Longitudinal analysis of risk of Non-Alcoholic Fatty Liver Disease (NAFLD) in adulthood.

Daniel J Cuthbertson,[1] Emily Brown,[1] Juha Koskinen,[2,3] Costan G. Magnussen,[2,4] Nina Hutri-Kähönen[5] Matthew Sabin[6,7] Päivi Tossavainen[8] Eero Jokinen[9] Tomi Laitinen[10] Jorma Viikari[11,12] Olli T Raitakari[2] Markus Juonala[6,11,12]

[1]Obesity and Endocrinology Research Group, Institute of Ageing and Chronic Disease, University of Liverpool, UK,
[2]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Finland
[3]Heart Center, Kymenlaakson keskussairaala, Kotka, Finland
[4]Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia
[5]Department of Pediatrics, University of Tampere and Tampere University Hospital, Tampere, Finland
[6]Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[7]The Royal Children's Hospital and University of Melbourne, Melbourne, Victoria, Australia
[8]Department of Pediatrics, PEDEGO Research Unit and Medical Research Center, Oulu University Hospital and University of Oulu, Oulu, Finland.
[9]Department of Pediatric Cardiology, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland.
[10]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland.
[11]Department of Medicine, University of Turku, Turku, Finland.
[12]Division of Medicine, Turku University Hospital, Turku, Finland.

Corresponding author & address for reprints: **Dr Daniel Cuthbertson**

Clinical Sciences Centre, Aintree University Hospital, Liverpool, L9 7AL

E-mail: daniel.cuthbertson@liverpool.ac.uk

Tel 0151 529 5911,

Fax 0151 529 5888

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase

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Abstract

Background & Aims We aimed to determine how childhood body mass index (BMI) and metabolic health, along with change in BMI between childhood and adulthood, determine the risk of adult non-alcoholic fatty liver disease (NAFLD).

Methods Data from 2,020 participants aged 3-18 years at baseline, followed up 31 years later, was examined to assess the utility of four childhood metabolic phenotypes (metabolic groups I: normal BMI, no metabolic disturbances; II: normal BMI, one or more metabolic disturbances; III: overweight/obese, no metabolic disturbances; IV: overweight/obese, one or more metabolic disturbances) and four life-course adiposity phenotypes (adiposity group 1: normal child and adult BMI; 2, high child, normal adult BMI; 3, normal child BMI, high adult BMI; 4, high child and adult BMI) in predicting adult NAFLD.

Results The risk for adult NAFLD was similar across all four groups after adjustment for age, sex, lifestyle factors and adult BMI. Risk of adult NAFLD was not increased among individuals overweight/obese in childhood but non-obese in adulthood. In contrast, overweight or obese adults, irrespective of their youth BMI status, had ~8-10 fold increased risk ($P<0.001$).

Conclusion Childhood overweight/obesity, not metabolic health, is associated with increased risk for adult non-alcoholic fatty liver disease. However, the increased risk associated with childhood overweight/obesity can be largely removed by obtaining a normal body mass index by adulthood.

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Keywords Non-alcoholic fatty liver disease, metabolic health, obesity, risk

Lay Summary

Non-alcoholic fatty liver disease or '*fatty liver*' is usually seen in people who are overweight/obese and is associated with other metabolic complications (high blood pressure, high cholesterol) and long-term health problems. Using data from the Cardiovascular Risk in Young Finns study we assessed how body weight (lean/overweight/obese) or having metabolic complications during childhood increases the risk of developing fatty liver in adulthood. Obesity, rather than metabolic complications alone, is the major risk factor for developing fatty liver. However, individuals who were overweight/obese during childhood but became lean as adults do not have a higher risk of fatty liver in adulthood.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in Western society with a recent meta-analysis of 8.5 million people from 22 countries suggesting a global prevalence of NAFLD of 25.2.[1] The prevalence of NAFLD is even higher in selected populations, such as individuals with components of the metabolic syndrome and type 2 diabetes.[2,3] Conversely, in patients diagnosed with NAFLD, obesity, type 2 diabetes and metabolic syndrome co-exist in 51%, 22.5% and 42.5% of cases.[1] Thus, NAFLD has been described as the hepatic manifestation of the metabolic syndrome.[4]

Obesity is the major risk factor for NAFLD. However, while many overweight or obese individuals have evidence of metabolic complications (hypertension, dyslipidaemia, insulin resistance), a proportion may not have such complications *i.e.* are metabolically healthy.[5] In the UK BioBank cohort, in which liver fat was characterised by magnetic resonance spectroscopy in 4,989 individuals, the proportion of overweight/obese individuals with normal liver fat was two-fold higher than that of overweight/obese individuals with higher liver fat (42.9 *vs.* 18.1%).[6] Liver fat tends to be associated with poor metabolic health such that metabolically unhealthy individuals, whether lean or obese, tend to have greater liver fat.[5,7]

Although the concept of metabolically healthy obesity is contentious, a number of studies have examined the association of metabolically healthy obesity with incident heart failure, cardiovascular disease and type 2 diabetes with conflicting results.[8-13] Few studies have examined the association between metabolic health status and obesity with incident NAFLD.[14-16] These analyses have been performed among adult populations. Thus, whether it is childhood weight status, or the associated metabolic phenotype, that increases the risk of NAFLD remains unclear.

The main aim of this study was to assess the relative ability of childhood (age 3-18 years) weight status (normal, overweight or obese) and presence or absence of metabolic complications (i.e. being

metabolically healthy *vs*. unhealthy), in a large, community-based cohort of young adults, to determine the relative risk of developing NAFLD in adulthood. In addition, we aimed to evaluate the combined effects of child and adult weight status to assess whether the influence of child overweight or obesity is reversible.

Patients and Methods

Participants and Study Design

The Cardiovascular Risk in Young Finns Study is an ongoing multicentre study examining precursors of atherosclerosis in children and adolescents. It was first launched in 1980 when 3,596 participants, aged between 3-18 (3, 6, 9, 12, 15 and 18) years, were randomly selected from the national register, across Finland, to produce a representative sample of Finnish children. Thereafter, follow-up studies have been conducted regularly in 3-year interval until 1992. The most recent 31-year follow-up (2011) among 2,042 participants included clinical examination, blood samples, questionnaires and liver ultrasound examinations.

The sample for the present analysis included those who had participated in the 1980 survey (baseline assessment) and who had liver ultrasound examinations performed in 2011. We have previously shown that participants who attended follow-up studies shared characteristics similar to those who attended at baseline.[17,18] We included in our analysis the following baseline characteristics: age, gender, BMI, blood pressure, glucose, high-density lipoprotein (HDL)-cholesterol and triglycerides concentrations. For the follow-up in 2011, we included age, gender, BMI, blood pressure, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), HDL-cholesterol and triglycerides concentrations. In addition, we included data on alcohol use, diet, physical activity and liver ultrasound results. During the follow up period there have been 110 deaths recorded: 36 deaths from endogenous causes and 74 deaths from external causes (Supplementary Figure 1). The study was approved by local ethics committees of Turku, Tampere, Oulu, Kuopio and Helsinki, and written informed consent was obtained from all of the study participants or their parents if they were under 18 years of age.

Clinical Measurements

In childhood and adulthood, height and weight were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Childhood BMI was adjusted

for age and sex by calculating a BMI-SD score. Blood pressure was measured from the brachial artery with a standard sphygmomanometer with cuff size chosen according to arm circumference.

Questionnaires were used to obtain data on smoking and physical activity. The physical activity index was calculated as previously described.[19] Adulthood alcohol consumption data were acquired by standardised questionnaires. Alcohol intake was defined as consumed alcohol units per week (16 g of alcohol). Information on dietary habits was obtained with a detailed food frequency questionnaire providing an estimate of food consumption in grams/day.

Data collected from national hospital discharge registries confirmed that none of the participants had viral or auto-immune liver disease.

Biochemical Measurements

Venous blood samples were drawn after an overnight fast for determination of serum lipid levels, insulin and CRP. Serum insulin was measured with immunoassay. All lipid and lipoprotein determinations were performed on serum using standard methods, as described previously.[18,20] Low density lipoprotein cholesterol concentration was calculated by the Friedewald formula in participants with triglycerides <4.0 mmol/l.

Genetic Analysis

In the present study, we used the SNPs rs738409, near the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene and rs58542926, near the transmembrane 6 superfamily member 2 (*TM6SF2)* gene associated with fatty liver in previous genome-wide association analyses as the genetic marker for susceptibility for fatty liver, as previously described.[21] Genotyping was performed with the custom-built Illumina BeadChip 670K.

Ultrasound Measurements

Presence of hepatic steatosis NAFLD was determined by ultrasound imaging of the liver using a validated protocol and Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA)

with 4.0 MHz adult abdominal transducers.[22] The evaluation of fatty liver was done by one ultrasonographer, and the clinical diagnosis (fatty liver or normal liver) was based on visual assessment of the hepatic-renal ratio, liver parenchymal brightness and the visibility of vessel walls and the diaphragm.[21]

Classification of Metabolic Disturbances, Metabolic Syndrome and Overweight at Youth

In the absence of diagnostic criteria for paediatric metabolic abnormalities, we generated age- and sex-specific percentiles of systolic and diastolic blood pressures, HDL-cholesterol, triglyceride, and glucose levels from prevalence data from the National Cholesterol Education Program, Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents as previously described.[22] Participants were categorised as having a metabolic disturbance in youth if he/she had any of the following: systolic blood pressure $\geq 90^{th}$ percentile and/or diastolic blood pressure $\geq 90^{th}$ percentile, insulin $\geq 90^{th}$ percentile, triglycerides $\geq 90^{th}$ percentile, or HDL-cholesterol $\leq 10^{th}$ percentile.[22]

Participants were categorised into four metabolic groups on the basis of i) ***youth BMI category*** using age- and sex-specific international BMI centiles to extrapolate cut-points at ages 3 to 18 that correspond to adult BMI of 25 kg/m^2 (overweight) and 30 kg/m^2 (obese)[23] and ii) ***metabolic status***. *Metabolic Group I,* the reference group, included participants with normal BMI and no metabolic disturbances *i.e.* metabolically healthy; *Metabolic Group II* included participants with normal BMI, but who had one or more metabolic disturbances, *i.e.* metabolically unhealthy; *Metabolic Group* III included participants who were overweight/obese with no metabolic disturbances, *i.e.* metabolically healthy; and *Metabolic Group* IV included participants who were overweight/obese, with one or more metabolic disturbances *i.e.* metabolically unhealthy.

Classification of Adiposity Groups

Participants were grouped into four separate adiposity groups as previously described[24] according to their BMI in childhood and as adults. For the definitions of childhood overweight and obesity, we

used age-specific and sex-specific international BMI percentiles to extrapolate cut-off points for those aged 3 to 18 years that correspond to adult BMIs of 25 (overweight) and 30 kg/m^2 (obese).[23] The groupings were as follows: *Adiposity Group 1*, participants with a normal BMI in childhood and normal BMI as adults; *Adiposity Group 2*, participants who were overweight or obese in childhood but had a normal BMI as adults; *Adiposity Group 3*, participants with a normal BMI in childhood who were overweight as adults and *Adiposity Group 4*, participants who were overweight or obese in childhood and overweight as adults.

Statistical Methods

Statistical analyses were performed with SAS 9.4. Statistical significance was inferred as a P value of less than 0.05. The normality assumptions of the residuals were assessed by examining histograms of the residuals and normal probability plots. The residuals were normally distributed. Values for plasma triglycerides and alcohol consumption were log transformed to correct for skewness. Baseline and clinical characteristics across each of the metabolic/BMI groups are presented as mean±SD for continuous variables or percent for categorical variables. ANOVA was used to compare characteristics among the study groups. Age and sex adjusted logistic regression model was used to calculate odds ratios and 95% confidence intervals (model 1 in the text). Further adjustments were done by including adult lifestyle habits (model 2 in the text) and adult BMI (model 3 in the text) as covariates in the models.

Thereafter additional adjustments for adulthood alcohol consumption (portions per week), smoking (yes/no) and physical activity, and finally also for adulthood BMI were performed.

Results

Participant Characteristics

Participant characteristics are provided in Table 1, sub-divided into four metabolic groups according to their baseline BMI (normal *vs*. overweight/obese) and the number of associated metabolic risk factors (none *vs*. ≥1). The proportion of participants with NAFLD in adulthood for each group was Metabolic Group I: 16.9%, Group II: 20.4%, Group III: 26.6%, and Group IV: 40.5%. We observed no difference in BMI change from childhood to adulthood between Group I and IV. Details of BMI change measured from two time points has been presented in the online supplement.

Association of Youth BMI Status and Metabolic Health on Adult NAFLD

The risk of adult NAFLD compared according to their youth phenotype is shown in Table 2. The odds ratios of NAFLD were significantly greater in overweight/obese participants (Metabolic group III and IV) compared with normal BMI participants (Metabolic group I), with the highest odds ratio among the metabolically unhealthy, overweight/obese group (Metabolic group IV). These differences were evident after adjustment for adult alcohol consumption (portions per week), smoking (yes/no) and physical activity (Table 2, Model 2). However, when adjusted for adult BMI, the odds ratios between all four groups were not significantly different from the reference group (Table 2, Model 3). Age-stratified analyses (Supplementary Table 1) were largely consistent with those shown in Table 2. We also compared those with normal BMI but metabolic disturbances (Metabolic group II) with those who were overweight or obese but metabolically healthy (Metabolic group III). No significant differences between the groups were observed (age and sex adjusted OR 1.53 95 % CI 0.90-2.62, P=0.11). When comparing participants with normal BMI but metabolic disturbances (Metabolic group II) with those who were overweight or obese and had metabolic disturbances, we observed a significantly higher odds ratio for adult NAFLD between the groups (age and sex adjusted OR 2.54 95 % CI 1.51-4.29, P=0.0005). However, this association was not observed after additional adjustment for adulthood BMI (P > 0.10).

There were no significant differences in the odds ratios for adult NAFLD when those overweight or obese but metabolically healthy youth (metabolic group III) were compared with those who were overweight or obese and had metabolic disturbances (metabolic group IV) (Supplementary Figure 1, lower).

Association of Child Metabolic Syndrome on Risk of Adult NAFLD

We examined the effect of child metabolic syndrome status at baseline on the subsequent risk of developing NAFLD in adulthood. The odds ratio of subsequent NAFLD was higher in those with childhood metabolic syndrome versus those with no childhood metabolic syndrome, even after adjustment for age and sex (Table 3, Model 1), adulthood alcohol consumption, smoking and physical activity (Table 3, Model 2). However, after further adjustment for adult BMI, there was no difference in risk of NAFLD between those with or without child metabolic syndrome (Table 3, Model 3).

Association of BMI Transition from Childhood to Adulthood on Risk of Adult NAFLD

When using a BMI cut-off of 25 kg/m^2, participants who were overweight/obese as adults (adiposity groups 3 and 4) had approximately 8-fold increased odds ratio of developing NAFLD, compared to the normal BMI group (group 1), regardless of childhood adiposity status (Table 4A). Contrary, those who were not overweight/obese in adulthood (group 2) had similar odds ratio for NAFLD compared to the normal BMI group (group 1). If a BMI cut-off of 30 kg/m^2 was applied, similar results were found: participants who were obese (adiposity groups 3 and 4) had an 8-10 fold increased odds of developing NAFLD, compared with the normal BMI group (group 1), regardless of childhood adiposity status (Table 4B). Contrary, those who were not obese as adults (group 2) had similar risk for NAFLD compared with the normal BMI group (group 1).

Discussion

The results of this study demonstrate that obesity status in childhood, independent of metabolic health, is predictive of NAFLD, suggesting measurement of body mass index, rather than that of associated metabolic risk factors, provides sufficient information for risk assessment of NAFLD. With the strong tracking of BMI from childhood to adulthood, obesity seems to be the major factor in the development of adulthood NAFLD although reversal of childhood overweight/obesity status in adulthood was not associated with an increased risk of NAFLD suggesting childhood BMI as a modifiable risk factor for NAFLD.

We have previously examined individual childhood predictors of adult fatty liver using the Young Finns Study cohort and demonstrated being small for gestational age, high serum insulin, high BMI and genetic variants in PNPLA3 and TM6SF2 as being important risk factors.[21] We have also demonstrated that metabolic aberrations appear to precede the development of fatty liver in young adults, up to 10 years before diagnosis. The most striking metabolic abnormalities related to high concentrations of triglyceride-related lipid measures and branched chain amino acids.[25] The latest findings further extend our understanding of the contributory factors for development of NAFLD in adulthood.

The findings of the present study provide important novel information concerning the combined effects of childhood obesity and metabolic risk factors on adult NAFLD. We evaluated the effects of metabolically healthy *vs.* unhealthy obesity and explored whether the effects of childhood obesity and metabolic factors are mediated by adult obesity. These data strongly support the premise that it is obesity rather than metabolic health as the main driver for NAFLD. Initial assessments of the relative risk of NAFLD according to the four different metabolic groups, suggested metabolic phenotype was relevant, with the greatest odds ratio in the metabolically unhealthy obese, even after adjustment for age, sex, alcohol, smoking and physical activity. However, after adjustment for adulthood BMI any differences in relative risk were no longer evident. Consistent with these data,

the presence or absence of childhood metabolic syndrome did not influence the relative risk of NAFLD when adulthood BMI status was considered. In contrast, those who were overweight/obese (whether a BMI cut-off of 25 or 30 kg/m^2 was adopted) in adulthood had a significantly greater increased risk of NAFLD, compared to the normal BMI population, regardless of childhood adiposity status. The relative risk was greater in overweight/obese individuals who had been overweight/obese as children than in those individuals who had been of normal BMI in childhood.

Significantly, the risk of NAFLD among overweight or obese children, who became non-obese by adulthood, was similar to those who were never obese, and this finding is consistent with our previous findings for the risks of other cardio-metabolic conditions.[24] We have previously shown similar risks of type 2 diabetes, hypertension, dyslipidaemia and carotid-artery atherosclerosis in overweight or obese children who became non-obese by adulthood. Many studies examining obesity and its associated risks, examine the dose-response relationship between BMI and hazard ratios/incidence rates of the complications overlooking the impact of the duration of obesity. Duration of obesity is an independent risk factor for obesity-related complications[26] and data from the Framingham Heart Study reveal duration of obesity to also be a relevant risk factor for NAFLD with an incremental risk for every additional year of obesity. In this study, although we were unable to examine the duration of obesity in years for any given individual, we were able to demonstrate that children who were overweight or obese in childhood and who were of normal BMI when re-examined in adulthood were not more likely to have NAFLD.

There have been several studies performed to date among adult populations that have attempted to address the same question as the one posed in this study. Sung *et al*. undertook a cross-sectional assessment of 14,384 South Koreans and found that metabolically healthy obese individuals were at risk of fatty liver but attenuated risk of pre-clinical atherosclerosis, although this study did not address the relative contributions of weight versus metabolic factors in development of NAFLD.[14] Chang *et al*. examined 77,425 individuals, who were free of NAFLD or associated metabolic abnormalities at

baseline, and re-examined them after 4.5 years of follow up, found an adjusted hazards ratio of 2.15 (2.06-2.26) and 3.55 (3.37-3.74) for developing NAFLD in overweight and obese respectively, compared with normal BMI, individuals.[15] The association between BMI and hazard ratio of NAFLD was progressive across the whole range of BMI values suggesting that it is obesity *per se*, independent of co-existing insulin resistance or metabolic abnormalities, that increases the risk of NAFLD. In contrast, Lee *et al.*, examined 3,045 individuals without NAFLD and diabetes at baseline and re-evaluated them after 4 years. Their findings of a lower risk of NAFLD in metabolically healthy obese *versus* metabolically unhealthy non-obese led to the conclusion that metabolic health is more important than obesity in the development of NAFLD.[16] However, contrary to the present analyses, their study cohort consisted of adults (mean age 44 years).

The strengths of this study are the large, randomly selected cohort, who have undergone careful metabolic characterisation, have been followed up after a significant interval of 31 years and with their initial assessment occurring in childhood/early adulthood. The availability of accurate information on lifestyle factors, particularly alcohol intake and physical activity patterns that would otherwise confound analysis is also advantageous.

We must acknowledge certain limitations to this study. First, we did not have data on liver enzymes measured in childhood. Secondly, because our study cohort was a homogeneous ethnic group, the generalisability of our results is limited to Caucasians. Thirdly, the loss of original participants during the long-term follow-up is also recognised. Non-participants were younger and more often male in childhood than participants. Therefore, the rates of adult fatty liver in our cohort might be an underestimation of the real rates. However, a detailed analysis of participants who dropped out was performed after the 21-year follow-up study in 2001 (n=2283) and the findings suggest the present study cohort is representative of the original study population.[18] The application of ultrasonography to detect incident fatty liver will underestimate the true prevalence of NAFLD. Although ultrasound has been validated against the gold standard method of liver fat quantification of magnetic resonance

spectroscopy, it is generally accepted it will detect only moderate or severe steatosis and mild steatosis will not be detectable.[27] Large scale epidemiological studies have applied these gold-standard measures but with obvious implications of cost, feasibility and resource availability.[28,29] Furthermore, no information is available from liver ultrasound on the staging (steatosis, steatohepatitis or fibrosis etc.) within the NAFLD spectrum. Future research directions for this cohort will be to apply non-invasive biomarkers of liver fibrosis (e.g. using the Fib 4 or NAFLD fibrosis score) or even further imaging (e.g. using elastography) to assess for fibrosis.[30] Concerning the changes in BMI status between child and adult measurements, due to lack of comprehensive serial data, we were unable to examine in detail the timing of BMI normalisation. We observed that the influence of child overweight or obesity was reversible with respect to NAFLD. However, it is possible to have different associations in certain genetic groups based on e.g. PNPLA3 genotype. Within our cohort the number of subjects in several PNPLA3/adiposity subgroups did not allow us to conduct properly powered analyses to investigate this.

In conclusion, we demonstrate that obesity, and not the presence of obesity-related metabolic abnormalities, is the overwhelming risk factor for the development of NAFLD in adulthood. The risks are greatest if obesity has been evident since childhood but can be minimised, with risks of NAFLD returning to baseline, if childhood obesity is addressed and the individual returns back to normal BMI in adulthood. Weight loss, through lifestyle intervention, must remain the therapeutic priority for prevention of development and progression of NAFLD.

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Table 1 Clinical characteristics of participants at baseline (1980) and follow up (2011).

Metabolic Group	**Metabolic Group I (Reference)** (Normal BMI, no metabolic factors)	**Metabolic Group II** (Normal BMI, ≥1 metabolic factor)	**Metabolic Group III** (Overweight/obese, no metabolic factors)	**Metabolic Group IV** (Overweight/obese, ≥1 metabolic factor)	P-value*
Baseline data 1980	N=1455	N=402	N=79	N=84	
Age (years)	10.7±5.1	11.5±4.7	10.3±4.4	12.1±4.1	0.005
Sex (male)	46%	44%	44%	51%	0.68
BMI (kg/m^2)	17.4±2.6	18.0±2.7	21.9±3.2	23.7±3.9	<0.0001
Systolic blood pressure (mmHg)	111±11	116±12	115±10	122±10	<0.0001
Diastolic blood pressure (mmHg)	68±9	69±10	71±8	73±9	<0.0001
Triglycerides (mmol/l)	0.58±0.20	0.88±0.40	0.61±0.22	1.12±0.51	<0.0001
HDL-cholesterol (mmol/l)	1.62±0.29	1.38±0.31	1.61±0.27	1.35±0.33	<0.0001
Insulin (IU/L) (LOGe corrected values	6.5±2.9	10.7±1.9	8.9±1.7	15.6±1.8	<0.0001
Genetic data					
Variant in PNPLA3 (CC/CG/GG)%	59/36/5	58/36/6	63/36/1	53/41/6	0.57
Variant in TM6SF2 (CC/CT/TT)%	89/11/0	89/11/0	83/17/0	81/18/1	0.03

Follow-up data 2011					
NAFLD (%)	17	20	27	40	<0.0001
BMI (kg/m^2)	25.8±4.7	26.8±4.7	32.1±6.9	32.1±6.79	<0.0001
Physical activity index	9.1±1.9	9.0±1.9	8.7±2.2	9.0±2.0	0.28
Serum ALT (U/L)	17.9±21.1	18.1±13.9	18.7±13.9	19.7±12.2	0.45
Triglycerides (mmol/l)	1.27±1.20	1.5±1.14	1.27±0.65	1.59±0.96	<0.0001
HDL-cholesterol (mmol/l)	1.35±0.33	1.22±0.31	1.31±0.30	1.21±0.34	<0.0001
Insulin (IU/L)	6.6±2.1	6.7±2.1	7.9±2.3	12.0±3.1	<0.0001
Alcohol consumption (dose/day)	0.82 (0.05)	0.82 (0.06)	0.94 (0.18)	0.66 (0.16)	0.64
Meat consumption (g/day)	141±85	146±84	149±80	131±69	0.42
Fruit consumption (g/day)	160±146	174±84	139±110	190±149	0.25
Fish consumption (g/day)	49±39	53±45	47±29	51±42	0.37
Vegetable consumption (g/day)	165±129	169±133	142±98	234±208	0.06
Smoking (%)	14	14	25	18	0.05

* P-values from comparing across study groups

Table 2 Odds ratios (OR) and 95% confidence intervals (CI) of adult NAFLD according to youth metabolic phenotype.

			Model 1		**Model 2**		**Model 3**	
Metabolic Group	**n/N**	**%**	**OR (95 % CI) (adjusted*)**	**P-value (adjusted*)**	**OR (95 % CI) (adjusted†)**	**P-value (adjusted†)**	**OR (95 % CI) (adjusted‡)**	**P-value (adjusted‡)**
Metabolic Group I	246/1,455	17	Reference		Reference		Reference	
Metabolic Group II	82/402	20	1.23 (0.92-1.64)	0.16	1.35 (0.98-1.86)	0.062	1.06 (0.66-2.17)	0.75
Metabolic Group III	21/79	27	1.95 (1.13-3.34)	0.016	2.22 (1.26-3.96)	0.0031	0.60 (0.31-1.16)	0.13
Metabolic Group IV	34/84	40	3.13 (1.94-5.06)	<0.0001	3.62 (2.15-6.09)	<0.0001	1.19 (0.66-2.16)	0.56

* Adjusted for age and sex

† Further adjusted for adulthood alcohol consumption (portions per week), smoking (yes/no) and physical activity

‡ Further adjusted for adulthood BMI

Metabolic Group I = Reference (normal BMI, no metabolic disturbances), Group II (normal BMI, ≥ 1 metabolic disturbances), Group III (overweight/obese, no metabolic disturbances), Group IV (overweight/obese, ≥ 1 metabolic disturbances).

Metabolic disturbances (≥90^{th} % systolic blood pressure and diastolic pressure, ≥90^{th} % insulin ≥90^{th} %, triglycerides, ≤ 10^{th} percentile HDL-cholesterol)

n/N number of cases/total number of participants within the group

OR (95 % CI), Odds ratios 95% confidence intervals

Table 3 Odds ratios (OR) and 95% confidence intervals (CI) for adult NAFLD according to child metabolic syndrome (MetS) status

		Model 1		Model 2		Model 3	
Metabolic syndrome (1980)	**n/N**	**OR (95 % CI adjusted*)**	**P-value (adjusted*)**	**OR (95 % CI adjusted†)**	**P-value (adjusted†)**	**OR (95 % CI adjusted‡)**	**P-value (adjusted‡)**
No	271/1,534	**Reference**					
Yes	112/486	1.48 (1.14-1.92)	0.0031	1.57 (1.17-2.08)	0.0019	0.89 (0.64-1.24)	0.50

* Adjusted for age and sex

† Further adjusted for adulthood alcohol consumption (portions per week), smoking (yes/no) and physical activity

‡ Further adjusted for adulthood BMI

n=NAFLD cases in adulthood

N=Number of participants according to MetS status

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) of adult NAFLD according to adiposity group in youth and adulthood comparing **A) normal BMI** *vs.* **overweight/obese** i.e. a BMI of 25 kg/m^2 used as cut-off point. **B) non-obese** *vs.* **obese** i.e. BMI of 30 kg/m^2 cut-off point,

A)

Adiposity Group	n/N	**Model 1***		**Model 2****	
		OR (95 % CI)	P-value	OR (95 % CI)	P-value
Adiposity Group 1	48/858	**Reference**		**Reference**	
Adiposity Group 2	1/20	1.02 (0.13-7.97)	0.98	1.13 (0.14-8.92)	0.91
Adiposity Group 3	280/999	5.79 (4.17-8.03)	<0.0001	6.14 (4.23-8.90)	<0.0001
Adiposity Group 4	54/143	9.35 (5.91-14.80)	<0.0001	11.3 (6.77-18.72)	<0.0001

B)

		Model 1*		**Model 2****	
Adiposity Group	n/N	OR (95% CI)	P-value	OR (95% CI)	P-value
Adiposity Group 1	180/1,540	Reference		Reference	
Adiposity Group 2	6/68	0.82 (0.34-1.98)	0.67	0.89 (0.34-2.31)	0.80
Adiposity Group 3	148/317	7.86 (5.86-10.54)	<0.0001	8.72 (5.86-12.10)	<0.0001
Adiposity Group 4	49/95	8.07 (5.09-12.80)	<0.0001	9.69 (5.86-16.01)	<0.0001

n/N number of NAFLD cases/total number of participants in the group
* Age- and sex adjusted P-values for the comparison to Adiposity group 1 (reference)
** Further adjusted for adulthood alcohol consumption, physical exercise and smoking

Adiposity groups were: Adiposity group 1, normal childhood and adulthood BMI; group 2, high childhood, normal adulthood BMI; group 3, normal childhood BMI, high adulthood BMI, group 4, high childhood and adulthood BMI. Normal and high BMI were defined using international age-specific and sex-specific BMI cutoff points for overweight and obesity in childhood, and BMI cutoff points of 25 and 30 were used for adults