Fatty Liver Among Adolescent Offspring of Women With Type 1 Diabetes (the EPICOM Study)

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OBJECTIVE

Intrauterine exposure to maternal type 1 diabetes is associated with a less favorable metabolic profile later in life. Nonalcoholic fatty liver disease is the hepatic manifestation of a cluster of metabolic abnormalities linked to insulin resistance. This study aimed to evaluate the effect of maternal pregestational type 1 diabetes on the presence of fatty liver in offspring and the association between maternal BMI, glycemic control during pregnancy, offspring metabolic risk factors, and offspring level of soluble CD163 (sCD163) (a marker of macrophage activation) and risk of fatty liver.

RESEARCH DESIGN AND METHODS

This study was a prospective nationwide follow-up study of offspring (*n* = 278) of mothers with pregestational type 1 diabetes between 1993 and 1999 and matched control subjects (*n* = 303). Mean age at the time of follow-up was 16.7 years (range 13.0–20.4 years). We used the fatty liver index (FLI) and waist-to-height ratio (WHtR) to evaluate the presence of fatty liver among the offspring. An FLI ≥60 or WHtR >0.469 were used as cutoff points for fatty liver.

RESULTS

More type 1 diabetes–exposed offspring had high FLI and WHtR indices compared with unexposed control subjects. We found significant associations between increasing maternal prepregnancy BMI, being born large for gestational age, offspring level of sCD163, as well as offspring metabolic risk factors (decreasing adiponectin and HDL cholesterol and increasing leptin, HOMA of insulin resistance, and HOMA of insulin secretion) and degree of fatty liver.

CONCLUSIONS

Intrauterine exposure to maternal type 1 diabetes and higher maternal prepregnancy BMI may predispose to fatty liver in the offspring. Offspring metabolic risk factors, including sCD163 levels, are associated with indices of fatty liver.

Along with the worldwide increase in obesity, the prevalence of nonalcoholic fatty liver disease (NAFLD) is rising, and in the pediatric population, NAFLD is now considered one of the leading causes of hepatic disease (1). Offspring of women with diabetes have an increased risk of developing type 2 diabetes, prediabetes, and obesity (2). This applies to both offspring of mothers with pregestational diabetes as well as gestational diabetes mellitus, and our research group has previously published data from the EPICOM (Epigenetic, Genetic, and Environmental Effects on Growth, Cognitive Functions, and Metabolism in Offspring of Women

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With Type 1 Diabetes) study, where adolescent offspring of mothers with type 1 diabetes had a similar risk (3–5). The mechanisms behind this are unknown. However, as maternal glucose easily passes the placenta and results in intrauterine hyperglycemia and fetal hyperinsulinemia, the hypothesis of fetal programming is considered a likely explanatory model (6). NAFLD is closely linked to diabetes, obesity, and dyslipidemia and covers a broad spectrum of liver diseases, spanning from simple steatosis to nonalcoholic steatohepatitis (7). Therefore, it is plausible that intrauterine exposure to maternal pregestational diabetes increases the offspring's risk of later NAFLD.

The pathogenesis of NAFLD is not fully understood, but it is hypothesized that specialized macrophages (Kupffer cells) play an important role (8,9). During inflammation, the activation of macrophages results in shedding of CD163, a hemoglobin-haptoglobin scavenger receptor. This can be measured as soluble CD163 (sCD163) and has been linked to NAFLD in both children and adults (10–12). Intrauterine conditions leading to the development of NAFLD have been sparsely studied. Patel et al. (13) described a clear increase in the degree of histologically proven hepatic steatosis in stillborn offspring of women with pregestational diabetes or gestational diabetes mellitus. However, they found no association between degree of steatosis and type of maternal diabetes or maternal glycemic control during pregnancy (13). In studies of neonates, researchers found an association between gestational diabetes mellitus and offspring intrahepatocellular fat, as well as a linear increase in offspring intrahepatocellular fat content with increasing maternal BMI (14,15). Furthermore, an increased risk of ultrasound-diagnosed fatty liver was reported in adolescent offspring of mothers with diabetes or maternal glycosuria (16).

Here, we use the fatty liver index (FLI) described by Bedogni et al. (17) and waist-to-height ratio (WHtR) to evaluate the presence of fatty liver among the offspring (18). Therefore, the overall aim of this study was to evaluate the effect of maternal pregestational type 1 diabetes on the presence of fatty liver in the offspring. Furthermore, we sought to describe any association between maternal glycemic control during pregnancy, maternal prepregnancy BMI, offspring level of sCD163, and metabolic risk factors and later fatty liver in the offspring.

RESEARCH DESIGN AND METHODS

The EPICOM study is a prospective nationwide follow-up study comprising offspring of women with pregestational type 1 diabetes and matched control subjects. The study has been described in detail previously (3). The cohort consists of 278 offspring of women with type 1 diabetes born between 1993 and 1999 and reported to the Danish Diabetes Association Birth Register and 303 control offspring. Only singletons and first child per mother, if the mother had more than one child during the study period, were included in the EPICOM cohort. The control subjects were matched according to sex, date of birth, and postal code as a marker of socioeconomic status. The participants were studied after an overnight fast, and the clinical examination was performed between April 2012 and October 2013 at three hospitals in Denmark (Aarhus, Copenhagen, and Odense). The protocol is in accordance with the Declaration of Helsinki and approved by the regional ethical committee (M-20110239). The clinical trial registration number is NCT01559181 (ClinicalTrials.gov).

Clinical Examination

All measurements except for height were performed three times, and the mean value was used for the analyses. We measured height to the nearest 0.1 cm and weight (in kilograms) to the nearest 0.1 kg. Abdominal circumference was measured using a tape measuring to the nearest 0.5 cm midway between arcus costae and crista iliaca after exhalation, and hip circumference corresponds to the widest measure around the hips. We estimated pubertal stage based on breast development and pubertal hair in girls and genital development and testicular volume in boys (Tanner stage).

Oral Glucose Tolerance Test

We performed a standard 2-h oral glucose tolerance test (OGTT) by using a glucose load of 1.75 g/kg body weight up to a total of 75 g. Venous blood was drawn from an antecubital vein at time 0, 30, and 120 min after the glucose administration. At these time points, plasma glucose, serum insulin, glucagon-like peptide 1 (GLP-1), and glucagon were measured. The OGTT was not performed if the adolescent had previously been diagnosed with diabetes.

We defined diabetes and prediabetes according to the World Health Organization 1999 criteria (19). Diabetes was defined as fasting plasma (p)-glucose $\geq$7.0 mmol/L and/or a 2-h p-glucose $\geq$11.1 mmol/L. Prediabetes was defined as the presence of impaired fasting glucose (IFG) (fasting p-glucose $\geq$6.1 and $<$7.0 mmol/L with 2-h p-glucose $<$7.8 mmol/L) and/or impaired glucose tolerance (IGT) (fasting p-glucose $<$7.0 mmol/L with a 2-h p-glucose $\geq$7.8 mmol/L and $<$11.1 mmol/L). Insulin sensitivity was evaluated by HOMA of insulin resistance (HOMA-IR) (20,21) and insulin secretion by HOMA of insulin secretion (HOMA-β) (20,21).

Biochemical Analyses

Glucose was measured in venous plasma with a hexokinase-glucose-6-phosphate dehydrogenase assay (Abbott Diagnostics, Abbott Park, IL). Serum insulin was measured by ELISA using dual-monoclonal antibodies (ALPCO Diagnostics, Salem, NH). Lipids were measured by enzymatic calorimetric analysis (Abbott), and γ-glutamyl transferase (GGT) was analyzed in venous plasma using the ADVIA Chemistry XPT system. Serum adiponectin and leptin were measured using in-house time-resolved immunofluorometric assay based on monoclonal antibodies (R&D Systems, Minneapolis, MN). GLP-1 and glucagon were measured during the OGTT, and all samples were extracted in a final concentration of 70% ethanol before GLP-1 measurement. Total GLP-1 was measured using a radioimmunoassay specific for the COOH terminus of the GLP-1 molecule (antibody code 89390) and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite (22). For glucagon measurements, we used a C-terminally directed antiserum (code 4305), therefore measuring glucagon of pancreatic origin (23). Sensitivity for both assays was $<$1 pmol/L, and intra-assay coefficient of variation $<$10%. GLP-1 measurements were available for the following: t = 0 min: 301, t = 30 min:

296, and t = 120 min: 299 participants. Glucagon measurements were available for the following: t = 0 min: 218, t = 30 min: 215, and t = 120 min: 217 participants. sCD163 was measured by an in-house ELISA, and the results have been partially presented before, but not in relation to fatty liver disease (24,25). Analyses of maternal HbA_{1c} between 1993 and 1999 were measured with local assays. Correction was made to a common standard (normal range of standard assay, 0.044–0.064) by multiplying the HbA_{1c} value with a correction factor as previously described (mean of the reference values for a standard assay divided by the mean of the reference values for the given assay) (26).

Calculations

FLI was calculated as described by Bedogni et al. (17):

$$\text{FLI} = \left(\frac{e^{0.953 * \log_e(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log_e(\text{GGT}) + 0.053 * \text{AC} - 15.745}}{1 + e^{0.953 * \log_e(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log_e(\text{GGT}) + 0.053 * \text{AC} - 15.745}} \right) \times 100$$

where AC is waist circumference. An FLI $<$30 can be used to rule out NAFLD (sensitivity 87%; negative likelihood ratio = 0.2), and an FLI $\geq$60 can be used to rule in NAFLD (specificity 86%; positive likelihood ratio = 4.3) (17). Therefore, we grouped the exposed offspring and their control subjects into three groups: FLI $<$30, FLI $\geq$30 and $<$60, and FLI $\geq$60. A WHtR $>$0.469 can also be used to rule in NAFLD (sensitivity 70.1%; specificity 76.9%; positive likelihood ratio = 3.0), and we therefore divided the participants into two groups using this cutoff point (18). FLI has been validated in a cohort of 596 adults (age 18–75 years) of European descent and WHtR among children and adolescents in Taiwan (n = 1,210, age 10–19 years) using ultrasound to diagnose NAFLD (17,18). FLI has further been validated against MRS (27).

Birth weight SD scores (bwSDS) were calculated using intrauterine growth curves adjusted for gestational age and sex (28). Being born small for gestational age (SGA) or large for gestational age (LGA) was defined as follows: SGA = bwSDS less than -1.28 and LGA = bwSDS >1.28.

Statistics

Continuous variables with symmetric distribution are presented as means and SD or range, and continuous variables with skewed distribution as medians and interquartile range (IQR). Differences in FLI and WHtR between type 1 diabetes–exposed offspring and their control subjects were analyzed using the rank sum test. We constructed violin plots for maternal prepregnancy BMI, offspring birth weight, adiponectin, leptin, HDL cholesterol, sCD163, HOMA-IR, and HOMA-β by FLI groups and WHtR groups to illustrate data distribution in the groups. Maternal BMI, adiponectin, leptin, fasting glucose, HOMA-IR, and HOMA-β were log transformed because of skewed distribution. The type 1 diabetes–exposed offspring and their matched control subjects were grouped according to FLI group and WHtR group and analyzed using Pearson χ^2 or Fisher exact test when appropriate. Differences between WHtR groups with regard to maternal BMI, offspring birth weight, SGA, LGA, adiponectin, leptin, HDL cholesterol, sCD163, fasting glucose, HOMA-IR, HOMA-β, GLP-1, and glucagon were analyzed using either Student t test or rank sum test. Differences between FLI groups and maternal BMI, offspring birth weight, SGA, LGA, adiponectin, leptin, HDL cholesterol, sCD163, fasting glucose, HOMA-IR, HOMA-β, GLP-1, and glucagon were analyzed using one-way ANOVA with Student t test as post hoc analysis. Bonferroni was used for multiple comparisons. We used two-way ANOVA to detect any possible effects between intrauterine type 1 diabetes exposure and FLI on the above-mentioned variables. We also used one-way ANOVA to study any association between maternal glycemic control in pregnancy and offspring FLI group in offspring of women with type 1 diabetes. Associations between maternal BMI, offspring birth weight, adiponectin, leptin, HDL cholesterol, sCD163, fasting glucose, HOMA-IR, HOMA-β, GLP-1, and glucagon and WHtR were analyzed using a linear regression model yielding WHtR as outcome measure. Maternal prepregnancy BMI, offspring birth weight, adiponectin, leptin, HDL cholesterol, fasting glucose, HOMA-IR, HOMA-β, GLP-1, or glucagon were included as a continuous variable, and the regression coefficient corresponds to 1-unit change in the variable (e.g., change in WHtR if maternal prepregnancy BMI changes from 21 to 22). We adjusted the regression analyses for intrauterine type 1 diabetes exposure and offspring sex, age, and Tanner stage. We used linear regression analysis to study associations between maternal glycemic status in pregnancy and later WHtR and Student t test to analyze differences in maternal glycemic control in pregnancy in the two WHtR groups. In relevant analyses (ANOVA and regression analyses), we examined the residuals to check for violations of the assumptions for the regression analyses. Statistical analyses were done in Stata 13.1, and P values $<$0.05 were considered significant.

RESULTS

Through the Danish Diabetes Association registry and through medical records, we were able to retrieve information regarding pregnancy and birth for the majority of the type 1 diabetes–exposed offspring and their control subjects (Table 1). As previously reported by our group, the adolescent offspring of women with type 1 diabetes were more metabolically unhealthy, having, among other characteristics, higher BMI, and more had prediabetes compared with their matched control subjects (3).

Difference in FLI and WHtR Between Offspring Born to Women With Type 1 Diabetes and Control Subjects

It was possible to calculate WHtR for all, except for one, and FLI could be generated for a total of 264 type 1 diabetes–exposed offspring and 288 control subjects. Overall, offspring of women with type 1 diabetes had higher FLI (median [IQR] 5.3 [2.5–13.7] vs. 3.6 [2.1–6.8], P = 0.001) (Fig. 1) and WHtR (0.44 [0.05] vs. 0.42 [0.04], P = $<$0.001). When grouped according to FLI and WHtR, significantly more offspring of women with type 1 diabetes had FLI $\geq$30 and $<$60 and FLI $\geq$60, or WHtR $>$0.469 (Table 2).

FLI and Offspring Metabolic Risk Factors

There was no difference in age in the different FLI groups. The FLI $\geq$30

Table 1—Baseline and follow-up characteristics of adolescent offspring of women with type 1 diabetes and matched control subjects

	Exposed to type 1 diabetes *n* = 278	Control subjects *n* = 303	*P* value
Baseline characteristics			
Maternal BMI at delivery (kg/m^2)	22.9 (21.3–25.1)	22.6 (20.6–24.8)	0.10
Parity	1.5 (1–5)	1.8 (1–6)	<0.001
Gestational age at birth (days)	260 (250–266)	280 (273–287)	<0.001
Birth weight (g)	3,562 (792)	3,564 (480)	0.13
SGA*	17 (7)	23 (10)	0.12
LGA†	150 (58)	15 (7)	<0.001
Maternal HbA_{1c} (mmol/mol) [%]			
Pregestational	61 (25–107) [7.7 (4.4–11.9)]		
First trimester	56 (21–89) [7.3 (4.1–10.3)]		
Second trimester	49 (49–91) [6.6 (4.6–10.5)]		
Third trimester	50 (29–92) [6.7 (4.8–10.6)]		
Pregestational albuminuria			
Normoalbuminuria	210 (76)		
Microalbuminuria	17 (6)		
Macroalbuminuria	13 (5)		
Status unknown	38 (14)		
Pregestational retinopathy			
No retinopathy	152 (55)		
Simplex	64 (23)		
Proliferative	20 (7)		
Status unknown	42 (15)		
Pregestational hypertension			
≤140/90 mmHg	247 (89)		
>140/90 mmHg	5 (2)		
Antihypertensive treatment	26 (9)		
Preeclampsia‡			
No preeclampsia	211 (76)		
Preeclampsia	56 (20)		
Status unknown	11 (4)		
Data at follow-up			
Male sex, *n* (%)	114 (41)	121 (40)	
Age (years)	16.7 (1.66)	16.8 (1.78)	0.37
BMI (kg/m^2)	23.1 (4.5)	21.6 (3.3)	<0.001
Abdominal circumference (cm)	74.9 (9.1)	71.4 (7.3)	<0.001
Systolic blood pressure (mmHg)	120 (11)	118 (10)	0.01
Diastolic blood pressure (mmHg)	66 (8)	64 (6)	0.03
HDL cholesterol (mmol/L)	1.39 (0.32)	1.44 (0.31)	0.04
LDL cholesterol (mmol/L)	2.2 (0.61)	2.2 (0.62)	0.55
HbA_{1c} (mmol/mol) [%]	34 (28–81) [5.3 (4.7–9.6)]	33 (22–40) [5.2 (4.2–5.8)]	0.23
OGTT 0 min GLP-1 (pmol/L)	8.8 (4.0)	8.7 (4.6)	0.78
OGTT 30 min GLP-1 (pmol/L)	12.4 (5.5)	10.7 (4.5)	0.006
OGTT 120 min GLP-1 (pmol/L)	10.7 (5.4)	10.4 (5.4)	0.79
OGTT 0 min glucagon (pmol/L)	6 (1–26)	7 (1–34)	0.58
OGTT 30 min glucagon (pmol/L)	4 (1–23)	3 (1–19)	0.24
OGTT 120 min glucagon (pmol/L)	2 (1–23)	2 (1–27)	0.69
AG (AG = diabetes + IFG + IGT), n (%)	41 (17)	24 (9)	0.009
HOMA-IR	2.2 (0.3–14)	2.0 (0.3–5.7)	0.01
HOMA-β	94 (54)	86 (33)	0.30
Adiponectin (mg/L)	11.4 (3.9)	11.8 (3.7)	0.14
Leptin (μg/L)	14.4 (1–161)	12.3 (0.6–80)	0.01
sCD163 (mg/L)	1.5 (1.2–1.9)	1.4 (1.1–1.7)	0.001

Data are presented as mean (SD) or median (range) unless otherwise specified. *P* values are generated using Student *t* test, Wilcoxon rank sum test, or χ^2. Baseline data are not available for mothers in the control group since they were matched at follow-up and not monitored during pregnancy. *Defined according to Marsal formul: SGA = bwSDS less than −1.28. †Defined according to Marsal formula: LGA = bwSDS >1.28. ‡Preeclampsia was defined as BP >140/90 mmHg and proteinuria 2+ on a urine protein test strip (equal to 1.0 g/L).

and <60 and FLI ≥60 groups included more females, but the difference was not statistically different between the three groups (Table 3). Alanine transaminase (ALAT), which is not included in FLI but is often used as a crude indicator of fatty liver disease, was found to increase with FLI group. Systolic and diastolic blood pressure at follow-up increased with FLI

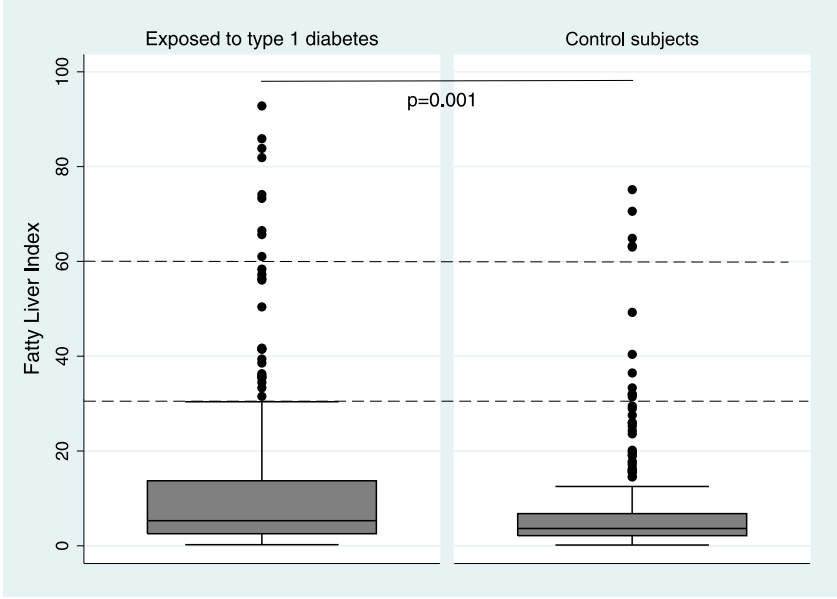

Figure 1—FLI in offspring of women with type 1 diabetes and matched control subjects. The box plot displays median and IQR. The whiskers are upper and lower adjacent value.

group, and for systolic blood pressure, the difference between FLI groups seemed to be affected by intrauterine type 1 diabetes exposure (P = 0.03).

Adiponectin significantly decreased whereas leptin and sCD163 increased with FLI group (Supplementary Fig. 1 and Table 3). As leptin levels differ between sexes, we performed a supplementary analysis dividing the offspring according to sex. This did not change the overall results (Table 3). For lipids, HDL cholesterol decreased with FLI group (Supplementary Fig. 1 and Table 3) whereas LDL cholesterol increased proportionally with increasing FLI group (Table 3). For GLP-1 and glucagon, we found no association with FLI group.

We found no association between either abnormal glucose tolerance (AG) (defined as having diabetes, IFG, or IGT) and FLI group or fasting glucose and FLI group (not shown). Both HOMA-IR and HOMA-β increased significantly with FLI group (Supplementary Fig. 1 and Table 3).

For 391 subjects, we had information on maternal prepregnancy BMI and found a positive association with increasing FLI group (Supplementary Fig. 1 and Table 3). We did not find an association between gestational age or birth weight and FLI group. We found an association between being born LGA and FLI group, an association that persisted after stratifying for intrauterine type 1 diabetes exposure (P = 0.03). There was no difference in number of SGA offspring in the FLI groups.

In the group of offspring of women with type 1 diabetes, we had information on pregestational HbA_{1c} for 222 mothers, first trimester HbA_{1c} for 242, second trimester HbA_{1c} for 249, and third trimester HbA_{1c} for 242. There was no association between maternal glycemic status and later offspring FLI group (not shown).

Overall, several risk factors were associated with FLI group (Table 3). However, among these risk factors, only systolic blood pressure and LGA were also associated with exposure to type 1 diabetes.

Table 2—Comparison of indicators of fatty liver among offspring of mothers with type 1 diabetes and control subjects

	n	Exposed to type 1 diabetes	Control subjects	*P* value
FLI <30	513	237	276	0.02*
FLI ≥30 and <60	25	18	7	
FLI ≥60	14	9	5	
WHtR ≤0.469	489	213	276	<0.001†
WHtR >0.469	91	64	27	

*Fisher exact test. †Pearson χ^2 test.

WHtR and Offspring Metabolic Risk Factors

In opposition to FLI, we found age to be significantly higher among those having a WHtR >0.469 (17.1 years [1.8] vs. 16.7 years [1.7], P = 0.02). Also, more females were present in the group with WHtR >0.469 (71% vs. 29%, P = 0.01).

We found both systolic and diastolic blood pressure to be higher if WHtR >0.469 (systolic pressure 122 mmHg [10.7] vs. 118 mmHg [10.4], $P < 0.001$; diastolic pressure 69 mmHg [8.5] vs. 64 mmHg [6.5], $P < 0.001$). ALAT was also found to be higher among those with WHtR >0.469 (median [IQR] 19 units/L [15–24] vs. 16 units/L [12–20], $P < 0.001$).

The level of adiponectin was significantly lower if WHtR >0.469 (median [IQR] 9.8 mg/L [8.1–11.4] vs. 11.3 mg/L [9.2–14.1], $P < 0.001$) (Supplementary Fig. 2), and the leptin level was significantly increased (39.4 μg/L [17.8–62.4] vs. 10.8 μg/L [3.7–21.1], $P < 0.001$). Also, the sCD163 level was increased in the participants with high WHtR (1.75 mg/L [0.6] vs. 1.49 mg/L [0.5], $P < 0.001$). For lipids, we found HDL cholesterol to be decreased among those with WHtR >0.469 (1.3 mmol/L [0.3] vs. 1.4 mmol/L [0.3], $P < 0.001$) (Supplementary Fig. 2), whereas LDL cholesterol levels were significantly higher if WHtR >0.469 (median [IQR] 2.4 mmol/L [2.0–3.0] vs. 2.1 mmol/L [1.7–2.5], $P < 0.001$). As with FLI, we found no association between level of GLP-1 or glucagon during the OGTT, AG, or fasting glucose and WHtR (AG and fasting glucose results not shown). Both HOMA-IR and HOMA-β were significantly higher if WHtR >0.469 (median [IQR] 3.0 [2.1–4.2] vs. 2.0 [1.5–2.6], $P < 0.001$; and 113.3 [81.4–149.4] vs. 78.8 [59.4–101.4], $P < 0.001$) (Supplementary Fig. 2).

As with FLI, maternal prepregnancy BMI was increased in the high-WHtR group (Supplementary Fig. 2 and Supplementary Table 1) and more were LGA (Supplementary Table 1). We did not find any association between gestational age, birth weight, or SGA and WHtR >0.469.

Table 3—Maternal and metabolic risk factors in subjects (offspring exposed to type 1 diabetes and control subjects) with low (FLI <30), intermediate (FLI ≥30 and <60), and high (FLI ≥60) likelihood of fatty liver disease

	FLI <30	FLI ≥30 and < 60	FLI ≥60	*P* values between groups	*P* values between type 1 diabetes–exposed and control subjects*
Maternal prepregnancy BMI (kg/m^2)	22.8 (21.0–24.8)	23.0 (21.2–26.4)	25.8 (23.5–27.3)†	†0.003	0.72
Birth weight (g)	3,552 (682)	3,560 (493)	3,894 (501)	NS	0.89
Gestational age at birth (days)	269 (259–280)	264 (250–268)	265 (261–272)	NS	0.19
SGA‖ (yes/no)	37/391	1/20	0/12	0.77	0.33#
LGA¶ (yes/no)	136/292	12/9	9/3	0.001	0.03#
Follow-up					
Sex (male/female)	208/305	12/13	3/11	NS	
Age (years)	16.6 (15.3–18.1)	17.7 (16.3–18.5)	17.5 (16.5–19.2)	NS	0.62
Systolic blood pressure (mmHg)	117 (111–125)	128 (123–135)†	121 (120–125)	<0.001†	0.03
Diastolic blood pressure (mmHg)	64 (60–69)‡	68 (63–74)	72 (69–76)†	0.003‡, <0.001†	0.07
Adiponectin (mg/L)	11.2 (9.1–14.0)‡	9.6 (8.9–10.8)	8.6 (6.2–10.4)†	0.04‡, 0.002†	0.99
Leptin (μg/L)	11.9 (4.1–24.0)‡	31.3 (16.3–58.8)	79.1 (40.8–95.8)†	<0.001‡, <0.001†	0.65
Leptin (male)	3.3 (2.0–6.1)‡	15.5 (8.6–18.2)§	38.5 (36.5–96.1)†	<0.001‡, §0.011, <0.001†	0.68
Leptin (female)	20.7 (13.2–32.6)‡	58.8 (51.5–72.1)	85.6 (48.7–95.8)†	<0.001‡, <0.001†	0.49
HDL cholesterol (mmol/L)	1.4 (0.31)‡	1.3 (0.32)	1.04 (0.23)†	0.03‡, <0.001†	0.71
LDL cholesterol (mmol/L)	2.1 (0.60)	2.3 (0.73)§	2.9 (0.69)†	0.03§, <0.001†	0.14
ALAT (units/L)	16 (12–20)‡	19 (15–33)	22 (19–38)†	0.03‡, <0.001†	0.74
sCD163 (mg/L)	1.51 (0.5)	1.76 (0.6)	2.05 (0.6)†	<0.001†	0.20
HOMA-IR	2.0 (1.6–2.7)‡	3.1 (2.6–4.2)	4.3 (3.5–5.5)†	<0.001‡, <0.001†	0.82
HOMA-β	80.9 (60.4–103.8)‡	112.1 (90.1–144.3)§	168.4 (118.7–245.0)†	<0.001‡, 0.02§, <0.001†	0.49
GLP-1, *t* = 0 min (pmol/L)	8.9 (4.36)	6.8 (3.49)	8.7 (3.39)	NS	0.90
GLP-1, *t* = 30 min (pmol/L)	11.5 (5.19)	11.3 (4.48)	11.4 (2.83)	NS	0.36
GLP-1, *t* = 120 min (pmol/L)	10.7 (5.54)	9.1 (3.75)	11.4 (3.71)	NS	0.53
Glucagon, *t* = 0 min (pmol/L)	6 (4–9)	6 (3–8)	4.5 (3.5–6.5)	NS	0.70
Glucagon, *t* = 30 min (pmol/L)	4 (2–6)	5 (1–6)	5 (3–6)	NS	0.41
Glucagon, *t* = 120 5min (pmol/L)	2 (1–5)	1 (1–5)	2 (1–9)	NS	0.11

Data displayed are mean (SD), median (IQR), or number. One-way ANOVA was used to compare groups and Student *t* test for post hoc analysis. *Two-way ANOVA was used to compare type 1 diabetes–exposed offspring with control subjects. †Significantly different from FLI <30. ‡Significantly different from FLI ≥30 and <60. §Significantly different from FLI ≥60; Bonferroni correction was used for multiple comparison. ‖Defined according to Marsal formula: SGA = bwSDS less than −1.28. ¶Defined according to Marsal formula: LGA = bwSDS >1.28. #Pearson χ^2 or Fisher exact test was used to compare groups and stratified by type 1 diabetes exposure status.

Among offspring of mothers with type 1 diabetes, a WHtR >0.469 was associated with significantly higher maternal HbA_{1c} in the third trimester (52 vs. 49 mmol/L, *P* = 0.04) but not pregestational or first or second trimester HbA_{1c} (not shown).

Analyzing WHtR as a continuous variable, we found WHtR to be positively associated with maternal prepregnancy BMI, offspring systolic and diastolic blood pressure, leptin, sCD163, LDL cholesterol, HOMA-IR, and HOMA-β, whereas WHtR was negatively associated with offspring HDL cholesterol and adiponectin (Supplementary Table 2). All these associations persisted after adjusting for intrauterine type 1 diabetes exposure and offspring sex, age, and Tanner stage. There was no association between WHtR as a continuous variable and GLP-1, glucagon, or fasting glucose.

CONCLUSIONS

In the current study, we report that offspring of women with type 1 diabetes have higher FLI and WHtR than their matched control subjects, indicating that more of them are at risk for having fatty liver disease. We found that the presence of elevated FLI and WHtR, and thus a high risk of fatty liver disease, was associated with insulin resistance, inappropriate lipid levels, altered levels of adipokines, sCD163, and higher blood pressure. Also, maternal prepregnancy BMI and being born LGA were positively associated with a higher risk of indices associated with fatty liver.

To our knowledge, this is the first study to explore the risk of fatty liver in offspring of women with type 1 diabetes. Fatty liver among adolescent offspring of women with gestational diabetes mellitus and/or high maternal prepregnancy BMI has been studied before (16,29), but the results of these studies have been diverging. Bellatorre et al. (29) described offspring hepatic fat fraction (estimated by MRI) being associated with maternal prepregnancy BMI but not gestational diabetes mellitus status, and Patel et al. (16) described an association between offspring fatty liver (defined by ultrasound assessment) and maternal

diabetes/glycosuria but not maternal prepregnancy BMI (after adjustment for offspring current fat mass). In the current study, we found an association between offspring risk of fatty liver and both maternal type 1 diabetes and prepregnancy BMI. Maternal HbA_{1c} in the third trimester was significantly higher in the group of offspring with WHtR >0.469. However, we did not find any association between maternal glycemic status and FLI group or WHtR as a continuous variable. This could indicate that the presence of indices of fatty liver among offspring of women with type 1 diabetes is a consequence of a metabolically unhealthy mother, rather than intrauterine hyperglycemia itself.

Previously, the association between fatty liver (using FLI) and insulin resistance, coronary heart disease, and early atherosclerosis has been evaluated in a cohort of adults without diabetes aged 30–60 years (the Relationship Between Insulin Sensitivity and Cardiovascular Disease [RISC] study) (30). As in our study, a high FLI was associated with an unhealthy metabolic profile with reduced insulin sensitivity (using the euglycemic-hyperinsulinemic clamp) and, furthermore, a higher risk of coronary heart disease (30). In comparison, we found similar results in our study where the participants have an average age of 17 years. In the RISC study, more men than women were represented in the group with the highest FLI. The opposite applied to the EPICOM cohort, where female offspring had the highest FLI and WHtR. In the EPICOM study, we have previously described reduced insulin sensitivity among the oldest offspring of women with type 1 diabetes (17+ years), and especially among the female participants (31). We also described adiponectin to be significantly lower among the type 1 diabetes–exposed female offspring from the EPICOM cohort, indicating sex differences among the type 1 diabetes–exposed offspring (32).

The pathogenesis behind the development of NAFLD in offspring exposed to an adverse intrauterine environment has been studied in animal models. In mice, a high-fat diet during gestation increased the risk of NAFLD in the offspring, and high-fat diet after weaning increased the risk of progression to nonalcoholic steatohepatitis in the offspring. Hepatic mitochondrial electron transport chain enzyme complex activity was reduced among the offspring exposed to a high-fat diet during gestation, and hepatic gene expression of lipogenesis, oxidative stress, and inflammatory pathways was upregulated (33). In nonhuman primates, maternal high-fat feeding during gestation also increased liver triglycerides in the offspring along with upregulation of pathways for hepatic de novo lipid synthesis and inflammation (34). During pregnancy, subcutaneous fat is not available for lipid storage until the third trimester. Therefore, Brumbaugh and Friedman (36) hypothesized that the fetus uses other organs like the liver for excess energy storage, laying the foundation for the development of NAFLD later in life (35).

Consistent with previous studies, we found adipokine levels to differ with FLI and WHtR. Lower levels of adiponectin have been described in patients with NAFLD, and even though leptin levels are more controversial, a recent meta-analysis found leptin to be higher among NAFLD patients (37,38). A positive association between levels of sCD163 and NAFLD (NAFLD defined either by ultrasound, by index, or biopsy-verified NAFLD) has previously been described, and we therefore included this variable to validate our findings (10–12). Our study, in accordance with previous studies, found sCD163 to differ with the degree of fatty liver, here expressed as FLI and/or WHtR group. However, although the level of sCD163 correlates with biopsy-verified NAFLD and progression of NAFLD in adults, the picture among children is less clear (12,39).

In our study, we had access to GLP-1 measurements for approximately half the participants and glucagon for one-third of the patients. Prior studies have described an impaired incretin effect and fasting hyperglucagonemia in patients with NAFLD, but in our study, we did not find an association between GLP-1 or glucagon levels and increasing FLI or WHtR (40). This could be due to the limited number of subjects analyzed but also the young age of the participants.

An obvious limitation of our study is the use of FLI and WHtR to define fatty liver. Ultrasound assessment, hepatic MRI, or liver biopsy were not a part of the EPICOM study setup. However, considering reports of a 9.6% prevalence of fatty liver in the U.S. and risk of end-stage liver disease induced by NAFLD, it is important to consider surrogate measures of NAFLD (41,42). FLI is only validated in adults (age 18–75 years), but since the mean age in the EPICOM cohort was ~17 years and ~90% of our cohort had achieved pubertal development corresponding to Tanner stage 4 or 5, we find the use of the FLI justified. In contrast, WHtR is validated among children and adolescents. Therefore, we chose to describe both FLI and WHtR. The results are similar, but the use of the two indexes is not without limitations. Contrary to WHtR, only systolic blood pressure and LGA in the FLI groups were associated with maternal type 1 diabetes. This dissimilarity could display a power problem as only a few participants have high FLI. Also, the WHtR cutoff for fatty liver is solely described in the study by Lin et al. (18), and it is therefore unknown if the same cutoff is applicable to our Danish population. A review describes a cutoff for WHtR of 0.5 as a universal sex-neutral cutoff for cardiometabolic disease (43). Therefore, one could speculate that the cutoff defined by Lin et al. (18) is too low, but for now, no other studies in adolescents have defined another WHtR cutoff for fatty liver disease. And as a consequence of the absence of diagnostic imaging of fatty liver disease in the EPICOM cohort, we chose to use the indexes available. Another limitation is the lack of information on alcohol consumption in our group. However, our group is young, and we have no reason to assume that offspring of women with type 1 diabetes consume larger amounts of alcohol than their control subjects. Our study participants were not tested for hepatitis, which is associated with hepatic steatosis and level of sCD163, but none of the participants reported having hepatitis upon inclusion in the EPICOM study.

The study has several strengths. It is the largest cohort comprising offspring of women with type 1 diabetes. The phenotype is well described, and we have extensive information regarding pregnancy and birth, including information on maternal glycemic control in pregnancy and maternal prepregnancy BMI.

In conclusion, our findings suggest that intrauterine exposure to maternal type 1

diabetes and higher maternal prepregnancy BMI may predispose to fatty liver. In addition, offspring metabolic risk factors were associated with indices of fatty liver disease.

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