0.10		
Total lipids in small LDL (mmol/L)	575	0.338	0.083	605	0.340	0.087	1180	0.339	0.085	17	7 0.439	0.135	1148	0.425	0.114	1325	0.427	0.11		
Total lipids in very large HDL (mmol/L)	575	0.482	0.196	605	0.495	0.184	1180	0.488	0.189	17	7 0.320	0.189	1148	0.497	0.229	1325	0.474	0.23		
Total lipids in large HDL (mmol/L)	575	0.874	0.291	605	0.859	0.275	1180	0.866	0.282	1,	/ 0.509	0.335	1148	0.900	0.382	1325	0.849	0.39		
Total lipids in medium HDL (mmol/L)	575	0.917	0.127	605	0.871	0.126	1180	0.894	0.128	1,	/ 0.828	0.241	1148	0.9/1	0.175	1325	0.952	0.19		
Total lipids in small HDL (mmol/L)	575	1.039	0.103	605	0.997	0.115	1180	1.018	0.111	17	7 1.055	0.254	1148	1.085	0.138	1325	1.081	0.15		
Lipoprotein particle size																				
Mean diameter for VLDL particles (nm)	575	37.063	1.633	605	37.238	1.557	1180	37.152	1.591	17	7 38.527	1.737	1148	36.943	1.599	1325	37.152	1.70		
Mean diameter for LDL particles (nm)	575	23.587	0.103	605	23.628	0.109	1180	23.608	0.107	17	7 23.487	0.093	1148	23.573	0.100	1325	23.562	0.10		
Mean diameter for HDL particles (nm)	575	10.081	0.233	605	10.102	0.221	1180	10.092	0.226	17	7 9.798	0.244	1148	10.068	0.262	1325	10.032	0.27		
Cholesterol																				
Serum total cholesterol (mmol/L)	575	3.576	0.620	605	3.596	0.643	1180	3.586	0.629	17	7 4.161	0.885	1148	4.234	0.828	1325	4.225	0.83		
Total cholesterol in VLDL (mmol/L)	575	0.438	0.188	605	0.472	0.189	1180	0.455	0.189	17	7 0.826	0.395	1148	0.592	0.265	1325	0.623	0.29		
Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	575	0.944	0.269	605	0.994	0.271	1180	0.970	0.270	17	7 1.452	0.472	1148	1.219	0.383	1325	1.250	0.40		
Total cholesterol in LDL (mmol/L)	575	1.130	0.330	605	1.149	0.341	1180	1.139	0.334	17	7 1.492	0.498	1148	1.460	0.442	1325	1.464	0.44		
Total cholesterol in HDL (mmol/L)	575	1.503	0.274	605	1.453	0.266	1180	1.477	0.270	17	7 1.217	0.350	1148	1.556	0.363	1325	1.511	0.37		
Total cholesterol in HDL2 (mmol/L)	575	1.035	0.254	605	0.988	0.246	1180	1.011	0.250	17	7 0.751	0.327	1148	1.072	0.335	1325	1.030	0.35		
Total cholesterol in HDL3 (mmol/L)	575	0.468	0.024	605	0.466	0.024	1180	0.467	0.024	17	7 0.466	0.035	1148	0.483	0.033	1325	0.481	0.03		
Esterified cholesterol (mmol/L)	572	2.516	0.447	604	2.517	0.460	1176	2.517	0.452	17	6 2.941	0.636	1147	2.975	0.593	1323	2.971	0.59		
Free cholesterol (mmol/L)	572	1.062	0.179	604	1.079	0.186	1176	1.070	0.182	17	6 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	17	7 1.686	0 809	1148	1 1 2 9	0 755	1325	1 190	0.78		
Triglycerides in VI DL (mmol/L)*	575	0.582	0.885	605	0.648	0.830	1180	0.615	0.858	17	7 1 249	0.927	1148	0.694	0.945	1325	0 750	0.70		
Triglycerides in LDL (mmol/L)	575	0.113	0.02/	605	0.123	0.027	1180	0.118	0.026	17	7 0 153	0.040	11/18	0.054	0.044	1325	0.157	0.04		
Triglycerides in HDL (mmol/L)	575	0.129	0.021	605	0.126	0.020	1180	0.133	0.020	17	7 0 161	0.047	11/18	0.150	0.040	1325	0.153	0.04		
Total phosphoglycoridos (mmol/L)	575	1 622	0.030	604	1,620	0.030	1176	1 6 2 6	0.050	17	1 965	0.047	1140	1 026	0.040	1222	1 010	0.04		
	572	1.032	0.240	604	1.620	0.201	1170	1.020	0.250	17	1.865	0.554	1147	1.926	0.340	1323	1.918	0.54		
Ratio of triglycerides to phosphoglycerides	572	0.526	0.253	604	0.569	0.277	11/6	0.548	0.265	17	6 0.946	0.587	1147	0.580	0.279	1323	0.628	0.35		
Phosphatidylcholine & other cholines (mmol/L)	572	1.691	0.240	604	1.687	0.267	11/6	1.689	0.253	17	5 1.8//	0.320	1147	1.978	0.336	1322	1.965	0.33		
Sphingomyelins (mmol/L)	572	0.348	0.061	604	0.349	0.064	1176	0.348	0.062	1,	5 0.370	0.070	1147	0.397	0.078	1322	0.394	0.07		
Total cholines (mmol/L)	572	2.005	0.256	604	1.997	0.264	1176	2.001	0.259	1,	5 2.185	0.334	1147	2.317	0.351	1322	2.299	0.35		
Apolipoproteins																				
Apolipoprotein A1 (g/L)	575	1.509	0.159	605	1.484	0.151	1180	1.497	0.155	17	7 1.461	0.178	1148	1.589	0.205	1325	1.572	0.20		
Apolipoprotein B (g/L)	575	0.682	0.135	604	0.706	0.136	1179	0.694	0.135	17	7 0.955	0.245	1148	0.812	0.196	1325	0.831	0.20		
Ratio of apolipoprotein B to apolipoprotein A	575	0.455	0.097	604	0.479	0.098	1179	0.467	0.098	17	7 0.660	0.178	1148	0.518	0.136	1325	0.537	0.15		
Fatty acids																				
Total fatty acids (mmol/L)	570	9.215	1.697	604	9.370	1.730	1174	9.294	1.709	17	3 11.850	2.723	1145	10.917	2.392	1318	11.034	2.44		
Estimated degree of unsaturation	570	1.212	0.056	604	1.196	0.065	1174	1.204	0.061	17	3 1.179	0.070	1145	1.212	0.066	1318	1.208	0.06		
22:6, docosahexaenoic acid (mmol/L)	570	0.078	0.028	604	0.074	0.029	1174	0.076	0.028	17	3 0.118	0.051	1145	0.111	0.039	1318	0.112	0.04		
18:2, linoleic acid (mmol/L)	570	2.539	0.456	604	2.592	0.464	1174	2.566	0.459	17	3 2.919	0.567	1145	2.880	0.584	1318	2.885	0.58		
Omega3 fatty acids (mmol/L)	570	0.309	0.086	604	0.296	0.083	1174	0.302	0.085	17	3 0.451	0.160	1145	0.400	0.117	1318	0.406	0.17		
Omega6 fatty acids (mmol/L)	570	3,077	0.493	604	3,094	0.492	1174	3.086	0.491	17	3 3 553	0.648	1145	3,507	0.627	1318	3,513	0.63		
Polyunsat fatty acids (mmol/L)	570	3 386	0.565	604	3 390	0.561	1174	3 388	0.560	17	3 4 004	0 775	1145	3 907	0 721	1318	3 919	0.02		
Monounsat fatty acide: 16:1-18:1 (mmol/L)	570	2 500	0.505	604	2.550	0.501	1174	2 544	0.500	17	2 2 5 2 0	1.059	11/5	3.907	0.721	1210	3 125	0.72		
Saturated fatty acids (mmol/L)	570	2.300	0.052	604	2.307	0.001	1174	2.344	0.040	17	5 5.52U	1.000	1145	2,000	0.917	1210	2.122	0.94		
Service and the deliver of the service of the servi	570	J.J∠ŏ	U.042	004	3.393	0.082	11/4	3.302	0.001	1/	o 4.325	1.088	1145	3.930	0.932	1319	3.979	0.95		

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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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
lsoleucine (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	
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	
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	
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	
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	
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	
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	
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	
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	
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	
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	

Ethanol can be introduced in to a sample from disinfectants used during blood collection/processing of sample.

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

Metabolic subgroup		Differences	by age (Adult	ts - 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 <i>,</i> 0.890)	< 0.001	<0.001	2.534
Total lipids in very large VLDL (mmol/L)*	0.922	(0.700 <i>,</i> 1.145)	< 0.001	<0.001	3.031
Total lipids in large VLDL (mmol/L)*	0.648	(0.502 <i>,</i> 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 <i>,</i> 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 <i>,</i> 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	-0 1/17	(-0.263 -0.021)	0.013	0.014	1 622
Mean diameter for LDL particles (nm)	-0.147	(-0.203, -0.031)	0.013	0.014	1.000 0.10 <i>6</i>
Moon diamotor for HDL particles (nm)	-0.044	(-0.052, -0.037)	<0.001	<0.001	0.106
iviean 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 <i>,</i> 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 <i>,</i> 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 Al	0.055	(0.046, 0.064)	<0.001	<0.001	0.127
	0.000	(0.0.0, 0.004)	-0.001	-0.001	5.127
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
· · ·					

2	Eatty acid ratios					
3	Patie of 22:6 decosphereancie acid to total fatty acide (%)	0 200	(0,100,0,228)	<0.001	<0.001	0 202
4	Patio of 18:2 lipolois asid to total fatty asids $(\%)$	1.079	(0.190, 0.220)	<0.001	<0.001	0.292
5	Ratio of 18.2 infolet actuate to total fatty actus (%) Ratio of amogra 2 fatty acids to total fatty acids (%)	-1.079	(-1.331, -0.827)	<0.001	<0.001	5.567
6	Ratio of omegas fatty acids to total fatty acids (%)	0.440	(0.405, 0.490)	<0.001	<0.001	0.095
7	Ratio of onlyunsat, fatty acids to total fatty acids (%)	-0.552	(-1.227, -0.737)	<0.001	<0.001	3.290
/	Ratio of polyunsat, fatty acids to total fatty acids (%)	-0.340	(-0.794, -0.298)	<0.001	<0.001	3.307
8	Ratio of monourisat. ratty acids to total fatty acids (%)	0.741	(0.347, 0.334)	<0.001	<0.001	1 962
9	Natio of Saturated fatty acids to total fatty acids (20,	-0.195	(-0.328, -0.002)	0.004	0.005	1.805
10	Glycolysis related					
11	Glucose (mmol/L)*	-0.002	(-0.014.0.009)	0 700	0 709	0 147
12	Lactate (mmol/L)	-0.180	(-0.215, -0.144)	<0.001	<0.001	0.177
13	Pyruvate (mmol/L)	-0.007	(-0.009, -0.005)	<0.001	<0.001	0.029
14	Citrate (mmol/L)	-0.017	(-0.018, -0.016)	< 0.001	<0.001	0.019
15	Glycerol (mmol/L)	-0.011	(-0.015, -0.008)	< 0.001	< 0.001	0.023
16			(,			
10	Amino acids					
1/	Alanine (mmol/L)	0.013	(0.009, 0.017)	< 0.001	<0.001	0.060
18	Glutamine (mmol/L)	-0.023	(-0.027, -0.019)	<0.001	<0.001	0.060
19	Glycine (mmol/L)	0.007	(0.003, 0.010)	<0.001	<0.001	0.049
20	Histidine (mmol/L)	0.001	(0.000, 0.002)	0.005	0.006	0.009
21	Isoleucine (mmol/L)	0.003	(0.002, 0.004)	< 0.001	<0.001	0.019
22	Leucine (mmol/L)	0.004	(0.003, 0.006)	<0.001	<0.001	0.021
23	Valine (mmol/L)	0.009	(0.006, 0.011)	< 0.001	<0.001	0.039
24	Phenylalanine (mmol/L)	0.005	(0.005, 0.006)	< 0.001	<0.001	0.010
27	Tyrosine (mmol/L)	0.001	(-0.000, 0.002)	0.100	0.105	0.014
25						
20	Ketone bodies					
27	Acetate (mmol/L)*	0.101	(0.084, 0.117)	<0.001	<0.001	0.235
28	Acetoacetate (mmol/L)*	-0.004	(-0.086, 0.078)	0.922	0.922	1.022
29	3hydroxybutyrate (mmol/L)*	-0.064	(-0.100, -0.028)	0.001	0.001	0.493
30						
31	Fluid balance					
32	Albumin (signal area)	-0.004	(-0.004, -0.004)	<0.001	<0.001	0.005
33	Creatinine (mmol/L)	0.016	(0.015, 0.017)	<0.001	0.001	0.012
34	1					
35	Inflammation	0.062	(0.047, 0.078)	<0.001	<0.001	0.217
36	Giveoprotein acetyis, mainy afacid giveoprotein (mmor)	0.062	(0.047, 0.078)	<0.001	<0.001	0.217
30 27						
3/	* Metabolite has been log transformed					
38	^ Benjamini-Hochberg adjusted p-value					
39	# Associations in Figure 2 are presented in SD-units. The conversion	factor provided (u	nweighted standard d	eviation of eac	h metabolite me	easure) can be
40	used to convert the association in absolute concentration to SD unit	s by dividing by th	e conversion factor. W	here metabolit	e has been log t	ransformed
41	conversion factor is standard deviation of log transformed metaboli	te			0	
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Supplementary table 3: Differences in mean metabolite levels in girls compared to boys in absolute concentration units.

Metabolic subgroup		Differences for ch	ildren (Female - Ma	le)	
-	Estimate	95% CI	pvalue	Adj_p-value^	Conversi factor (SI
Lipoprotein subclass lipids					Tactor (5)
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	0.45
Free cholesterol (mmol/L)	0.013	(-0.008, 0.034)	0.211	0.284	0.184
Glycerides and phospholipids	0 101	(0.056, 0.145)	-0.001	<0.001	0.20
Serum total triglycerides (mmol/L)*	0.101	(0.056, 0.145)	<0.001	<0.001	0.39
Triphyserides in LDL (mmol/L)	0.123	(0.062, 0.187)	<0.001	<0.001	0.55
Trighycerides in EDL (mmol/L)	0.003	(0.008, 0.012)	<0.001	<0.001	0.02
Total photophorphytoridas (mmol/L)	0.007	(0.004, 0.011)	0.001	0.202	0.02
Patio of trighterides to phosphoglycorides	-0.018	(-0.047, 0.010)	0.200	0.202	0.24
Ratio of trigrycendes to phosphogrycendes	0.031	(0.020, 0.083)	0.001	0.005	0.27
Serving and a service of the service	-0.011	(-0.040, 0.018)	0.447	0.534	0.25
Springomyelins (mmol/L)	-0.001	(-0.009, 0.008)	0.706	0.757	0.06
rotal cholines (himoly)	-0.017	(-0.048, 0.013)	0.208	0.554	0.25
Apolipoproteins					
Apolipoprotein A1 (g/L)	-0.030	(-0.048, -0.013)	0.001	0.002	0.15
Apolipoprotein B (g/L)	0.027	(0.012, 0.042)	0.001	0.002	0.13
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	1.65
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	0.45
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	0.48
Polyunsat. fatty acids (mmol/L)	0.023	(-0.041, 0.086)	0.483	0.567	0.55
Monounsat. fatty acids; 16:1, 18:1 (mmol/L)	0.105	(0.034, 0.176)	0.004	0.009	0.62
Saturated fatty acids (mmol/L)	0.073	(-0.000, 0.146)	0.052	0.085	0.63
Fatty acid ratios					
Ratio of 22:6 docosahexaenoic acid to total fatty acids (%)	-0.049	(-0.078, -0.019)	0.001	0.003	0.25
Ratio of 18:2 linoleic acid to total fatty acids (%)	0.197	(-0.173, 0.567)	0.297	0.379	3.23
Ratio of omega3 fatty acids to total fatty acids (%)	-0.145	(-0.213, -0.078)	<0.001	0.000	0.59
Ratio of omega6 fatty acids to total fatty acids (%)	-0.302	(-0.658, 0.054)	0.096	0.142	3.10
Ratio of polyunsat. fatty acids to total fatty acids (%)	-0.447	(-0.830, -0.065)	0.022	0.042	3.34
Ratio of monounsat. fatty acids to total fatty acids (%)	0.499	(0.202, 0.796)	0.001	0.003	2.60
Ratio of saturated fatty acids to total fatty acids (%)	-0.051	(-0.251, 0.148)	0.614	0.689	1.74
Chucohucic related					
Glucose (mmol/L)*	-0.013	(-0.026.0.001)	0.061	0.098	0.11
Lactate (mmol/L)	-0.015		0.001	0.038	0.11
Durivate (mmol/L)	-0.045	(-0.037, 0.007)	0.088	0.133	0.45
ryiuvale (IIIIIIII/L) Citrata (mmal/L)	-0.001	(-0.004, 0.002)	0.524	0.000	0.024
Citrate (mmol/L)	0.007	(0.005, 0.009)	<0.001	<0.001	0.01
Giycerol (mmol/L)	0.006	(0.002, 0.010)	0.004	0.009	0.02
Amino acids					
Alanine (mmol/L)	0.011	(0.004, 0.017)	0.002	0.004	0.05
Glutamine (mmol/L)	0.023	(0.018, 0.029)	<0.001	<0.001	0.05
Chusing (mmgl/L)	0.010	(0.006, 0.014)	<0.001	<0.001	0.03
Givenie (minor)	01010	()			
Histidine (mmol/L)	0.001	(-0.000, 0.002)	0.075	0.118	0.008

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1						
2	Leucine (mmol/L)	-0.002	(-0.005, -0.000)	0.022	0.041	0.018
2	Valine (mmol/L)	-0.007	(-0.011, -0.003)	0.001	0.003	0.036
3	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
4						
5	Ketone bodies					
5	Acetate (mmol/L)*	-0.030	(-0.048, -0.011)	0.002	0.005	0.166
6	Acetoacetate (mmol/L)*	-0.058	(-0.172, 0.055)	0.313	0.393	0.992
7	3hydroxybutyrate (mmol/L)*	0.041	(-0.019, 0.101)	0.178	0.248	0.513
8	Fluid balance					
0	Albumin (signal area)	-0.001	(-0.001, -0.000)	0.037	0.064	0.005
2	Creatinine (mmol/L)	0.000	(-0.001, 0.001)	0.624	0.690	0.007
10						
11	Inflammation					
11	Glycoprotein acetyls, mainly a1acid glycoprotein (mmol/L)	0.017	(-0.004, 0.038)	0.104	0.151	0.183
12						

* 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

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

	Metabolic subgroup		Differences for	adults (Femal	e - Male)	Conversi factor (SI 2.173 2.555 1.343 0.398 0.223 0.107 0.236 0.293 0.482 0.113 0.239 0.405 0.184 0.145					
		Estimate	95% CI	pvalue	Adj_p-value^	Conversio					
Lipoprotein subcl	ass lipids					100001 (00					
Total lipids in chyl	omicrons & 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	e VLDL (mmol/L)*	-0.900	(-1.107, -0.693)	<0.001	<0.001	1.343					
Total lipids in med	ium VLDL (mmol/L)	-0.325	(-0.385, -0.264)	<0.001	<0.001	0.398					
Total lipids in sma	I 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	e LDL (mmol/L)	-0.011	(-0.058, 0.035)	0.634	0.671	0.293					
Total lipids in med	ium LDL (mmol/L)	-0.028	(-0.057, 0.001)	0.058	0.076	0.182					
Total lipids in sma	I 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	e HDL (mmol/L)	0.395	(0.334, 0.455)	<0.001	<0.001	0.405					
Total lipids in med	ium HDL (mmol/L)	0.129	(0.101, 0.158)	<0.001	<0.001	0.184					
Total lipids in sma	I HDL (mmol/L)	0.002	(-0.021, 0.025)	0.850	0.874	0.145					
Lipoprotein partio	le size										
Mean diameter fo	r VLDL particles (nm)	-1.414	(-1.669, -1.159)	<0.001	<0.001	1.678					
Mean diameter fo	r LDL particles (nm)	0.081	(0.066, 0.096)	< 0.001	< 0.001	0.100					
Mean diameter fo	r HDL particles (nm)	0.278	(0.236, 0.320)	<0.001	<0.001	0.279					
Cholesterol											
Serum total chole	sterol (mmol/L)	0.112	(-0.018, 0.241)	0.091	0.116	0.817					
Total cholesterol i	n VLDL (mmol/L)	-0.187	(-0.230, -0.145)	< 0.001	< 0.001	0.275					
Remnant choleste	rol (nonHDL, nonLDL cholesterol) (mmol/L)	-0.184	(-0.244, -0.124)	< 0.001	< 0.001	0.383					
Total cholesterol i	n LDL (mmol/L)	-0.048	(-0.117, 0.021)	0.175	0.213	0.437					
Total cholesterol i	n HDL (mmol/L)	0.344	(0.286, 0.401)	<0.001	< 0.001	0.382					
Total cholesterol i	n HDL2 (mmol/L)	0.324	(0.271, 0.378)	<0.001	< 0.001	0.354					
Total cholesterol i	n HDL3 (mmol/L)	0.019	(0.014, 0.025)	< 0.001	< 0.001	0.034					
Esterified choleste	rol (mmol/L)	0.070	(-0.023, 0.163)	0.139	0.172	0.586					
Free cholesterol (I	nmol/L)	0.046	(0.009, 0.084)	0.016	0.023	0.238					
Glycerides and ph	ospholipids oridoo (mmol/L)*	0.244	(0.416 0.272)	-0.001	-0.001	0.409					
Tei-hourida ingiyo	endes (mmor/L)*	-0.344	(-0.416, -0.273)	<0.001	<0.001	0.468					
Trigiycerides in VL	DL (mmoi/L)*	-0.530	(-0.630, -0.429)	<0.001	<0.001	0.655					
Trigiycerides in LU	L (mmol/L)	0.008	(0.001, 0.014)	0.033	0.044	0.044					
Triglycerides in HL	pL (mmol/L)	-0.004	(-0.010, 0.002)	0.228	0.272	0.040					
l otal phosphoglyc	erides (mmol/L)	0.106	(0.052, 0.159)	<0.001	<0.001	0.340					
Ratio of triglyceric	es to phosphoglycerides	-0.289	(-0.337, -0.241)	<0.001	<0.001	0.318					
Phosphatidylcholi	he & other cholines (mmol/L)	0.138	(0.086, 0.190)	<0.001	<0.001	0.333					
Sphingomyelins (r	nmol/L)	0.032	(0.020, 0.045)	<0.001	<0.001	0.078					
lotal cholines (mr	nol/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 apolipopr	otein B to apolipoprotein Al	-0.126	(-0.148, -0.105)	<0.001	< 0.001	0.144					
Fatty acids		0 711	(1001 0 220)	10 001	-0.001	2 200					
Total fatty acids (r	nmoi/L)	-0.711	(-1.091, -0.330)	<0.001	<0.001	2.380					
Estimated degree	of unsaturation	0.031	(0.021, 0.042)	<0.001	<0.001	0.066					
22:6, docosahexa	noic 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 acid	s (mmol/L)	-0.035	(-0.054, -0.016)	<0.001	0.001	0.122					
Omega6 fatty acid	s (mmol/L)	0.004	(-0.094, 0.102)	0.936	0.936	0.612					
Polyunsat. fatty ad	ids (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 ac	ds (mmol/L)	-0.307	(-0.455, -0.159)	<0.001	<0.001	0.932					
Fatty acid ratios											
Ratio of 22:6 docc	sahexaenoic acid to total fatty acids (%)	0.064	(0.018, 0.110)	0.006	0.009	0.288					
Ratio of 18:2 linol	eic acid to total fatty acids (%)	1.527	(0.984, 2.070)	<0.001	< 0.001	3.430					
Ratio of omega3 f	atty acids to total fatty acids (%)	-0.038	(-0.152. 0.075)	0.508	0.570	0.710					
Ratio of omega6 f	atty 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	3.62					
Ratio of monouns	at. fatty acids to total fatty acids (%)	-1.456	(-1.9140.998)	< 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					
		,	,, 5.67.17			2.00					
Glycolysis related											
Glucose (mmol/L)	k	-0.071	(-0.097, -0.044)	< 0.001	<0.001	0.16					
Lactate (mmol/L)		-0.178	(-0.252, -0.105)	<0.001	<0.001	0.46					
Pyruvate (mmol/l		-0.007	(-0.013, -0.002)	0.004	0.006	0.032					

1						
2	Citrate (mmol/L)	-0.001	(-0.004, 0.001)	0.335	0.387	0.016
2	Giycerol (mmol/L)	-0.003	(-0.008, 0.002)	0.279	0.328	0.022
ر ۱	Amino acids					
4	Alanine (mmol/L)	-0.020	(-0.030, -0.011)	<0.001	<0.001	0.060
5	Glutamine (mmol/L)	-0.038	(-0.048, -0.028)	< 0.001	< 0.001	0.065
6	Glycine (mmol/L)	0.029	(0.020, 0.038)	< 0.001	< 0.001	0.059
7	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
8	Leucine (mmol/L)	-0.019	(-0.022, -0.016)	<0.001	< 0.001	0.022
9	Valine (mmol/L)	-0.029	(-0.035, -0.022)	<0.001	< 0.001	0.042
10	Phenylalanine (mmol/L)	0.000	(-0.002, 0.001)	0.576	0.637	0.010
10	Tyrosine (mmol/L)	-0.005	(-0.007, -0.003)	<0.001	<0.001	0.014
11						
12	Ketone bodies					
12	Acetate (mmol/L)*	-0.076	(-0.119, -0.033)	0.001	0.001	0.273
13	Acetoacetate (mmol/L)*	0.018	(-0.148, 0.184)	0.828	0.863	1.048
14	3hydroxybutyrate (mmol/L)*	-0.087	(-0.163, -0.011)	0.025	0.035	0.472
15						
16	Fluid balance		(0.000, 0.001)		0.004	0.005
10	Albumin (signal area)	-0.002	(-0.002, -0.001)	<0.001	<0.001	0.005
17	Creatinine (mmol/L)	-0.013	(-0.015, -0.012)	<0.001	<0.001	0.010
18	Inflammation					
19	Glycoprotein acetyls, mainly alacid glycoprotein (mmol/L)	-0.098	(-0.136, -0.061)	<0.001	<0.001	0.239
20			(,,			
 21						
21	* Matabalita has been log transformed					

* 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.

Notoholio outoroun							Mother						All Children						
	n	сс	95% CI	n 1	PCC*	95% CI	n	сс	95% CI	n	PCC*	95% CI	n	сс	95% CI	n	PCC		
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		
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		
otal lipids in large VLDL (mmol/L)* Fotal lipids in medium 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		
Fotal lipids in small 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	0.29	0.22 - 0.34	909	0.23		
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		
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		
Total lipids in large LDL (mmol/L) Total lipids in medium 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		
Total lipids in small LDL (mmol/L)	469	0.28	0.19 - 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 - 0.33	909	0.25		
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		
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		
Total lipids in medium HDL (mmol/L) Total lipids in small HDL (mmol/L)	469 469	0.22 0.23	0.13 - 0.30 0.14 - 0.31	433 433	0.20	0.11 - 0.29 0.13 - 0.31	518 518	0.12	0.03 - 0.20 0.12 - 0.29	476 476	0.13 0.20	0.04 - 0.22 0.11 - 0.28	987 987	0.17 0.21	0.11 - 0.23 0.15 - 0.27	909 909	0.17		
Lipoprotein particle size																			
Mean diameter for VLDL particles (nm) Mean diameter for LDL particles (nm)	469 469	0.30	0.22 - 0.38 0.13 - 0.31	433 433	0.32	0.23 - 0.40 0.11 - 0.29	518 518	0.27	0.19 - 0.35 0.22 - 0.38	476 476	0.25	0.16 - 0.33 0.24 - 0.40	987 987	0.29	0.23 - 0.35 0.20 - 0.31	909 909	0.28		
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		
Cholesterol	400	0.27	0.10 0.25	400	0.22	0.14 0.00	F 10	0.22	0.24 0.20	476	0.24	0.25 0.42	007	0.20	0.24 0.25	000	0.20		
Total cholesterol in VLDL (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	987 987	0.30	0.24 - 0.35	909	0.30		
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	0.25		
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	0.25		
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		
Total cholesterol in HDL2 (mmol/L) Total cholesterol in HDL3 (mmol/L)	469	0.30	0.16 - 0.32	433	0.31	0.14 - 0.39	518 518	0.25	0.16 - 0.32	4/6 476	0.23	0.15-0.32	987 987	0.28	0.19 - 0.33	dUd 202	0.27		
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		
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		
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		
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		
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		
Triglycerides in HDL (mmol/L) Total phoenbackwarides (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		
Ratio of triglycerides to phosphoglycerides	465	0.28	0.15 - 0.32	430	0.25	0.14 - 0.32	518	0.26	0.17 - 0.34	476	0.27	0.18 - 0.35	983	0.25	0.20 - 0.32	906	0.2		
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		
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		
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		
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		
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		
Ratio of apolipoprotein B to apolipoprotein Al	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		
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		
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		
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	0.2		
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	0.2		
Omegas fatty acids (mmol/L) Omega6 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	0.2		
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	0.2		
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	0.3		
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	0.2		
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	0.3		
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	0.1		
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	0.3		
Ratio of omega6 fatty acids to total fatty acids (%) Ratio of polyupsat, fatty acids to total fatty acids (%)	462	0.23	0.15 - 0.32	427	0.26	0.17 - 0.35	517 517	0.25	0.17 - 0.33	475	0.22	0.13 - 0.30	979	0.24	0.18 - 0.30	902	0.24		
Ratio of monounsat, fatty acids to total fatty acids (%)	462	0.29	0.21 - 0.38	427	0.30	0.22 - 0.39	517	0.28	0.25 - 0.56	475	0.24	0.23 - 0.40	979	0.32	0.26 - 0.33	902	0.2		
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	0.2		
Glycolysis related	400	0.05	0.02 0.15	400	0.05	0.01 0.55	F 10	0.14	0.05 0.00	176	0.15	0.05 0.00	086	0.15	0.05 0.53	000	0.1		
Lactate (mmol/L)	468 469	0.06	-0.03 - 0.15 -0.05 - 0.13	432 433	0.05	-0.04 - 0.14 -0.04 - 0.15	518	0.14	0.00 - 0.23	476	0.14	0.03 - 0.23	985 987	0.11	0.05 - 0.17 0.00 - 0.13	908 909	0.10		
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	0.1		
Citrate (mmol/L) Glycerol (mmol/L)	469 124	0.24 0.25	0.15 - 0.32 0.07 - 0.41	433 113	0.25 0.24	0.16 - 0.34 0.06 - 0.41	517 155	0.16 0.10	0.07 - 0.24 -0.05 - 0.26	475 137	0.16	0.07 - 0.25	986 279	0.18 0.16	0.12 - 0.24 0.05 - 0.28	908 250	0.19		
Amino acida						*									1.10				
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	0.2		
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	0.27		
Histidine (mmol/L)	468 468	0.22	0.13 - 0.30	432 432	0.25	0.15 - 0.33	517 518	0.19 0.21	0.10 - 0.27	475 476	0.18	0.09 - 0.27	985	0.20	0.14 - 0.26 0.15 - 0.27	907 908	0.2		
Isoleucine (mmol/L)	466	0.39	0.31 - 0.47	431	0.40	0.32 - 0.47	518	0.33	0.25 - 0.29	476	0.30	0.22 - 0.38	984	0.36	0.30 - 0.41	907	0.34		
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		
Valine (mmol/L) Phenylalanine (mmol/L)	469 469	0.39 0.32	0.31 - 0.46 0.23 - 0.39	433 433	0.38	0.30 - 0.46 0.22 - 0.39	516 518	0.27	0.19 - 0.35 0.21 - 0.37	474 476	0.26	0.17 - 0.34 0.18 - 0.35	985 987	0.33	0.27 - 0.38 0.24 - 0.36	907 909	0.3		
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	0.2		
Ketone bodies																			
Acetate (mmol/L)* Acetoacetate (mmol/L)*	468 469	0.11 0.13	0.02 - 0.20 0.04 - 0.22	433 433	0.08 0.14	-0.02 - 0.17 0.04 - 0.23	517 517	0.14 0.03	0.06 - 0.22 -0.05 - 0.12	475 475	0.14 0.01	0.06 - 0.23 -0.08 - 0.10	985 986	0.13	0.07 - 0.19 0.01 - 0.14	908 908	0.1		
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	0.2		
Fluid balance Albumin (signal area)	100	0.20	0 20 0 27	422	0.26	017 024	510	0.10	0.20 0.20	476	0.20	0.19 0.20	996	0.70	0.22 0.24	900	0.7		
Creatinine (mmol/L)	458	0.29	0.20 - 0.37	432	0.26	0.17 - 0.34	518 514	0.28	0.20 - 0.36	475	0.28	0.19 - 0.36	986 972	0.28	0.22 - 0.34	908 896	0.25		
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		
* log transformation has been an ited to access the																			
log constormation has been applied to metabolite																			

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.

2		purcui correta		een on parento		iono, acag				All Par	ents								
3	Metabolic subgroup	n	сс	Male (95% Cl	Child n	PCC*	95% CI	n	сс	Female 95% CI	Child n	PCC*	95% CI	n	сс	All Chi 95% Cl	ldren n	PCC*	95% CI
4	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
5	Total lipids in very large VLDL (mmol/L)* Total lipids in large VLDL (mmol/L)*	547 547	0.23 0.20	0.15 - 0.31 0.12 - 0.28	505 505	0.23	0.15 - 0.31 0.13 - 0.30	586 586	0.17 0.20	0.10 - 0.25 0.12 - 0.28	544 544	0.16 0.19	0.08 - 0.24 0.11 - 0.27	1133 1133	0.21	0.15 - 0.26 0.15 - 0.26	1049 1049	0.20 0.21	0.14 - 0.26 0.15 - 0.26
6	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) Total lipids in very small VLDL (mmol/L)	547 547	0.27	0.19 - 0.34 0.15 - 0.31	505 505	0.27	0.19 - 0.35 0.13 - 0.30	586 586	0.26	0.18 - 0.33 0.17 - 0.33	544 544	0.26	0.17 - 0.33 0.18 - 0.34	1133 1133	0.26	0.21 - 0.32 0.19 - 0.30	1049 1049	0.26	0.21 - 0.32 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
8	Total lipids in large LDL (mmol/L) Total lipids in medium LDL (mmol/L)	547	0.28	0.20 - 0.36	505	0.25	0.17 - 0.33	586	0.29	0.21 - 0.36	544 544	0.31	0.23 - 0.38	1133	0.29	0.23 - 0.34 0.23 - 0.34	1049	0.29	0.23 - 0.34
0	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
9	Total lipids in large HDL (mmol/L)	547	0.29	0.21 - 0.37	505	0.28	0.19 - 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
10	Total lipids in medium HDL (mmol/L) Total lipids in small HDL (mmol/L)	547 547	0.15 0.19	0.07 - 0.24 0.10 - 0.27	505 505	0.13 0.16	0.04 - 0.21 0.08 - 0.25	586 586	0.10 0.18	0.02 - 0.18 0.10 - 0.26	544 544	0.10 0.17	0.02 - 0.19 0.09 - 0.25	1133 1133	0.12 0.18	0.07 - 0.18 0.12 - 0.23	1049 1049	0.11 0.17	0.05 - 0.17 0.11 - 0.22
11	Lipoprotein particle size																		
12	Mean diameter for VLDL particles (nm) Mean diameter for LDL particles (nm)	547 547	0.29 0.19	0.21 - 0.36 0.11 - 0.27	505 505	0.30 0.17	0.22 - 0.38 0.08 - 0.25	586 586	0.22 0.27	0.14 - 0.29 0.19 - 0.34	544 544	0.19 0.29	0.11 - 0.27 0.21 - 0.36	1133 1133	0.25 0.23	0.20 - 0.31 0.17 - 0.28	1049 1049	0.24 0.23	0.19 - 0.30 0.17 - 0.28
13	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
14	Cholesterol Serum total cholesterol (mmol/L) Tatel cholesterol in V(D) (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
15	Remnant cholesterol in VLDL (mmol/L) Remnant cholesterol (nonHDL, nonLDL cholesterol) (mmol/L)	547	0.26	0.18 - 0.34 0.19 - 0.34	505	0.27	0.19 - 0.35 0.18 - 0.34	586	0.26	0.18 - 0.33 0.20 - 0.35	544 544	0.26	0.18 - 0.34 0.21 - 0.37	1133	0.26	0.22 - 0.32	1049	0.27	0.22 - 0.32
16	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
10	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
17	Total cholesterol in HDL3 (mmol/L) Esterified cholesterol (mmol/L)	547 543	0.25	0.17 - 0.33	505	0.22	0.14 - 0.30	586 584	0.23	0.15 - 0.31	544 542	0.23	0.14 - 0.30	1133	0.24	0.19 - 0.30	1049	0.22	0.16 - 0.28
18	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
19	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
20	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
21	Triglycerides in LDL (mmol/L) Triglycerides in HDL (mmol/L)	547 547	0.20	0.12 - 0.28 0.17 - 0.33	505 505	0.19	0.10 - 0.27 0.18 - 0.34	586 586	0.27	0.19 - 0.34 0.20 - 0.35	544 544	0.28	0.20 - 0.35 0.20 - 0.35	1133 1133	0.24	0.18 - 0.29 0.21 - 0.32	1049 1049	0.24	0.18 - 0.29 0.21 - 0.32
21	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
22	Ratio of triglycerides to phosphoglycerides Phosphatidylcholine & other cholines (mmol/L)	543 542	0.23	0.15 - 0.31 0.20 - 0.36	502	0.24	0.15 - 0.32	584 584	0.21	0.13 - 0.28 0.19 - 0.34	542 542	0.20	0.12 - 0.28 0.20 - 0.36	1127	0.22	0.21 - 0.27	1044	0.22	0.16 - 0.28 0.20 - 0.31
23	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
23		542	0.27	0.19 - 0.35	501	0.23	0.14 - 0.31	584	0.29	0.21 - 0.36	542	0.50	0.22 - 0.37	1120	0.28	0.22 - 0.33	1043	0.26	0.20 - 0.32
24	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
25	Apolipoprotein B (g/L) Batio of apolipoprotein B to apolipoprotein Al	547 547	0.27	0.19 - 0.35	505 505	0.27	0.19 - 0.35	585 585	0.28	0.20 - 0.35	543 543	0.30	0.22 - 0.37	1132 1132	0.28	0.23 - 0.33	1048 1048	0.29	0.23 - 0.34
26		547	0.20	0.10 0.00	505	0.27	0.10 0.54	505	0.22	0.15 0.25	545	0.20	0.11 0.10	1152	0.25	0.10 0.15	1010	0.24	0.10 0.25
27	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
27	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
28	22:6, docosahexaenoic acid (mmol/L) 18:2. linoleic acid (mmol/L)	537 537	0.23 0.24	0.15 - 0.31 0.16 - 0.32	496 496	0.21	0.13 - 0.30 0.16 - 0.33	583	0.33 0.27	0.26 - 0.40 0.19 - 0.34	541 541	0.33	0.25 - 0.40 0.21 - 0.37	1120 1120	0.28	0.23 - 0.34 0.21 - 0.32	1037 1037	0.27 0.28	0.21 - 0.32 0.22 - 0.33
29	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
20	Omega6 fatty acids (mmol/L) Polyunsat. fatty acids (mmol/L)	537	0.26	0.18 - 0.33 0.17 - 0.33	496 496	0.25	0.16 - 0.33	583	0.30	0.23 - 0.38 0.24 - 0.39	541 541	0.33	0.25 - 0.40 0.26 - 0.41	1120 1120	0.28	0.23 - 0.34 0.23 - 0.34	1037	0.29	0.24 - 0.35 0.24 - 0.35
30	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
31	saturateu ratty acius (mmor))	557	0.25	0.17 - 0.35	490	0.25	0.10 - 0.55	565	0.28	0.20 - 0.35	541	0.28	0.20 - 0.36	1120	0.27	0.21 - 0.32	1057	0.27	0.21 - 0.32
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
22	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
33	Ratio of omega3 fatty acids to total fatty acids (%) Ratio of omega6 fatty acids to total fatty acids (%)	537 537	0.32 0.24	0.24 - 0.39 0.16 - 0.32	496 496	0.32	0.23 - 0.39 0.18 - 0.34	583 583	0.40	0.33 - 0.47 0.15 - 0.30	541 541	0.38 0.20	0.31 - 0.45 0.12 - 0.28	1120 1120	0.36 0.24	0.31 - 0.41 0.18 - 0.29	1037 1037	0.35	0.29 - 0.40 0.17 - 0.28
34	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
35	Ratio of monounsat. Tatty acids to total fatty acids (%) Ratio of saturated fatty acids to total fatty acids (%)	537	0.27	0.19 - 0.34 0.14 - 0.30	496 496	0.27	0.19 - 0.35 0.14 - 0.31	583 583	0.30	0.23 - 0.37 0.19 - 0.34	541 541	0.29	0.21 - 0.37 0.17 - 0.33	1120	0.29	0.23 - 0.34 0.19 - 0.30	1037	0.28	0.22 - 0.34 0.19 - 0.30
36	Glycolysis related Glucose (mmol/1)*	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
37	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
57	Pyruvate (mmol/L) Citrate (mmol/L)	545 547	0.11 0.23	0.03 - 0.20 0.15 - 0.31	503 505	0.13 0.25	0.04 - 0.21 0.16 - 0.33	586 585	0.22	0.15 - 0.30 0.06 - 0.22	544 543	0.22	0.14 - 0.30 0.05 - 0.22	1131 1132	0.17 0.17	0.11 - 0.22 0.12 - 0.23	1047 1048	0.17 0.18	0.11 - 0.23 0.12 - 0.24
38	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
39	Amino acids				504	0.00		505	0.00	0.05 0.00	5.43	0.00			0.00			0.07	0.01 0.00
40	Alanine (mmol/L) Glutamine (mmol/L)	546 547	0.24	0.16 - 0.31 0.22 - 0.37	504 505	0.22	0.14 - 0.31 0.21 - 0.37	585 586	0.32	0.25 - 0.39 0.12 - 0.27	543 544	0.30	0.22 - 0.37	1131 1133	0.28	0.23 - 0.34 0.19 - 0.30	1047 1049	0.27	0.21 - 0.32 0.18 - 0.29
41	Glycine (mmol/L) Histidine (mmol/L)	545 545	0.21 0.21	0.13 - 0.29 0.13 - 0.29	503 503	0.24 0.21	0.15 - 0.32 0.12 - 0.29	585 586	0.19 0.22	0.11 - 0.27 0.14 - 0.29	543 544	0.19 0.20	0.11 - 0.27 0.12 - 0.28	1130 1131	0.20 0.21	0.15 - 0.26 0.16 - 0.27	1046 1047	0.21 0.21	0.15 - 0.27 0.15 - 0.26
40	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
42	Valine (mmol/L)	547	0.32	0.24 - 0.39	505	0.35	0.23 - 0.40	584	0.24	0.19 - 0.32	544	0.21	0.13 - 0.29	1135	0.28	0.23 - 0.35	1049	0.27	0.21 - 0.32
43	Phenylalanine (mmol/L) Tyrosine (mmol/L)	547 545	0.29 0.31	0.22 - 0.37 0.24 - 0.39	505 503	0.29 0.31	0.21 - 0.37 0.22 - 0.38	586 586	0.30 0.27	0.22 - 0.37 0.19 - 0.34	544 544	0.28 0.23	0.20 - 0.36 0.15 - 0.31	1133 1131	0.29 0.29	0.24 - 0.35 0.24 - 0.34	1049 1047	0.28 0.27	0.23 - 0.34 0.21 - 0.32
44	Ketone bodies																		
45	Acetate (mmol/L)* Acetoacetate (mmol/L)*	546 547	0.09 0.11	0.01 - 0.17 0.03 - 0.20	505 505	0.05 0.11	-0.03 - 0.14 0.03 - 0.20	585 585	0.15 0.04	0.06 - 0.22 -0.04 - 0.12	543 543	0.15 0.04	0.07 - 0.23 -0.05 - 0.12	1131 1132	0.12 0.07	0.06 - 0.17 0.02 - 0.13	1048 1048	0.11 0.07	0.05 - 0.17 0.01 - 0.13
46	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
47	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
48	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
49	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
50	• I																		

* 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).

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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



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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	2	14-15
		term in the title or the abstract		
		(b) Provide in the abstract an informative and balanced	2	9-13
		summary of what was done and what was found		25-32
Introduction		0		
Background/rationale	2	Explain the scientific background and rationale for the	4-5	Pg 4 (3-9 ,21-22,
		investigation being reported		31-32, 33-35)
				Pg 5 (5-7)
Objectives	3	State specific objectives, including any prespecified	5	8-11
		hypotheses		
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,	5-6	Pg 5 (17-20)
		including periods of recruitment, exposure, follow-up,		Pg 6 (3-32)
		and data collection		
Participants	6	(a) Cohort study—Give the eligibility criteria, and the	5	23-31
		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		
		(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,	7-9	Pg 7 (4-17)
		potential confounders, and effect modifiers. Give		Pg 8 (table 1, 9-
		diagnostic criteria, if applicable		16)
				Pg 9 (1-24)
Data sources/	8*	For each variable of interest, give sources of data and	7-9	Pg 7 (4-17)
measurement		details of methods of assessment (measurement).		Pg 8 (table 1, 9-
		Describe comparability of assessment methods if there is		16)
		more than one group		Pg 9 (1-24)
Bias	9	Describe any efforts to address potential sources of bias	9 -10	Pg 9 (31-35)
				$\mathbf{D} = 10 (10 + 10)$

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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	9-10 Pg	g 9 (28-35)
		analyses. If applicable, describe which groupings were chosen and why	P	g 10 (3-15)
Statistical methods	12	2 (a) Describe all statistical methods, including those used	9-10 Pg	g 9 (28-35)
		to control for confounding	Р	g 10 (3-15)
		(b) Describe any methods used to examine subgroups	9-10 P	g 9 (28-35)
		and interactions	P	g 10 (3-15)
		(c) Explain how missing data were addressed	9-10 Pg	g 9 (31-35)
			P	g 10 (16-18)
		(d) Cohort study—If applicable, explain how loss to	9 31	1-35
		follow-up was addressed		
		Case-control study If applicable, explain how matching		
		of cases and controls was addressed		
		(<u>e</u>) Describe any sensitivity analyses	10 16	5-18
Results			Page	Line numbe
Participants	13*	(a) Report numbers of individuals at each stage of study—eg	11 and	5-12
i unicipulits	15	numbers potentially eligible examined for eligibility, confirmed	figure 1	012
		eligible, included in the study, completing follow-up, and	8	
		analysed		
		(b) Give reasons for non-participation at each stage	11	8-12
			6	7-11
			figure 1	
		(c) Consider use of a flow diagram	figure 1	
Descriptive	14*	(a) Give characteristics of study participants (eg demographic,	12	2
data		clinical, social) and information on exposures and potential	(table 2)	
		confounders		
		(b) Indicate number of participants with missing data for each	figure 1	
		variable of interest	table 2	2
			sup Table	
			1	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and	NA	
	1 5 4	total amount)		
Outcome data	15*	Cohort study—Report numbers of outcome events or summary	sup table 1	
		Case existend study. Benerit numbers in each surround establish	NA	
		case-control study—Report numbers in each exposure category,	, NA	
		Cross sectional study. Poport numbers of outcome quants or	NA	
		summary measures	INA	
Main results	16	(a) Give unadjusted estimates and if applicable confounder-	13	21-24
	10	adjusted estimates and their precision (eq. 95% confidence	sunn tahlea	21-27
		interval). Make clear which confounders were adjusted for and	2-6	
		why they were included	- •	
		(b) Report category boundaries when continuous variables were	NA	
		categorized		
		<u> </u>		
	For p	eer review only - http://bmjoper?bmj.com/site/about/guide	elines.xhtml	

		(<i>c</i>) 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 informati	on			
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.