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ORIGINAL RESEARCH

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Prevalence of prediabetes and type 2 diabetes in children with obesity and increased transaminases in European German-speaking countries. Analysis of the APV initiative

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Summary

Background: Non-alcoholic fatty liver disease (NAFLD), prediabetes and type 2 diabetes mellitus are known to be closely linked with obesity as early as during childhood.

Objectives: The study aimed to determine the prevalence of prediabetes and T2DM in children with obesity with or without increased transaminases.

Methods: Data from the observational multicentre (n = 51), cross-sectional Adipositas Patienten Verlaufsbeobachtung registry were analyzed. Mild increase (mild group) was defined by alanine transaminase (ALT) >24 to ≤50 U/L and moderate to severe increase (advanced group) by ALT > 50 U/L. Prediabetes and T2DM were defined according to recent IDF/ISPAD guidelines.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; ALT, alanine amino transaminase; US, United States; NASH, non-alcoholic steatohepatitis; APV, Adipositas Patienten Verlaufsbeobachtung registry; OGTT, oral glucose tolerance test; min, minutes; mild increase group, mild group; IFG, impaired fasting glucose; BMI, body mass index; BMI-SDS, BMI as standard deviation score; HOMA-IR, homeostatic model assessment for insulin resistance; Matsuda-ISI, insulin sensitivity index-Matsuda; INS_{AUC}, area under the curve of insulin; INS_{peak}, insulin peak; HOMA-SC, homeostatic model assessment for insulin secretion; SD, standard deviation; %, percentage; χ^2-Test, chi-square test; OR, odds ratio; CI, confidence interval; NGT, normal glucose tolerance; ULN, upper limit of the norm.

Results: The prevalence of prediabetes and T2DM was 11.9% (95% CI: 11.0–12.8) and 1.4% (95% CI: 1.1–1.7) among all participants (n = 4932; male = 2481; mean age 12.9 ± 2.7 years; BMI-SDS 2.1 ± 0.5; Tanner stage 3.2 ± 1.5). The prevalence of impaired glucose metabolism (prediabetes and T2DM) was 13.8% (95% CI: 12.1–15.4) in the mild, 21.9% (95% CI: 18.8–25.1) in the advanced group, 10.7% (95% CI: 9.4–11.9) in the control group. Mild and advanced groups had greater odds ratios for prediabetes [1.42; 95% CI: 1.17–1.72, 2.26-fold; (1.78–2.86), respectively], the advanced group also for T2DM [2.39 (1.36–4.21)] compared to controls. While an increase in transaminases predominantly affected boys, girls within the advanced group had a higher T2DM prevalence than males (5.4 vs. male 2.1%).

Conclusions: Children with obesity and increased liver transaminases as surrogates of NAFLD should be screened for T2DM.

KEYWORDS

Blood glucose, childhood obesity, diabetes, impaired glucose metabolism, non-alcoholic fatty liver disease, prediabetes

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease among children and adults, and is known to be closely linked to obesity, sedentary lifestyle and hypercaloric diets.[1,2] The severity of NAFLD ranges from isolated steatosis to steatohepatitis, fibrosis and cirrhosis.[3] NAFLD is associated with several other metabolic comorbidities including dyslipidemia, hypertension and type 2 diabetes mellitus (T2DM).[4] Ultimately, patients with NAFLD carry an increased risk for cardiovascular disease ([5]). In severe cases, hepatocellular carcinoma and liver failure might develop in children with obesity-related NAFLD, requiring liver transplantation ([5,6]).

In addition, a strong association between insulin resistance, hepatic steatosis and impaired glucose metabolism has been shown to be present already in youth ([7]). Even adolescents with merely moderately elevated alanine transaminase (ALT) values were shown to have deteriorations in lipid- and glucose metabolism ([8]). Providing the only reliable available epidemiological data so far, a recent study demonstrated a prevalence of impaired glucose metabolism among children with histologically diagnosed NAFLD of around 30% (6.5% T2DM) in the United States (US)[9] as compared to only 5% of children without NAFLD (less than 1% T2DM) ([10,11]). Children with T2DM were shown to have greater risk of having non-alcoholic steatohepatitis (NASH), and thus greater long-term-risk for an adverse hepatic outcome.[9]

To date, there has been no study with sufficient sample size allowing an estimate of the prevalence of prediabetes and T2DM in children depending on the presence of NAFLD in European German-speaking countries. There is a substantial difference in the prevalence and incidence of pediatric T2DM between certain ethno-racial groups in the United States and cohorts from other regions worldwide.[12] In addition, a recent meta-analysis of adult data showed that the association between NAFLD and the risk of incident T2DM is stronger in some ethnic groups and that more severe forms of NAFLD seemed to be linked to an even greater risk of developing T2DM.[13] Thus, the aim of this large European multicentre cross-sectional study was to assess the prevalence of prediabetes and T2DM and in patients with varying degrees of increased transaminases as surrogates of NAFLD.

2 | MATERIALS AND METHODS

2.1 | The Adipositas Patienten Verlaufsbeobachtung registry

Data of the Adipositas Patienten Verlaufsbeobachtung (APV) registry were collected on the basis of German guidelines, defining how to diagnose and treat children and adolescents with overweight or obesity.[14] Standardised longitudinal documentation of patients with obesity was managed by a computer software using the visual FoxPro 9.0 compiler. Participation in this study was only allowed for specialised obesity treatment centres. Collected data were anonymised and sent to the department of epidemiology at the University of Ulm for central analysis. The consortium of the APV registry has access to all data and warrants its integrity. Ethical as well as data management guidelines corresponded to local standards.

2.2 | Selection of patients

This study included clinical and laboratory data collected in 51 centres in Germany, Austria and Switzerland from the APV database (www.a-p-v.de) ([15]). Between 2000 and 2018, 109.772 patient visits of patients with

overweight or obesity were documented. Totally, 7620 underwent an oral glucose tolerance test (OGTT) and 4932 also had fasting blood samples taken for biochemical analysis. The following inclusion criteria of patients were applied: (i) age 2–20 years, (ii) OGTT with venous blood glucose levels at 0 and 120 min and (iii) assessment of liver enzymes within one month of OGTT. Exclusion criteria were (i) underweight, (ii) syndromal or endocrine obesity and (iii)) metformin treatment. OGTT-derived measures of insulin sensitivity and secretion were analyzed in a subgroup of 1528 patients with complete glucose and insulin data sets.

2.3 | Definition of non-alcoholic fatty liver disease, prediabetes and type 2 diabetes mellitus

All participants were stratified based on their ALT value, as a surrogate for NAFLD, into (i) control group (ALT ≤ 24 U/L, *n* = 2506), (ii) mild increase group (mild group, ALT > 24 U/L – ≤ 50 U/L, *n* = 1760) and (iii) advanced group (ALT > 50 U/L, *n* = 666).[16] Prediabetes (*n* = 586) was defined by impaired fasting glucose (IFG) with a fasting glucose value between 5.6 mmol/L and ≤ 7 mmol/L and/or a 120 min blood glucose level in OGTT ≥ 7.8 mmol/L and < 11.1 mmol/L (IGT). T2DM (*n* = 69) was defined by a fasting glucose value ≥7 mmol/L and/or a 120 min OGTT value ≥11.1 mmol/L and exclusion of other diabetes types by local clinical physician.[17]

2.4 | Characterisation of weight status

Height and weight were assessed by standardised and calibrated scales and stadiometers wearing light clothing without shoes by trained staff. Normal weight was defined by a body mass index (BMI) smaller or equal to the 90th percentile, overweight above the 90th percentile and below or equal to the 97th percentile, obesity above the 97th percentile and less or equal to 99.7th percentile and morbid obesity above the 99.7th percentile, respectively, based on percentiles for German children and adolescents ([18,19]), and BMI was also expressed as standard deviation score (BMI-SDS).[20]

2.5 | Characterisation of insulin sensitivity and secretion

Insulin sensitivity was characterised by homeostatic model assessment for insulin resistance (HOMA-IR) and insulin sensitivity index-Matsuda (MATSUDA-ISI) ([21,22]). Insulin secretion was described by area under the curve of insulin (INS_{AUC}), insulin peak (INS_{peak}) and homeostatic model assessment for insulin secretion (HOMA-SC) ([22,23]).

2.6 | Statistical methods

All statistical analyses were performed with SAS 9.2 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA). A *p*-value of <0.01 was considered as statistically significant. Continuous parameters were described by mean and standard deviation (SD) and binary variables by percentages (%). To show differences in mean, significance levels were tested using Wilcoxon tests for continuous variables and chi-square tests (χ^2-test) for binominal data. Correlations were tested via Spearman´s correlation test. Multiple logistic regression models were used to identify the odds ratio (OR) and 95% Wald confidence interval (CI) of different NAFLD groups. OR was adjusted for sex, age and BMI-SDS.

3 | RESULTS

Characteristics of different transaminase groups in regard to pubertal stage, anthropometry, liver enzymes and measures of glucose metabolism (Table 1).

The demographic characteristics and concentrations of fasting glucose, fasting insulin and liver enzymes of the different transaminase groups are given in Table 1. Patients in the advanced group were older than those in the other groups. While there were more girls in the control group, more than half of the participants in the mild and almost 3 out of 4 participants of the advanced group were male. BMI and BMI-SDS values were higher among patients in the mild group and the advanced group as compared to those in the control group. While there was no difference in the prevalence of patients classified with obesity between transaminase groups, less patients with mild and advanced severity were classified with overweight and more of them with extreme obesity as compared to the controls. Tanner stage did not differ between subjects of different transaminase groups. As by definition, liver enzymes were the highest in the advanced group, followed by the mild and the control group, respectively.

Patients in the mild group as well as in the advanced group had higher glucose levels than in the control group during OGTT (Fig. 1). Compared to the control group, this difference was significant in the advanced group at baseline and at all time points of the OGTT. Only in the mild group, the fasting and 60 min glucose value had no significant difference in comparison to the control group. Comparing all three transaminase categories, blood insulin values were higher in both increase groups than in control patients at all time points of OGTT (Fig. 1).

Among patients with normal glucose tolerance (NGT), prediabetes and T2DM, 50% (95% CI: 48.5–51.5), 53.4% (95% CI: 49.4–57.5) and 46.4% (95% CI: 34.3–58.4), respectively, were male. In the NGT, prediabetes and T2DM group, 47.7% (95% CI: 46.2–49.1), 59.9% (95% CI: 55.9–63.9) and 53.6% (95% CI: 41.6–65.7) of the patients were in the mild group and 12.2% (95% CI: 11.2–13.1), 21.5% (95% CI: 18.2–24.6) and 29% (95% CI: 18.0–40.0) in the advanced group, respectively.

3.1 | Measures of insulin sensitivity and secretion

As indicated by HOMA-IR and MATSUDA-ISI, patients in the mild and advanced group were more insulin resistant than controls. Insulin

TABLE 1 Characteristics of patients in different transaminase groups (controls, mild increased transaminases group, advanced group)

	Control group	Mild increase group	Advanced group	*p*-Value*	*p*-Value**
Total (N)	2506	1760	666		
Mean age (years) (Std. Dev.).	12.7 (2.7)	12.8 (2.7)	13.6 (2.7)	.37	<.01
Male sex (%, 95% CI)	39.5 (37.6–41.5)	57.5 (55.2–59.8)	72.1 (68.7–75.5)	<.01	<.01
BMI (kg/m^2) (Std. Dev.)	30.4 (6)	32.3 (6.4)	34.7 (7.4)	<.01	<.01
BMI-SDS (Std. Dev.)	2.0 (0.5)	2.2 (0.5)	2.3 (0.5)	<.01	<.01
Tanner stage (Std. Dev.)	3.2 (1.6)	3.1 (1.5)	3.3 (1.5)	.26	.66
Normal weight (%, 95% CI)	5.4 (4.5–6.3)	2.4 (1.7–3.1)	1.4 (0.5–2.2)		
Overweight (%, 95% CI)	30.0 (28.2–31.8)	19.0 (17.1–20.8)	13.8 (11.2–16.4)		
Obesity (%, 95% CI)	53.3 (51.3–55.2)	59.3 (57.0–61.6)	55.9 (52.1–59.6)		
Extreme obesity (%, 95% CI)	11.3 (0.1–12.6)	19.3 (17.5–21.2)	29.0 (25.5–32.4)		
Fasting glucose (mmol/L)	4.71	4.70	4.86	.20	<.01
AST (U/L) (Std. Dev.)	22.8 (8.7)	29.5 (7.8)	54.1 (59.0)	<.01	<.01
ALT (U/L) (Std. Dev.)	17.4 (5.1)	33.4 (7.0)	88.0 (63.5)	<.01	<.01
GGT (U/L) (Std. Dev.)	16.7 (10.5)	22.9 (12.0)	40.9 (46.3)	<.01	<.01
HOMA-IR (Std. Dev.)	3.7 (3.7)	4.5 (3.3)	5.9 (5.2)		
MATSUDA-ISI (Std. Dev.)	2.6 (2.4)	2.1 (1.7)	1.7 (1.4)		
INS_{AUC} [(mass/volume) × time] (Std. Dev.)	20 834.1 (17 613.3)	21 605 (14 536.7)	26 076.5 (17 956.2)		
INS_{peak} (Um/L) (Std. Dev.)	98.0 (172.9)	115.2 (158.6)	172.2 (196.1)		
IGI (Std. Dev.)	−1.1 (150.1)	3.2 (3.0)	5.1 (24.6)		
HOMA-SC (Std. Dev.)	0.7 (0.5)	0.9 (0.5)	1.1 (0.6)		

Abbreviations: BMI, body mass index; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transpeptidase; BMI-SDS, *standardised* BMI; 95% CI, 95% confidence interval; HOMA-IR, homeostatic model assessment for insulin resistance; MATSUDA-ISI, indicates values which are comparable to rate of disappearance of plasma glucose measured by insulin clamp with glucose tracer; INSAUC, area under the curve insulin; INSpeak, insulin peak; HOMA-SC, homeo-static model assessment for insulin secretion; IGI, insulinogenic index; 0–30 INSAUC/GluAUC., 0–30 min area under the curve insulin over area under the curve glucose; 30–120 INSAUC/GluAUC., 30–120 min area under the curve insulin over area under the curve glucose; Std. Dev, standard deviation.

*p-Value: Differences between control and mild increase group.

**p-Value: Differences between control and advanced group.

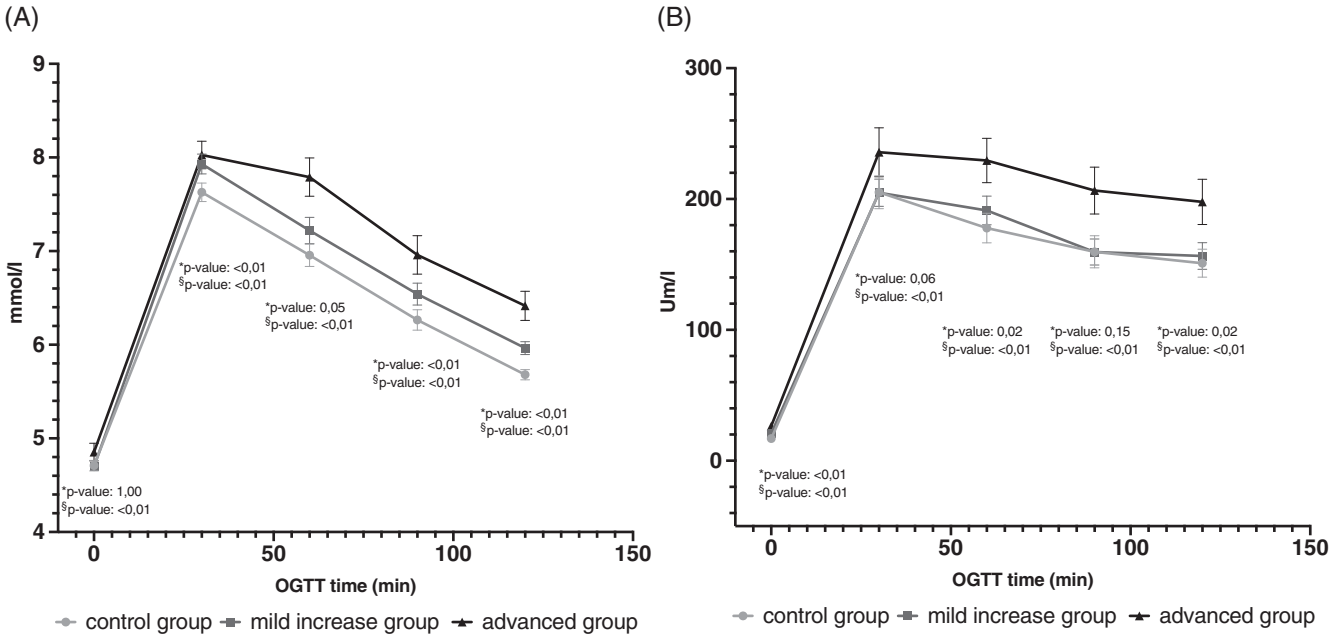

FIGURE 1 Blood glucose levels (a, mmol/L) and insulin level during oral glucose tolerance test (OGTT) (b, Um/L) in the control group, mild increased transaminase group and advanced group, shown with lower and upper 95% CI for mean

TABLE 2 Prevalence and 95% confidence interval of prediabetes and type 2 diabetes mellitus in different groups (controls, mild increase group and advanced group)

	Controls	Mild increase group	Advanced group	*p*-Value*	*p*-Value**
Total (N)	2506	1760	666		
Prediabetes (%, 95% CI)	9.4 (8.2–10.5)	12.8 (11.2–14.3)	18.9 (15.9–21.9)	<.01	<.01
♂ (%, 95% CI)	9.7 (7.8–11.5)	12.5 (10.4–14.5)	19 (15.4–22.5)		
♀ (%, 95% CI)	9.2 (7.7–10.6)	13.2 (10.8–15.7)	18.8 (13.1–24.5)		
T2DM (%, 95% CI)	1.3 (0.8–1.7)	1 (0.5–1.4)	3.0 (1.7–4.3)	.35	<.01
♂ (%, 95% CI)	1.1 (0.5–1.8)	1.1 (0.4–1.7)	2.1 (0.8–3.4)		
♀ (%, 95% CI)	1.4 (0.8–2.0)	0.8 (0.2–1.4)	5.4 (2.1–8.6)		

Abbreviations: OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.
**p*-Value: Differences between the controls and mild increase group.
***p*-Value: Differences between the controls and advanced group.

TABLE 3 OR and 95% CI of T2DM and prediabetes according to level of ALT

	T2DM (*n* = 69)					Prediabetes (*n* = 586)				
	Crude		Adjusted*			Crude		Adjusted*		
	OR	95% CI	OR	95% CI	*p*-Value**	OR	95% CI	OR	95% CI	*p*-Value**
Advanced group	**2.4**	1.4–4.2	**2.2**	1.2–4	0.01	**2.3**	1.8–2.9	**1.9**	1.5–2.4	<.01
Mild increase group	**0.8**	0.4–1.4	**0.7**	0.4–1.4	.02	**1.4**	1.2–1.7	**1.3**	1.1–1.6	<.01
Controls	**1**	–	**1**	–	–	**1**		**1**	–	–

Note: Logistic regression models were used to estimate the OR and 95% CI, adjusted for age, sex and BMI-SDS.
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; BMI-SDS, BMI as standard deviation score; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.
**p*-Value: Difference in adjusted odds ratios for T2DM between different groups with elevated transaminases compared to control group.
***p*-Value: Difference in adjusted odds ratios for prediabetes between different groups with elevated transaminases compared to control group.

secretion described by HOMA-SC was higher in both increase groups as compared to controls. INS_{AUC}, INS_{peak}, and insulinogenic index (IGI) tended to be higher with rising ALT-values, but did not reach significance (Table 1). In addition, ALT values were negatively correlated with MATSUDA-ISI ($r = -0.26$; $p < 0.01$) and positively with HOMA-IR ($r = 0.23$; $p < 0.01$), HOMA-SC ($r = 0.24$; $p < 0.01$) and peak insulin ($r = 0.21$, $p < 0.01$) in the entire cohort.

3.2 | Prevalences of prediabetes and type 2 diabetes mellitus in children within different transaminase groups

The prevalence of prediabetes and T2DM was 11.9% (95% CI: 11.0–12.8) and 1.4% (95% CI: 1.1–1.7) among all participants, respectively (Table 2). The prevalence of prediabetes was significantly higher in the advanced group as compared to the control group. Patients in the advanced group had a significantly higher prevalence of T2DM than controls. Prevalence of prediabetes increased with deteriorating transaminase degree in both girls and boys. However, girls within the advanced group had a higher T2DM prevalence than males within the same group. There was a significant difference in the prevalence of prediabetes between boys and girls of all groups, while there was no difference in the prevalence of T2DM between boys and girls referring to all groups.

3.3 | Odds ratios of prediabetes and type 2 diabetes mellitus in children within different transaminase groups

Children in the mild and the advanced group had significantly greater odds ratios of being classified with prediabetes as compared to controls, whereby adjustment for age, sex and BMI-SDS had little influence (Table 3). Children in the advanced group had greater odds ratios of being classified with T2DM compared to children within controls. These odds ratios remained similar after adjustment. There was no significant difference in the risk for T2DM between the mild and the control group.

4 | DISCUSSION

Our cross-sectional analysis of data derived from one of the largest pediatric European obesity multicentre studies shows that children with increased liver transaminases as surrogates of NAFLD show

higher prevalences for prediabetes and T2DM as compared to those with normal transaminases.

Current estimated worldwide healthcare cost in diabetes treatment and complications of more than USD 720 billion and an approximate 50% increase in the number of people with diabetes by 2045 underscore the serious global challenge of the diabetes pandemic.[24] Until less than 30 years ago, T2DM was regarded an adult disease.[25] However, since the early 1990s, a trend of increasing cases of T2DM in children has been witnessed, particularly in North America, paralleling the staggering increase in the prevalence of obesity.[25] However, while it is projected that by 2050 there may be a fourfold increase in the prevalence of youth-onset T2DM in the United States, in a recent follow-up survey of a cross-sectional study, Neu *et al.* reported that the prevalence of youth-onset T2DM in Germany remained stable over the past 10 years, and that the majority of youth-onset T2DM cases were part of specific ethnic minorities ([26,27]). Given the asymptomatic nature at T2DM followed by rapid progression with beta-cell function deteriorating by 15–20% per year when diagnosed in young people and complications arising within 5 years, identification of individuals at high risk is crucial.[28] Youth with NAFLD have recently been shown to have substantially higher risk of T2DM than youth in general, with as many as one in three children with NAFLD having abnormal glucose metabolism in a U.S. cohort ([9,29,30]). In a 5-year follow-up of adults with newly diagnosed diabetes, hepatocellular lipid content was higher in patients assigned to a severe insulin-resistant cluster as compared to patients with mild age-related diabetes, mild obesity-related diabetes, severe autoimmune diabetes and severe insulin-deficient diabetes at baseline.[31] Therefore, the results of our study underscore the need for youth with obesity and elevated liver enzymes to undergo comprehensive metabolic assessment including an OGTT.

We based our diagnostic criteria for increased ALT values on those suggested by Schwimmer *et al.* and considered elevations up to two times the upper limit of the norm (ULN) as surrogate of 'mild NAFLD'. While no increased risk for T2DM could be detected in this group, an increase in the odds ratios for prediabetes by ~30% was evident. Three percent of youth within the advanced group (ALT 2- to 4.5-fold the ULN) were diagnosed with T2DM and 22% with abnormal glucose metabolism in our cohort. These patients had two times the odds ratios for T2DM. The cross-sectional nature of our study prevents us from drawing any conclusions in regard to direction of causality. Serum liver enzyme concentrations are considered to have a relatively poor sensitivity and specificity in the diagnosis of NAFLD.[32] However, our data are in line with recently published systematic reviews and metanalyses on prospective studies in adult populations. Ballestri *et al.* pooled data of more than 117.000 adults diagnosed with NAFLD by serum liver enzymes which demonstrated an increased incident risk of T2DM with a pooled relative risk of 1.97 for ALT (last vs. first quartile or quintile).[33] Similar results were shown by Mantovani *et al.*, who analysed data of almost 300.000 adult individuals of whom 30% had been diagnosed with NAFLD by imaging methods.[13] Further, Sung and coworkers demonstrated that patients with NAFLD had twice the risk of developing T2DM compared to patients without NAFLD during five years of follow-up, while resolution of fatty liver prevented them from developing T2DM.[34] In keeping with this, a recent retrospective study demonstrated a strong and independent association between NAFLD improvement and reduced incidence of T2DM in adult patients.[35] In addition, an elevated risk of incident T2DM was even shown for patients with normal weight and NAFLD as compared to patients with normal to overweight without NAFLD.[36] To date, published adult data regarding the association between histologically confirmed-NAFLD and the risk of incident T2DM are rare, with no such longitudinal data available in the pediatric age group. In a retrospective analysis of Swedish adults, approximately 80% of individuals with biopsy-proven NAFLD developed T2DM (58%) or pre-diabetes (20%) within a 14-year follow-up period.[37] These data provide evidence of biological plausibility that NAFLD may increase risk of incident T2DM.

Our study confirms previous studies that pediatric NAFLD is associated with decreased insulin sensitivity and a compensatory insulin response.[38] In NAFLD, accumulation of intrahepatic fat predominantly results from increased influx of esterified free fatty acids from maladapted white adipose tissue (60%), de-novo lipogenesis (25%) and diet (15%) and drives hepatic gluconeogenesis and insulin resistance already in childhood.[39,40] In addition, oxidised fatty acids have been suggested to be involved in the pathogenesis of T2DM in adolescents with obesity by mediating hepatic injury and impairing beta-cell function. Further, hepatic insulin resistance has been shown to trigger the synthesis of several proinflammatory mediators and prodiabetogenic hepatokines (e.g., fetuin-A, fetuin-B, fibroblast growth factor-21, retinol-binding protein 4, selenoprotein P) that may favour the development of T2DM.[41,42]

In line with data from previous studies, our results show significant differences in NAFLD risk between both sexes with a clear male predominance.[43,44] However, among youth with prediabetes and T2DM, there were significantly more girls than boys. In addition to this general female predominance in abnormal glucose metabolism, girls within the advanced group exhibited more than double the prevalence in T2DM than boys (5.4% vs. 2.1%). These data are in line with those published by Newton *et al.*: among 675 U.S. adolescents with histologically proven NAFLD, 71.1% were male.[9] Contrary to this, girls with increased transaminases were significantly more likely to have T2DM than boys (13.7% vs. 3.5%). Although the cause for this gender difference remains elusive, it seems relevant that girls with NAFLD are more likely to have additional associated comorbidities such as arterial hypertension.[45]

In general, during reproductive age, males were shown to have a higher prevalence and severity of NAFLD compared to females. On the contrary, postmenopausal women are known to carry a greater risk of NAFLD than men, suggesting a protective role of oestrogens. Further, the role of NAFLD as a sexual dimorphic disease is also supported by animal models that demonstrate a disposition of male individuals to more advanced fatty liver disease compared to females that is linked to a state of subclinical inflammation and increased hepatic morbidity.[46] A study applying a computational model concluded that due to sex-specific metabolic demands, female and male

livers are metabolically diverse and therefore differently regulated.[47] In addition, a recent review stated that most published clinical and epidemiological studies fail to examine sex differences appropriately' and suggested to consider sex differences, sex hormones, age and other reproductive information in clinical investigation and gene association studies of NAFLD 'in order to fill current gaps and implement precision medicine for patients with NAFLD'.[46]

There are some strengths and limitations that need to be acknowledged. The primary strength of this study is its large sample size of patients. In addition, the availability of fasting and OGTT data improves the validity of the diagnosis of T2DM. Nevertheless, although investigators at the different study sites of the APV study group are requested to adhere to most recent T2DM screening recommendations, which includes islet cell antibody testing, some uncertainty in regard to accurate diagnostic testing remains.[48] The same applies to the exclusion of other causes of elevated liver enzymes such as Wilson's disease, celiac disease, autoimmune or other metabolic diseases including alcohol abuse, which may not have been adequately excluded by clinicians in all patients entered into the APV study database. NAFLD was diagnosed based on elevated liver enzyme levels although the gold standard of diagnosis is liver biopsy. Thus, there may be some limitations in detecting NAFLD in children with obesity having completely normal liver enzyme levels in the present study. However, since widely used 'normal' ranges of liver transaminases may underestimate the presence of NAFLD, we chose to use biology-based thresholds providing higher sensitivity and sufficient specificity.[16] Similarly, OGTT data in part reflect diagnostic management strategies of the study centres, which may not always adhere to current diagnostic guidelines as outlined above. There is also no centralised measurement of laboratory parameters, particularly glucose and insulin, and we have to acknowledge differences in assay procedures. Further, information about race and ethnicity were not available. Finally, given the high prevalence of prediabetes in our sample, calculated OR may overestimate the relative risk of prediabetes.

In conclusion, children with increased transaminases as surrogates of NAFLD have a two to threefold higher risk of prediabetes and T2DM as compared to controls. While NAFLD in youth predominantly affects boys, girls with NAFLD are at higher risk for T2DM. This study confirms that youth with NAFLD, in particular when associated with obesity, should undergo a detailed clinical assessment of glucose metabolism, independent of the background epidemiology of T2DM.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared.

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