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SHORT COMMUNICATION

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Elevated transaminases potentiate the risk for emerging dysglycemia in children with overweight and obesity

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Summary

Background: There is evidence that nonalcoholic fatty liver disease (NAFLD) increases the risk for dysglycemia in children in cross-sectional studies. However, the extent to which NAFLD may confer the risk for dysglycemia in longitudinal studies remains uncertain.

Objectives: We investigated whether elevated levels of alanine aminotransferase (ALT) as a proxy for NAFLD can serve as a predictor for future dysglycemia among children.

Methods: We performed survival analysis up to 11 years of follow-up on longitudinal data of 510 children with overweight and obesity from the Leipzig Childhood Cohort.

Results: Children with overweight/obesity and elevated ALT values had a more than 2-fold increased risk (hazard ratio 2.59, 95% confidence interval 1.49 to 4.50; $P < 0.01$) for future dysglycemia independent of age, sex and BMI-SDS.

Conclusions: Elevated transaminases are an early predictor for glycemic deterioration. Hence, NAFLD should further be addressed as a risk factor and therapeutic target for the early prevention of type 2 diabetes.

KEYWORDS
childhood obesity, dysglycemia, early-onset diabetes, nonalcoholic fatty liver disease, prediabetes, type 2 diabetes

1 | INTRODUCTION

The early development of sustained obesity in childhood[1] not only increases the risk for dysglycemia early in childhood but also the course of type 2 diabetes (T2D) appears to be more aggressive in children compared with adults.[2] Particularly in children, manifestation of T2D is often asymptomatic, which leads to a delay of diagnosis.[2] In view of the fact that children with T2D lose around 15 to 20% of their beta cell function per year, a late diagnosis entails long-term damage and necessitates premature insulin treatment.[2] Therefore, it is important to identify the children at highest risk for emerging dysglycemia. Nonalcoholic fatty liver disease (NAFLD) was associated with a higher risk for prediabetes and T2D in cross-sectional studies.[3,4] In addition, more severe forms of NAFLD seemed to be linked to

Abbreviations: ALT, alanine aminotransferase; BMI-SDS, body mass index SD score; CI, confidence interval; HOMA-IR, homeostasis model assessment for insulin resistance; HR, Hazard ratio; INS_{AUC}, area under the insulin curve during OGTT; INS_{peak}, highest insulin during OGTT; IQR, interquartile range; Matsuda-ISI, insulin sensitivity index according to Matsuda during OGTT; NAFLD, Nonalcoholic fatty liver disease; OGTT, oral glucose tolerance testing; SD, standard deviation; T2D, type 2 diabetes.

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an even greater risk of developing T2DM.[4] The extent to which NAFLD may confer the risk for prediabetes and T2D in longitudinal observations remains to be proven. In this study, we longitudinally investigated if children with obesity and elevated alanine aminotransferase (ALT) values as a proxy for NAFLD are prone to develop dysglycemia.

2 | METHODS

We included 510 children and adolescents with overweight and obesity (defined as a body mass index SD score [BMI-SDS] ≥1.28) from the Leipzig Childhood Cohort, comprising data from the LIFE Child (NCT02550236),[5] the Leipzig Childhood Obesity (NCT04491344)[6] and the Leipzig Atherobesity Childhood Cohort (NCT01605123)[7] (see Figure S1 for more details on the selection of study population). Participants were free of medications and diseases affecting glucose metabolism at baseline. All studies were approved by the local ethics committee. Written informed consent was provided by the legal guardian and the child itself from the age of 12 years on prior to study participation. All participants underwent oral glucose tolerance testing (OGTT) and assessment of ALT at baseline and at the follow-up visit (median follow-up period of 2.9 years, Table 1) between 1999 and 2019. For OGTT, subjects ingested 1.75 g/kg body weight dextrose (maximum 75 g) after a 10 hour overnight fast. Venous blood samples were drawn directly before glucose challenge and 30, 60, 90 and 120 minutes afterward. Glucose response was classified according to the criteria of the American Diabetes Association (ADA)[8] into impaired fasting glucose (IFG, ≥5.6 mmol/L), diabetic fasting glucose (DFG, ≥7.0 mmol/L), impaired glucose tolerance (IGT, 2-hour glucose ≥7.8 mmol/L) and diabetic glucose tolerance (2-hour glucose ≥11.1 mmol/L). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as a measure of hepatic insulin resistance, the insulin sensitivity index according to Matsuda (Matsuda-ISI) and area under the insulin curve during OGTT (INS_{AUC}) were calculated as measures of peripheral insulin resistance/sensitivity as described elsewhere.[9-11] ALT as a surrogate marker for NAFLD was used to stratify all participants into two different groups at baseline: (i) ALT≤24 U/L, n = 281 (control group) and (ii) ALT>24 U/L), n = 229 (elevated group).[12] The risk for emerging dysglycemia was assessed by Kaplan–Meier analyses and cox proportional hazard regression. Dysglycemia was defined according to ADA criteria as having IFG or DFG, in combination with IGT or DGT, and/or treatment with metformin. Height and weight were assessed by standardized and calibrated scales without shoes and clothing. BMI was transformed to sex- and age-specific SD scores (SDS) based on reference values from the German population.[13] Statistical analysis was performed using R software package R version 3.2.5 (R Foundation for Statistical Computing, Vienna Austria; http://www.R-project.org/).

3 | RESULTS

A total number of 510 children and adolescents with overweight and obesity were included in this study (Table 1). Among all participants, aged 2 to 19 years at baseline, 62 experienced dysglycemia during 11 years of follow-up (Table S1).

Children with overweight/obesity, who presented with elevated ALT, but without dysglycemia at baseline, had a significantly increased risk for glycemic deterioration compared with the control group (log-rank test: P-value<0.001). Approx. 50% vs 25% of children had developed dysglycemia after 8 years (Figure 1). Elevated ALT as a proxy of NAFLD more than doubled the risk for dysglycemia among children with overweight independently of BMI-SDS, age and sex (hazard ratio 2.59, 95% CI 1.49 to 4.50; $P < 0.01$, Table 1). We repeated the cox proportional hazard regression with the full linear spectrum of ALT levels at baseline and confirmed ALT levels as a significant predictor for emerging dysglycemia independently of age, sex and BMI-SDS (log-rank test: P-value<0.001). Of note, insulin sensitivity was already diminished along with elevated ALT levels at baseline (Table 1). Thereafter, especially hepatic insulin resistance (HOMA-IR) increased further with time within the elevated ALT group, whereas peripheral insulin resistance (INS_{AUC}, Matsuda-ISI) remained stable or deteriorated only slightly (Figure S2).

4 | DISCUSSION

Our results highlight, that even though the degree of obesity is related to the emergence of dysglycemia, the presence of elevated ALT values as a proxy for NAFLD confers an additional and independent risk for progressively deteriorating glucose metabolism already in childhood. Our findings are in line with a smaller study showing the emergence of diabetes in children with (biopsy-proven) NAFLD within a period of 4.5 years,[14] even though presence of diabetes relied on phone interview in that study. In addition, a recent study based on data from German and British adults concluded that participants with fatty liver disease and insulin resistance clustered to a subphenotype with the highest risk for emerging T2D during a 10-20 year follow-up period.[15] In line with those findings, we were able to show that deterioration among the elevated ALT group was especially pronounced for hepatic insulin resistance (HOMA-IR) rather than for peripheral insulin resistance (INS_{AUC}, Matsuda-ISI). These results support the role of NAFLD as a driver for hepatic insulin resistance and hence dysglycemia.

There is clinical evidence that a resolution of NAFLD during follow-up of adult patients lowers the risk of future T2D development independent of potential cofounders like BMI and weight loss.[16-18] This can be explained by reduced secretion of hepatokines and proinflammatory mediators through reduced lipotoxicity which affects both NAFLD status and T2DM incidence risk.[19] Of course, lifestyle intervention alone may beneficially impact the risk of T2DM.[20] Still, it cannot be disregarded that an improvement in liver transaminase and NAFLD status reduces the T2DM risk.[18] Unfortunately, it is impossible to show causal relationship over time from previous studies, as many studies were designed as cross-sectional cohort studies using odds ratios.[18,21]

As such, the strength of this study is its longitudinal set-up with detailed metabolic phenotyping in a large sample size, which has not

TABLE 1 Characteristics of patients at baseline and cox proportional hazard model

	Total group	Control group (ALT ≤24 U/L)	Elevated group (ALT >24 U/L)	P-value
No. (%)	510	281 (55.10)	229 (44.90)	
Follow-up time, median (IQR), years	2.07 (2.38)	2.18 (2.68)	2.01 (2.23)	0.05
Age, mean (SD), years	11.30 (2.96)	11.80 (3.16)	11.15 (3.08)	0.04
No. male (%)	227 (44.51)	104 (37.14)	123 (53.48)	0.54
BMI-SDS, mean (SD)	2.47 (0.55)	2.42 (0.54)	2.55 (0.56)	<0.001
ALT, mean (SD), U/L	23.40 (22.73)	18.60 (3.73)	33.89 (27.63)	<0.001
Fasting glucose, mean (SD), mmol/L	5.17 (0.46)	5.18 (0.47)	5.16 (0.46)	0.36
2-hour glucose, mean (SD), mmol/L	6.56 (1.18)	6.44 (1.08)	6.63 (1.26)	<0.001
HOMA-IR, mean (SD)	3.13 (3.23)	2.75 (1.83)	3.71 (4.27))	<0.001
Matsuda-ISI, mean (SD)	2.54 (2.41)	3.17 (2.56)	1.98 (2,05)	<0.001
$INS_{AUC,}$ mean (SD), pmol/L x h	1417.68 (1597.51)	1174.75 (894.70)	1808.23 (2065.41)	<0.001
	Cox proportional hazard model			
	Simple regression		**Multiple regression**	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Elevated ALT = yes	2.32 (1.39-3.88)	<0.001	2.59 (1.49-4.50)	<0.001
Age, per year	1.12 (1.03-1.22)	<0.001	1.16 (1.07-1.27)	<0.001
BMI-SDS	1.38 (0.92-2.04)	0.12	1.28 (0.83-1.98)	0.26
Sex = male	0.93 (0.56-1.55)	0.79	0.86 (0.51-1.47)	0.59

Note: Statistical differences between the control and elevated group at baseline were determined by Wilcoxon rank-sum test. SI conversion factors: To convert ALT to μkat/L, multiply values by 0.0167. Hazard ratios (HRs) regarding the onset of glycemic failure were calculated with cox proportional hazard regression for each indicated predictor in an univariate model and for all predictors together in a multivariable model. CI, confidence interval; HOMA-IR, homeostasis model assessment for insulin resistance, INS_{AUC}, area under the insulin curve during OGTT, IQR, interquartile range, SD, standard deviation, Matsuda-ISI, insulin sensitivity index according to Matsuda during OGTT.

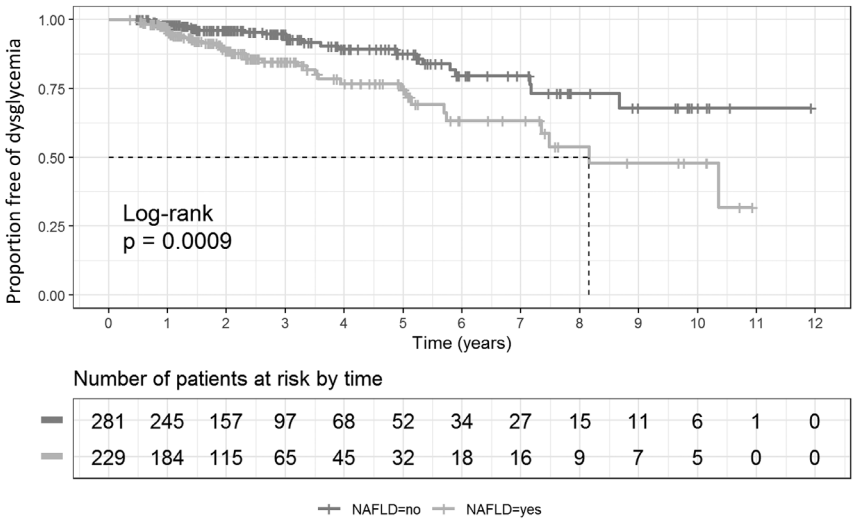

FIGURE 1 Kaplan–Meier estimation regarding time until onset of dysglycemia among children with overweight/obesity and elevated transaminases (NAFLD = yes, light gray) and with normal range transaminases (NAFLD = no, dark gray). Ticks on survival curves indicate censored cases, the dashed line represents median time of the elevated group until diagnosis of dysglycemia. Differences between survival curves were estimated with log-rank test. N = 510

been performed in children for this objective before. The statistical analysis of this study considers other risk factors showing a causal relationship between elevated ALT values and impaired glucose metabolism independent of risk factors like BMI-SDS, age and sex. As a weakness, the single measurement of ALT without an additional imaging may be suboptimal for the diagnosis of NAFLD. The gold standard to diagnose NAFLD would be a biopsy, which is, however, not easily accessible test due to the risk of possible complications especially in children.[22] However, it has been shown that the applied ALT cutoff has a sufficient concordance with the presence of NAFLD and provides a high sensitivity and sufficient specificity for NAFLD.[12] Also, the result of the present study is more useful in clinical practice because transaminase values are easily accessible even in the primary care. As another limitation, we used only a single OGTT for the assessment of dysglycemia. Therefore, we required two pathological OGTT criteria (eg, both IFG and IGT) in our analysis for the diagnosis

of (pre)diabetes, which is in line with current ADA guidelines. Furthermore, we have not considered additional risk factors for glycemic failure, for example, family history, physical activity, dietary habits or changes in the transaminase status over time. The next step could be the evaluation of a decision diagram for physicians at which ALT value children with obesity should undergo screening for impaired glucose metabolism. Further clinical research is warranted to test if a resolution of NAFLD reduces the risk of future T2D in children.

In conclusion, children with elevated ALT values as a proxy for NAFLD are more than two times more likely to progress to a prediabetic state within less than 10 years implying that elevated ALT values in the absence of other causes of underlying hepatopathies are an independent risk factor for early deterioration of glucose metabolism already in childhood. Hence, NAFLD should be addressed as a risk factor and therapeutic target in children with obesity.

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CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this article to disclose.

AUTHOR CONTRIBUTIONS

Florian Koutny and **Robert Stein** were involved in the study design, statistical analysis, generation of figures and tables, data interpretation and writing and reviewing the manuscript. **Daniel Weghuber** was involved in the study design, data interpretation as well as editing and reviewing the manuscript. **Antje Körner** was involved in the study design and data interpretation, supervising data collection, funding acquisition and editing and reviewing the manuscript. **Wieland Kiess** was involved in supervising data collection, funding acquisition and editing and reviewing the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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