Supplemental Material

Liver fat and Cardio-metabolic Risk Factors among School Age Children

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Table 1. Comparison of Child Characteristics between Children Included and Not Included in the Analyses

Characteristics	Participants (n = 3,170)	Non-participants (n = 965)	P-value
Age, mean (SD), years	9.8 (0.3)	9.8 (0.4)	0.100
Boys, n (%)	1563 (49.3)	505 (52.3)	0.100
Ethnicity, n (%), European	2118 (68.2)	576 (61.2)	<0.001
Birth weight, mean (SD), g	3446 (558)	3409 (531)	0.328
Body mass index, mean (SD), kg/m^2	17.5 (2.7)	17.8 (3.0)	0.011
Systolic blood pressure, mean (SD), mmHg	103.3 (8.0)	103.3 (8.1)	0.489
Diastolic blood pressure, mean (SD), mmHg	58.6 (6.4)	58.8 (6.6)	0.611
Insulin, median (95% range), pmol/l	182.3 (35.2, 629.1)	174.3 (35.8, 740.4)	0.605
Glucose, mean (SD), mmol/l	5.3 (0.9)	5.2 (1.0)	0.044
HOMA-IR, median (95% range)	7.0 (1.1, 28.8)	6.7 (1.1, 32.4)	0.340
Total – cholesterol, mean (SD), mmol/l	4.3 (0.7)	4.3 (0.6)	0.378
HDL – cholesterol, mean (SD), mmol/l	1.5 (0.3)	1.5 (0.3)	0.524
LDL – cholesterol, mean (SD), mmol/l	2.3 (0.6)	2.3 (0.6)	0.417
Triglycerides, median (95% range), mmol/l	1.0 (0.4, 2.6)	1.0 (0.4, 2.4)	0.418
C-reactive protein, median (95% range), mg/l	0.3 (0.3, 5.7)	0.3 (0.3, 5.1)	0.279

Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and using χ^2-test for dichotomous variables. HOMA-IR was calculated using the formula: insulin resistance = (insulin (μU/L) x glucose (mmol/L)) / 22.5. LDL-cholesterol is calculated according to the Friedewald formula. HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; n, number; SD, standard deviation.

Table 2. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Insulin, Glucose, HDL-cholesterol and LDL-cholesterol

	Insulin, Glucose, HDL-cholesterol and LDL-cholesterol at 10 years in Standard Deviation Scores Difference (95% Confidence Interval)			
	Insulin	**Glucose**	**HDL-cholesterol**	**LDL-cholesterol**
	(n = 2,246)	(n = 2,252)	(n = 2,253)	(n = 2,242)
Liver Fat Fraction				
Basic model	0.14 (0.10;0.18)*	0.02 (-0.02;0.07)	-0.13 (-0.17;-0.09)*	0.09 (0.04;0.13)*
Confounder model	0.14 (0.09;0.18)*	0.03 (-0.01;0.07)	-0.11 (-0.15;-0.07)*	0.09 (0.05;0.13)*
BMI model	0.06 (0.02;0.11)*	0.05 (0.01;0.10)†	-0.05 (-0.10;-0.01)†	0.05 (0.00;0.09)†
Non-alcoholic Fatty Liver Disease				
Basic model	0.41 (0.16;0.66)*	-0.04 (-0.29;0.21)	-0.37 (-0.61;-0.12)*	0.30 (0.17;0.43)†
Confounder model	0.38 (0.13;0.64)*	-0.03 (-0.29;0.23)	-0.31 (-0.56;-0.06)†	0.31 (0.05;0.56)†
BMI model	0.13 (-0.12;0.38)	0.02 (-0.24;0.28)	-0.11 (-0.36;0.14)	0.16 (-0.09;0.42)

Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in insulin and glucose in SDS per SDS change in childhood liver fat fraction. *P-value <0.01, †P-value <0.05. Associations are adjusted for child age, sex, ethnicity in the basic models, further adjusted for maternal pre-pregnancy BMI and maternal education in the confounder models and additionally adjusted for childhood BMI at ten years of age in the BMI model. N, number; SDS, standard deviation scores.

Table 3. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Cardio-metabolic Risk Factors at School Age among Normal Weight and Overweight/Obese Children

	Cardio-metabolic risk factors at 10 years in Standard Deviation Scores Difference (95% Confidence Interval)					
	Systolic blood pressure	**Diastolic blood pressure**	**HOMA-IR**	**Total – cholesterol**	**Triglycerides**	**C-reactive protein**
Liver fat fraction						
Normal weight group	n = 2,323	n = 2,324	n = 1,729	n = 1,729	n = 1,727	n = 1,730
	0.09 (0.04;0.13)*	0.06 (0.01;0.11)†	0.07 (0.01;0.13)†	0.07 (0.01;0.12)†	0.15 (0.10;0.21)*	0.07 (0.02;0.12)*
Overweight group	n = 536	n = 536	n = 377	n = 382	n = 380	n = 383
	0.13 (0.07;0.19)*	0.08 (0.02;0.15)†	0.14 (0.07;0.22)*	0.13 (0.05;0.21)*	0.20 (0.12;0.27)*	0.20 (0.11;0.29)*
Non-alcoholic Fatty Liver Disease						
Normal weight group	n = 2,323	n = 2,324	n = 1,729	n = 1,729	n = 1,727	n = 1,730
	0.24 (-0.15;0.62)	0.45 (0.05;0.85)†	-0.11 (-0.55;0.32)	0.17 (-0.27;0.60)	0.47 (0.03;0.91)†	0.28 (-0.11;0.67)
Overweight group	n = 536	n = 536	n = 377	n = 382	n = 380	n = 383
	0.37 (0.10;0.63)*	0.31 (0.04;0.58)†	0.32 (0.00;0.63)	0.45 (0.11;0.80)*	0.49 (0.17;0.81)*	0.76 (0.37;1.14)*

Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood cardio-metabolic risk factors in SDS per SDS change in childhood liver fat fraction or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat). *P-value <0.01, †P-value <0.05. Associations are adjusted for child's age, sex, ethnicity, maternal pre-pregnancy BMI and maternal education. HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; n, number; SDS, standard deviation scores.

Table 4 Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Cardio-metabolic Risk Factors at School Age

	Cardio-metabolic risk factors at 10 years in Standard Deviation Scores Difference (95% Confidence Interval) (n = 3,170)					
	Systolic blood pressure	**Diastolic blood pressure**	**HOMA-IR**	**Total – cholesterol**	**Triglycerides**	**C-reactive protein**
Liver fat fraction						
Confounder model	0.17 (0.14;0.21)*	0.07 (0.03;0.11)*	0.13 (0.09;0.17)*	0.11 (0.07;0.15)*	0.21 (0.17;0.25)*	0.20 (0.16;0.24)*
BMI model	0.07 (0.03;0.10)*	0.05 (0.01;0.09)*	0.07 (0.02;0.11)*	0.09 (0.04;0.13)*	0.16 (0.12;0.21)*	0.12 (0.08;0.16)*
Visceral fat model	0.07 (0.03;0.11)*	0.05 (0.00;0.09)*	0.11 (0.06;0.17)*	0.08 (0.03;0.13)*	0.13 (0.08;0.18)*	0.10 (0.05;0.15)*
Non-alcoholic Fatty Liver Disease						
Confounder model	0.66 (0.45;0.87)*	0.38 (0.17;0.59)*	0.34 (0.08;0.59)*	0.45 (0.20;0.70)*	0.67 (0.42;0.93)*	0.96 (0.71;1.21)*
BMI model	0.29 (0.09;0.49)*	0.31 (0.10;0.53)*	0.11 (-0.14;0.37)	0.36 (0.10;0.61)*	0.48 (0.23;0.74)*	0.67 (0.42;0.91)*
Visceral fat model	0.20 (-0.03;0.44)	0.25 (0.00;0.49)*	0.20 (-0.10;0.49)	0.32 (0.03;0.62)*	0.18 (-0.11;0.47)	0.59 (0.31;0.87)*

Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood cardio-metabolic risk factors in SDS per SDS change in childhood liver fat fraction or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat). *P-value <0.01, †P-value <0.05. Confounder model adjusted for child's age, sex, ethnicity, maternal pre-pregnancy BMI and maternal education. BMI model: confounder model additionally adjusted for child BMI at 10 years. Visceral fat model: confounder model additionally adjusted for visceral fat mass at 10 years. HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; n, number; SDS, standard deviation scores.

Table 5. Associations of Liver Fat Fraction with Odds of Clustering of Cardio-metabolic Risk Factors without Visceral Fat Mass – Confounder Models

Liver Fat Fraction (%)	**Clustering of Cardio-metabolic Risk Factors without taking into account Visceral Fat Mass** (n = 3,170)
< 2.0	*Reference group*
2.0 – 2.9	1.36 (1.08;1.71)*
3.0 – 3.9	2.85 (1.94;4.20)*
4.0 – 4.9	2.78 (1.50;5.14)*
≥ 5.0	6.68 (3.48;12,81)*

Values are odds ratios (95% Confidence Intervals) analyzed in a subgroup of complete cases (n = 1,906) that reflect the odds of cardio-metabolic clustering without taking into account visceral fat mass, defined as having two or more out of high (>75th percentile) systolic or diastolic blood pressure, low (<25th percentile) HDL-cholesterol or high (>75th percentile) triglycerides, and high (>75th percentile) insulin for children with increasing liver fat fraction compared to the reference group (children with <2% of liver fat). *P-value <0.01. Associations are adjusted for child age, sex, ethnicity, maternal pre-pregnancy BMI and maternal education. N, number.

Figure 1. Study Participants Flow Chart

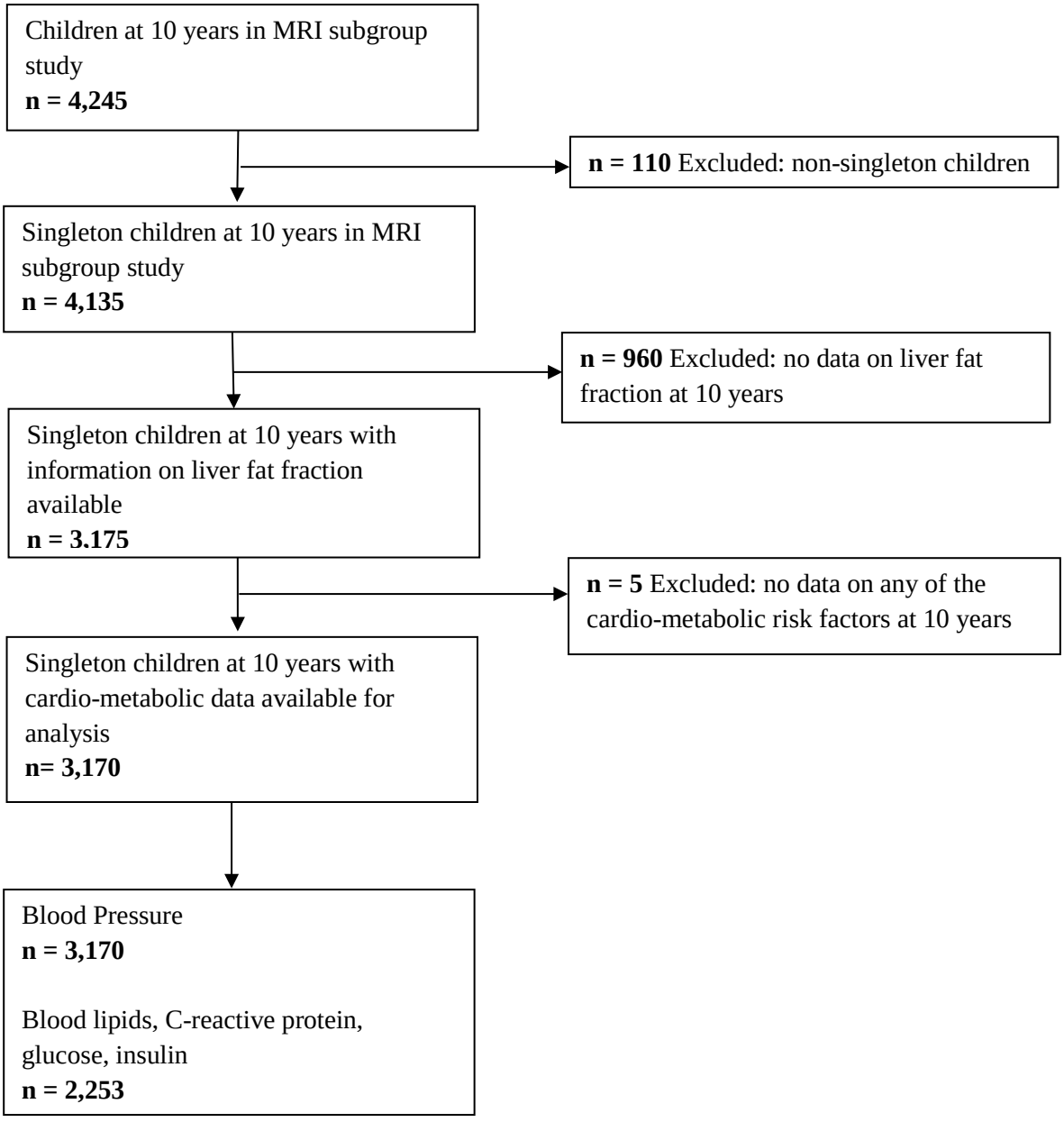
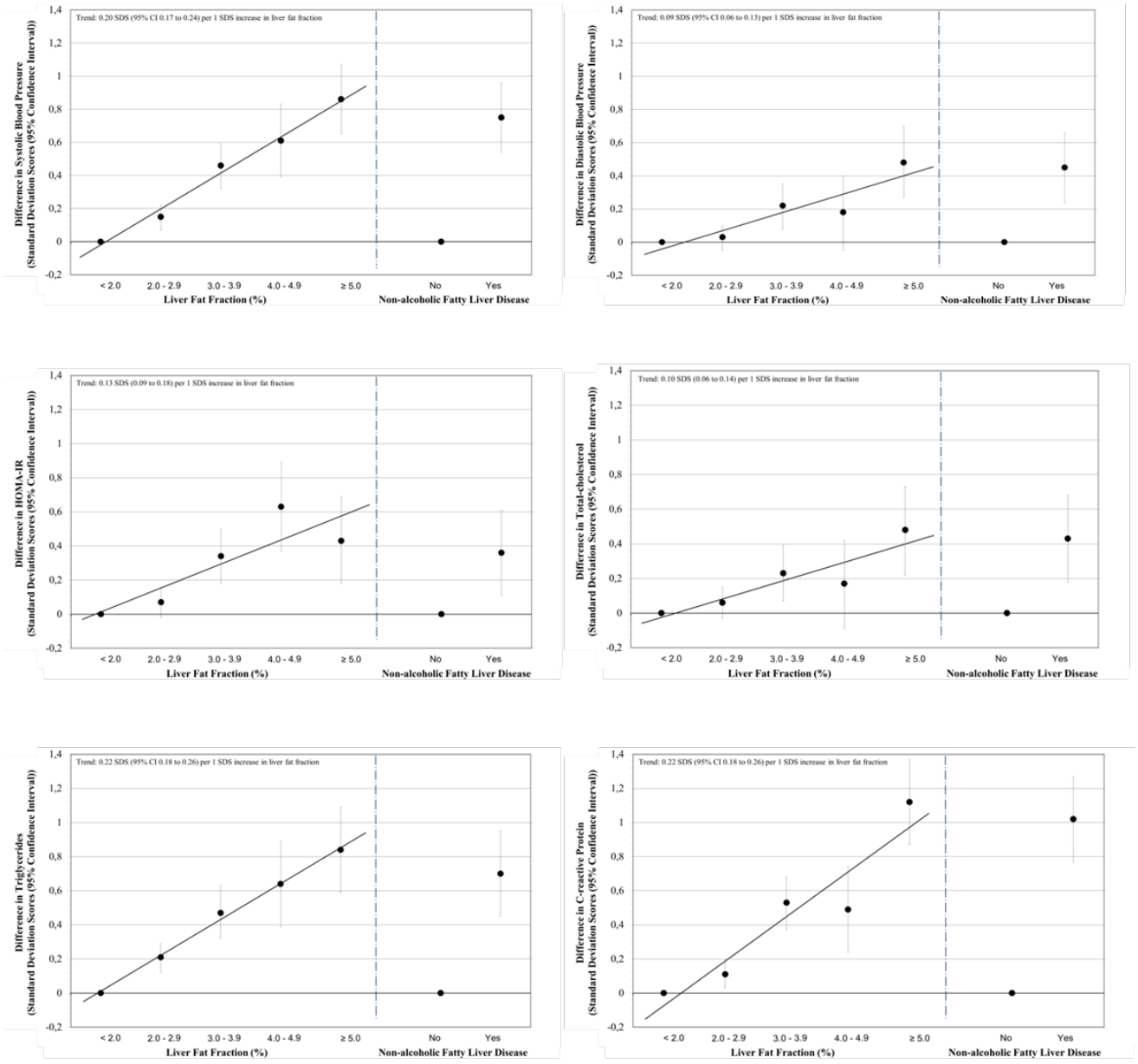
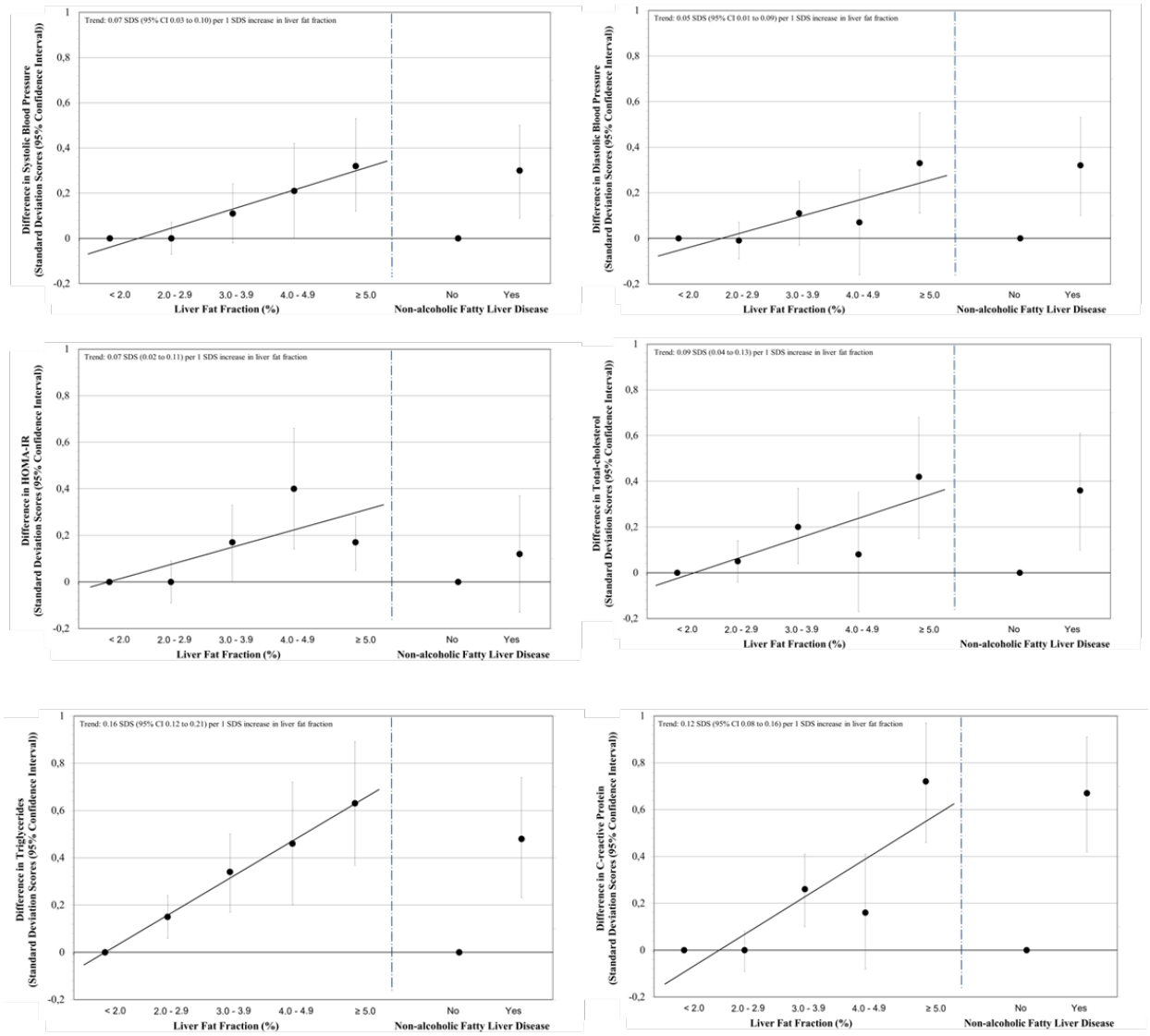


Figure 2. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Cardio-metabolic Risk Factors at School Age – Basic Models



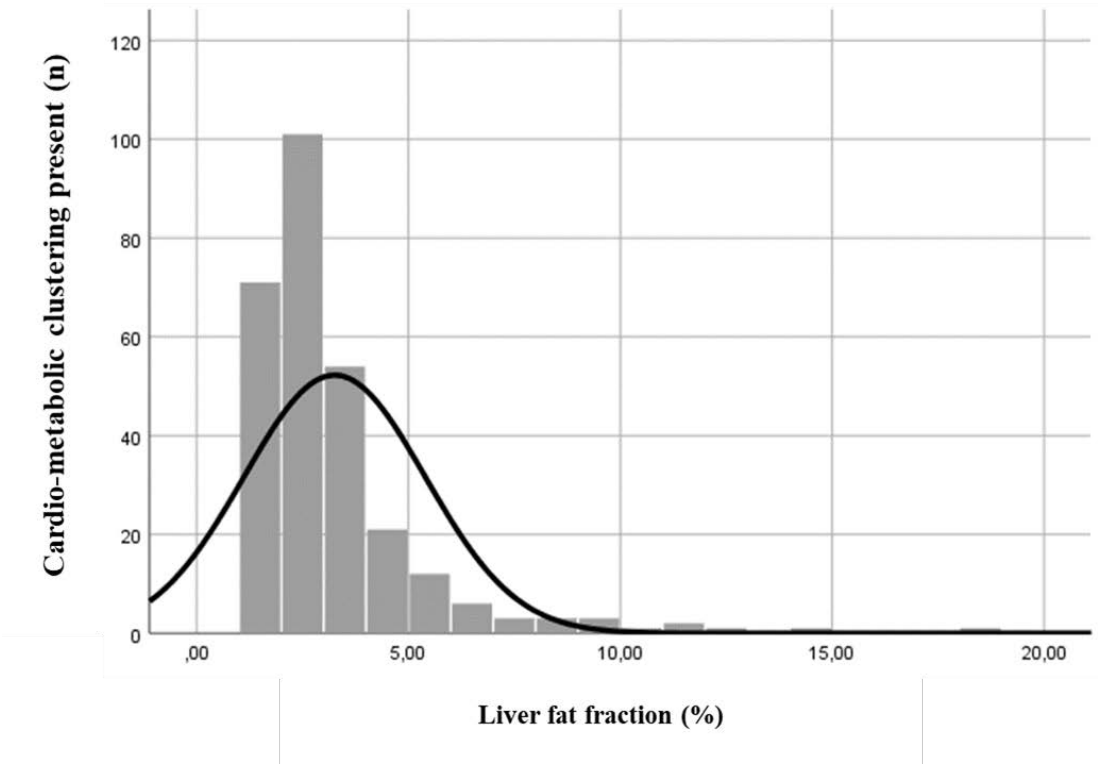
Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood cardio-metabolic risk factors in SDS per SDS change in childhood liver fat fraction as compared to the reference group (children with <2.0% of liver fat; left side of each figure), or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat; right side of each figure). Associations are adjusted for child age, sex, ethnicity. HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; SDS, standard deviation scores. Trend lines are given only when p-value for linear trend <0.05.

Figure 3. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Cardio-metabolic Risk Factors at School Age – Body Mass Index Models



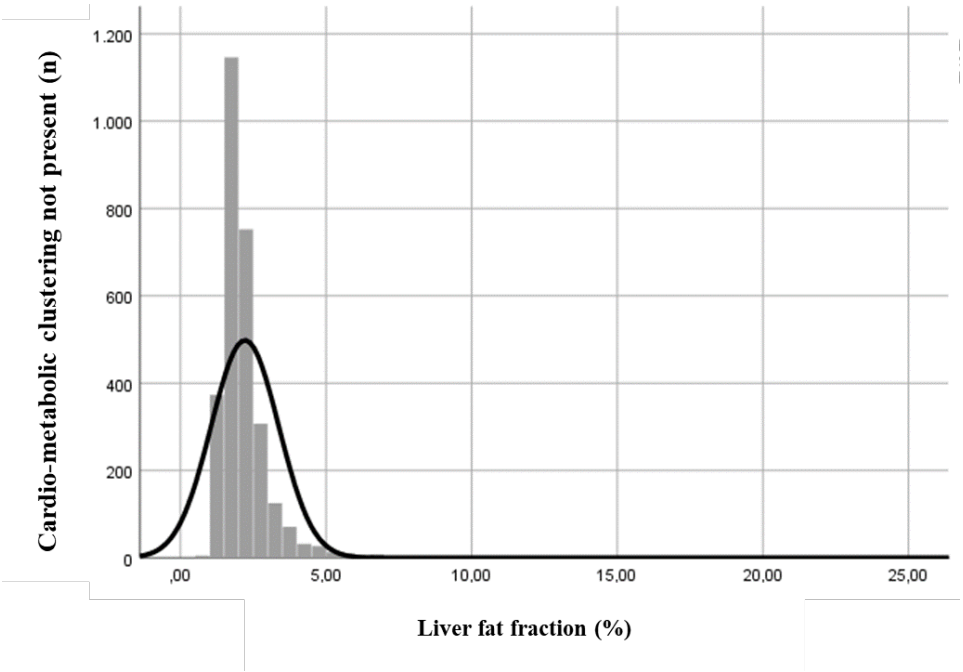
Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood cardio-metabolic risk factors in SDS per SDS change in childhood liver fat fraction as compared to the reference group (children with <2.0% of liver fat; left side of each figure), or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat; right side of each figure). Associations are adjusted for child age, sex, ethnicity, maternal pre-pregnancy BMI, maternal education and childhood BMI at ten years of age. HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; SDS, standard deviation scores. Trend lines are given only when p-value for linear trend <0.05.

Figure 4. Histogram of Liver Fat Continuously of Children with Cardio-metabolic Clustering



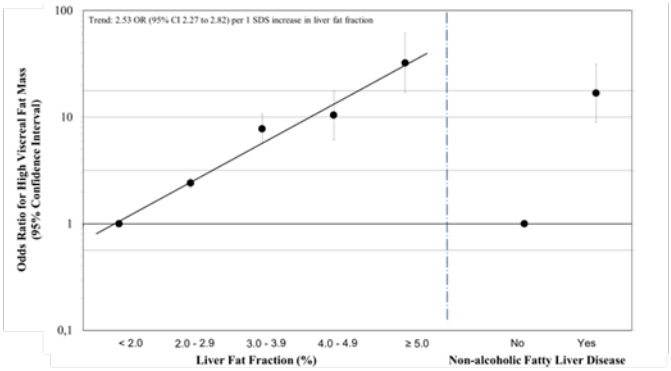
Histogram of liver fat continuously(%) for children with cardio-metabolic clustering present.

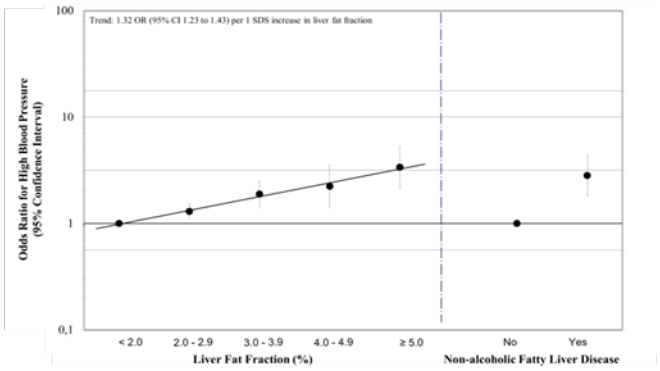
Figure 5. Histogram of Liver Fat Continuously of Children without Cardio-metabolic Clustering

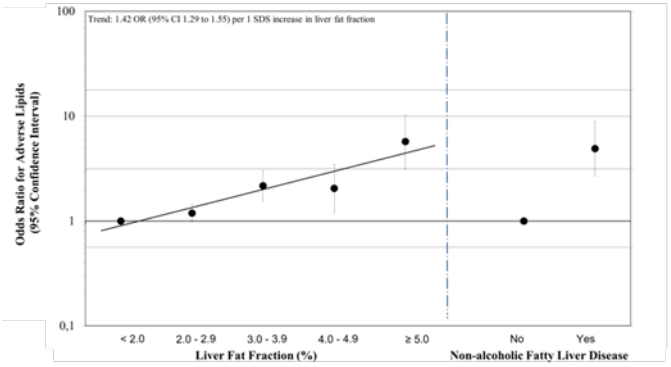


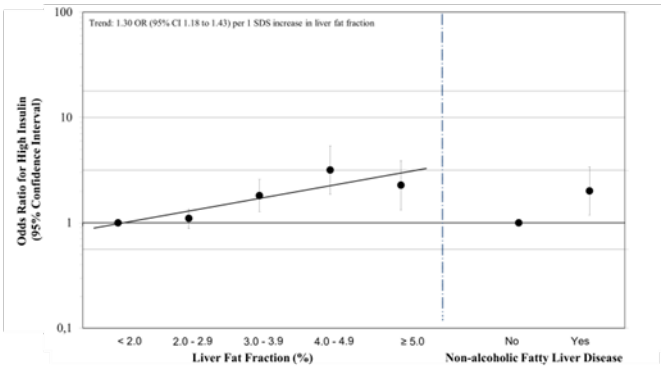
Histogram of liver fat continuously(%) for children without cardio-metabolic clustering present.

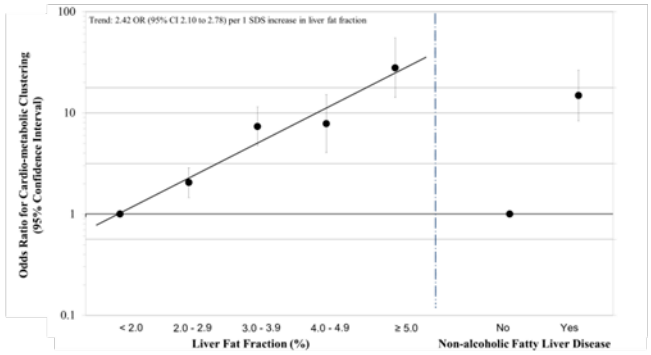
Figure 6. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Odds of Adverse Levels of Single and Clustered Cardio-metabolic Risk Factors at School Age – Basic Models





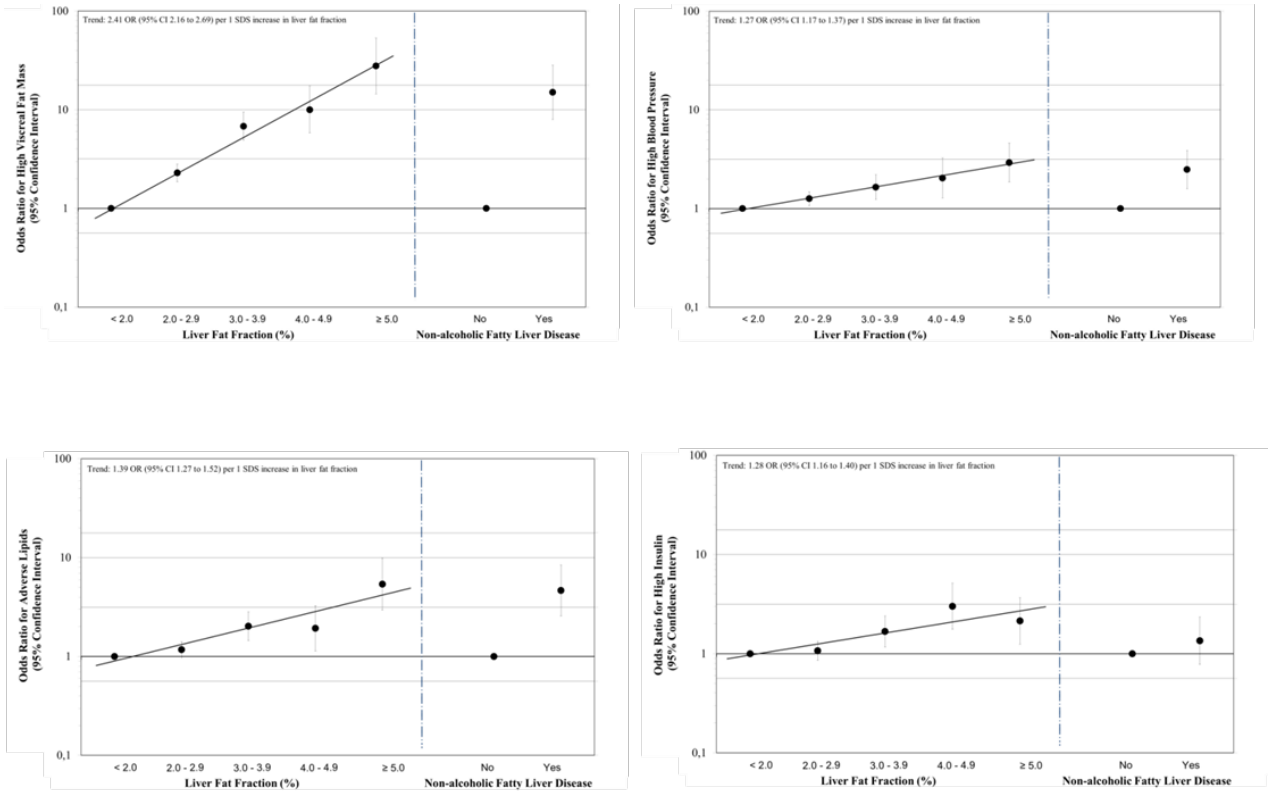






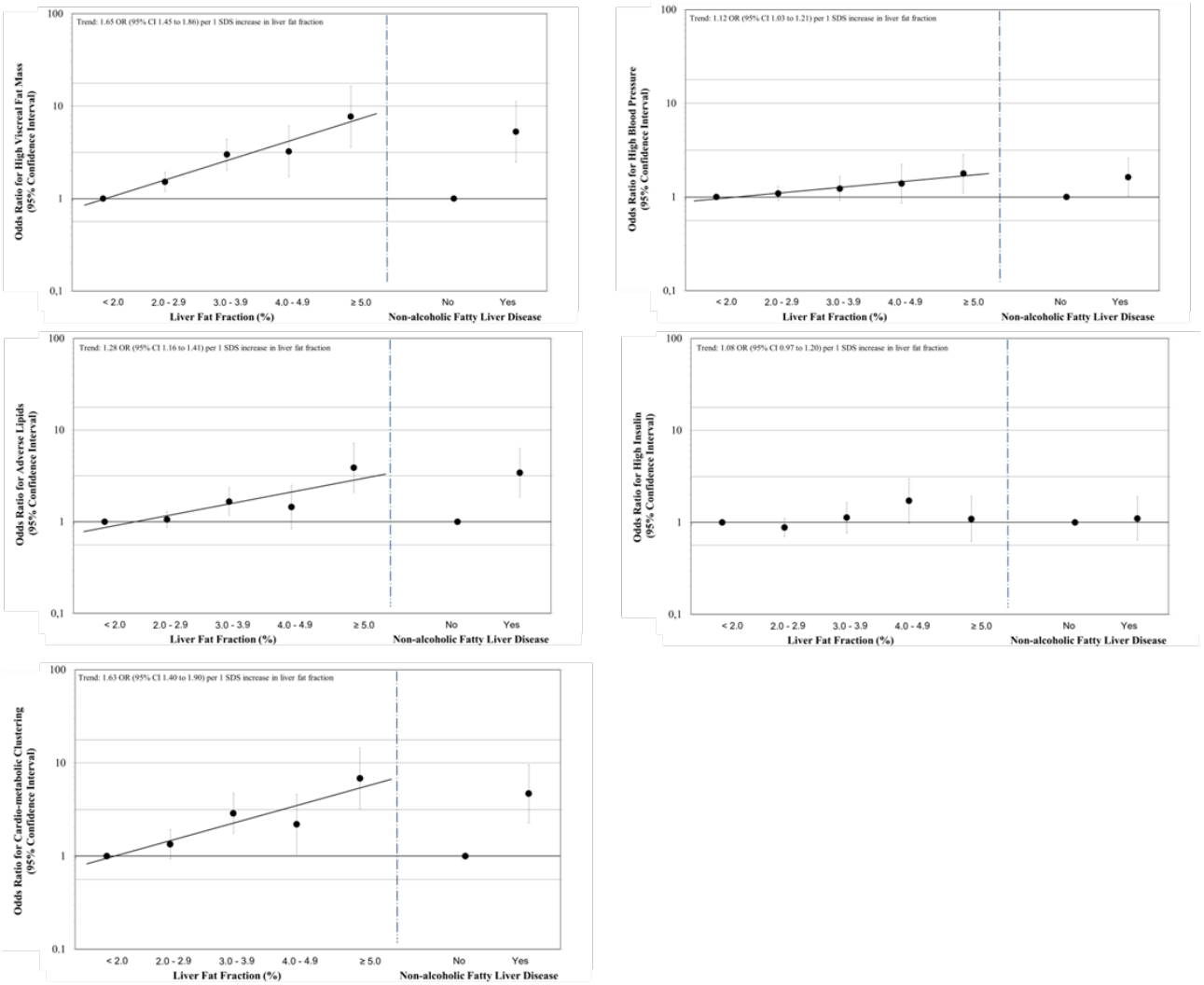
Values are odds ratios (95% Confidence Intervals) that reflect the risk of high (>75th percentile) visceral fat mass, high (>75th percentile) systolic or diastolic blood pressure, low (<25th percentile) HDL-cholesterol or high (>75th percentile) triglycerides, and high (>75th percentile) insulin and of cardio-metabolic clustering per SDS increase in liver fat fraction as compared to the reference group (<2.0%; left side of each figure), or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat; right side of each figure). Cardio-metabolic clustering was defined as having three or more of these risk factors and was analyzed in a subgroup of cases with complete data for all cardio-metabolic variables (n = 1,906). Associations are adjusted for child age, sex, ethnicity. OR, Odds Ratio; SDS, standard deviation scores. Trend lines are given only when p-value for linear trend <0.05.

Figure 7. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Odds of Cardio-metabolic Risk Factors at School Age – Confounder Models



Values are odds ratios (95% Confidence Intervals) that reflect the risk of high (>75th percentile) visceral fat mass, high (>75th percentile) systolic or diastolic blood pressure (shown as high blood pressure), low (<25th percentile) HDL-cholesterol or high (>75th percentile) triglycerides (shown as adverse lipids), and high (>75th percentile) insulin per increase in liver fat fraction as compared to the reference group (<2.0%; left side of each figure), or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat; right side of each figure). Associations are adjusted for child age, sex, ethnicity, maternal pre-pregnancy BMI and maternal education. SDS, standard deviation scores. Trend lines are given only when p-value for linear trend <0.05.

Figure 8. Associations of Liver Fat Fraction and Non-alcoholic Fatty Liver Disease with Odds of Adverse Levels of Single and Clustered Cardio-metabolic Risk Factors at School Age – Body Mass Index Models



Values are odds ratios (95% Confidence Intervals) that reflect the risk of high (>75th percentile) visceral fat mass, high (>75th percentile) systolic or diastolic blood pressure (shown as high blood pressure), low (<25th percentile) HDL-cholesterol or high (>75th percentile) triglycerides (shown as adverse lipids), and high (>75th percentile) insulin and of cardio-metabolic clustering per increase in liver fat fraction as compared to the reference group (<2.0%; left side of each figure), or for children with non-alcoholic fatty liver disease as compared to the reference group (children with <5% of liver fat; right side of the figure). Cardio-metabolic clustering was defined as having three or more of these risk factors and was analyzed in a subgroup of cases with complete data for all cardio-metabolic variables (n = 1,906). Associations are adjusted for child age, sex, ethnicity in the basic models, further adjusted for maternal pre-pregnancy BMI and maternal education in the confounder models and additionally adjusted for childhood BMI at ten years of age in the BMI model. OR, Odds Ratio; SDS, standard deviation scores. Trend lines are given only when p-value for linear trend <0.05.