RESEARCH

Steroid metabolomic signature of liver disease in nonsyndromic childhood obesity

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Abstract

Objective: Analysis of steroids by gas chromatography-mass spectrometry (GC-MS) defines a subject's steroidal fingerprint. Here, we compare the steroidal fingerprints of obese children with or without liver disease to identify the 'steroid metabolomic signature' of childhood nonalcoholic fatty liver disease.
Methods: Urinary samples of 85 children aged 8.5–18.0 years with BMI >97% were quantified for 31 steroid metabolites by GC-MS. The fingerprints of 21 children with liver disease (L1) as assessed by sonographic steatosis ($L1^{L}$), elevated alanine aminotransferases ($L1^{A}$) or both ($L1^{AL}$), were compared to 64 children without markers of liver disease (L0). The steroidal signature of the liver disease was generated as the difference in profiles of L1 against L0 groups.
Results: L1 comparing to L0 presented higher fasting triglycerides ($P = 0.004$), insulin ($P = 0.002$), INS/GLU ($P = 0.003$), HOMA-IR ($P = 0.002$), GGTP ($P = 0.006$), AST/SGOT ($P = 0.002$), postprandial glucose ($P = 0.001$) and insulin ($P = 0.011$). $L1^{AL}$ showed highest level of T-cholesterol and triglycerides ($P = 0.029$; $P = 0.044$). Fasting insulin, postprandial glucose, INS/GLU and HOMA-IR were highest in $L1^{L}$ and $L1^{AL}$ ($P = 0.001$; $P = 0.017$; $P = 0.001$; $P = 0.001$). The liver disease steroidal signature was marked by lower DHEA and its metabolites, higher glucocorticoids (mostly tetrahydrocortisone) and lower mineralocorticoid metabolites than L0. L1 patients showed higher 5α-reductase and 21-hydroxylase activity (the highest in $L1^{A}$ and $L1^{AL}$) and lower activity of 11βHSD1 than L0 ($P = 0.041$, $P = 0.009$, $P = 0.019$).
Conclusions: The 'steroid metabolomic signature' of liver disease in childhood obesity provides a new approach to the diagnosis and further understanding of its metabolic consequences. It reflects the derangements of steroid metabolism in NAFLD that includes enhanced glucocorticoids and deranged androgens and mineralocorticoids.

Key Words
- liver disease
- obesity
- steroid profile
- childhood
- childhood obesity

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Introduction

Nonsyndromic childhood obesity is associated with nonalcoholic fatty liver disease (NAFLD), a spectrum of conditions, ranging from steatosis to nonalcoholic steatohepatitis (NASH), and various degrees of fibrosis and cirrhosis (1). NAFLD is regarded as the hepatic manifestation of the metabolic syndrome (2). However, childhood obesity with no NAFLD is also complicated by the metabolic syndrome. Despite the growth of knowledge regarding obesity-related NAFLD in children, we still rely mostly on circulating levels of liver enzymes and ultrasonography imaging and some non-invasive tests (3, 4, 5, 6, 7). Liver biopsy in children with suspected NAFLD is recommended only for 'those where the diagnosis is unclear, where there is possibility of multiple diagnoses or before starting therapy with potentially hepatotoxic medications' (8).

The consequences of obesity-related NAFLD on liver metabolism are insufficiently understood (8). As steroid hormones are partially catabolized and conjugated by liver enzymes, we have anticipated that NAFLD would have its metabolic impact on steroid metabolism.

Here, we utilized our previously reported concept, arguing that an individual's urinary steroid metabolite profile represents a subject's unique metabolic fingerprint and offers means of metabolomic phenotyping at the individual level (9, 10). Thus, each individual has a unique 'steroidal fingerprint'. A cluster of similar 'steroidal fingerprints' related to a disease would be regarded as a 'steroid metabolomic disease signature' (10, 11), which represents the impact of a disease in people who differ in their phenotypes or have other health problems. We have previously clustered steroidal fingerprints of children with nonsyndromic obesity into five clusters with distinctive steroidal signatures (11).

Here, we analyzed the clinical data of a group of 85 patients with well-phenotyped nonsyndromic childhood obesity and defined those affected and those unaffected by NAFLD and/or elevated activities of liver enzymes. We generated steroidal disease signatures of the two groups and suggest that it might shed light on steroid-related metabolic sequelae of liver disease in childhood obesity.

Subjects and methods

Between March 2012 and August 2013, we examined a consecutive series of 117 obese Caucasian children and adolescents (BMI >97th centile). They were recruited from the patients referred to the Department of Pediatric Endocrinology, Medical University of Silesia, Katowice, Poland. After exclusion of younger participants (<8 years), syndromic obesity, chronic diseases, pharmacotherapy (also metformin) or precocious puberty, we included the remaining 85 patients (43 girls), aged 8.5–18.0 years (mean age 14.4, SD. 2.33, median 14.5 years).

All patients underwent a clinical assessment and diagnostic procedures that included general physical examination, anthropometric measurements of height, weight, waist and hip circumference and puberty assessment, as previously described (11). Morning fasting venous blood samples were collected to measure lipids, glucose (GLU), insulin (INS), TSH, fT4, cortisol and aminotransferases. Plasma total cholesterol (T Chol), high-density lipoprotein cholesterol (HDL-Chol) and triglyceride (TG) levels were analyzed enzymatically (Beckman Coulter). GLU and INS levels were also measured in an oral glucose tolerance test (OGTT, 1.75 g/kg, max 75 g). Enzymatic test (hexokinase method) was used for the quantitative determination of glucose (Beckman Coulter). INS was determined using a chemiluminescence immunoassay on Immulite 2000 analyzer (DPC, USA). Fasting INS/GLU ratio (FIGR) and homeostatic model assessment of INS resistance (R-HOMA, fasting GLU (mmol/L) × fasting INS (mIU/L)/22.5) were calculated as indices of insulin resistance. Cortisol was measured in the morning (08:00 h) and midnight using chemiluminescent immunoassay by Immulite 2000 analyzer (DPC, USA). Serum concentrations of fT4 and TSH were measured with a chemiluminescent immunometric assay (Siemens, Immulite 2000 Free T4 , Immulite 2000 Third Generation TSH). Gamma-glutamyl transpeptidase (GGTP), alanine (ALT/GPT) and aspartate aminotransferases (AST/SGOT) activities in the serum were assessed according to International Federation in Clinical Chemistry (Beckman Coulter).

Assessment of liver disease/NAFLD

Abdomen ultrasonography to evaluate the liver for hepatic steatosis features was performed with 5 MHz convex transducer (Logiq 5, GE Healthcare GmbH), according to the standards in pediatric population (12). The evidence of hepatic steatosis by abdominal ultrasound (hepatic echogenicity increased above the echogenicity of the adjacent right renal cortex and increase in fine echoes of liver parenchyma compared with intrahepatic vessel borders (7, 13)), and no causes for secondary hepatic fat accumulation, is defined here for NAFLD diagnosis (5, 7, 12). Any markers of liver dysfunction

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(elevated ALT >45 U/L – $L1^{A}$, NAFLD based on ultrasonography – $L1^{L}$, or both – $L1^{AL}$) where defined as liver disease – L1 as compared to L0 – without markers of liver disease.

Gas chromatography-mass spectrometry (GC-MS) of urinary steroids

Steroid metabolites in 24-h urine samples were analyzed by quantitative targeted GC-MS (9, 10, 11). Briefly, free and conjugated urinary steroids were extracted by solid phase extraction and conjugates were enzymatically hydrolyzed. After recovery of hydrolyzed steroids by solid phase extraction, known amounts of internal standards (5α-androstane-3α,17α-diol, stigmasterol) were added to each extract before formation of methyloxime-trimethylsilyl ethers. GC was performed using an Optima-1 fused silica column (Macherey–Nagel, Dueren, Germany) housed in an Agilent Technologies 6890 series GC that was directly interfaced to an Agilent Technologies 5975 inert XL mass selective detector. After calibration, values for the excretion of individual steroids were determined by measuring the selected ion peak areas against the internal standard areas.

Steroid metabolites' ratios, as described in our previous paper (11), were used to calculate the activity of the enzymes: 5α reductase (An/Et) (5αTHF/THF, 5αTHB/THB), 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) ((THF + αTHF)/THE), 3β-hydroxysteroid dehydrogenase-(3βHSD) ((THE + THF + αTHF)/P5T-17α) and 21-hydroxylase ((THE + THF + αTHF)/PT, (THE + THF + αTHF)/PO5α3α).

The study was conducted according to Helsinki declaration, and approved by the Ethics Committee of the Medical University of Silesia. Informed consent was

Table 1 Comparison of clinical (A) and chemical (B) phenotype of non-liver disease patients (L0) and patients with liver disease features (L1).

	Mean L0 (*n* = 64)	**S.D. L0**	**Mean L1** (*n* = 21)	**S.D. L1**	***P* value**
A. Clinical phenotype					
Sex (f/m)	36/28		7/14		0.001
Age (years)	14.4	2.3	14.0	2.4	NS
Weight (kg)	87.0	20.0	91.2	21.8	NS
Height (cm)	164.0	11.4	166.1	11.5	NS
hSDS	0.4	1.4	0.8	1.6	NS
BMI (kg/m^2)	32.0	5.2	32.8	5.4	NS
BMI z score IOTF	2.7	0.5	2.8	0.5	NS
Waist (cm)	100.6	11.5	102.1	11.6	NS
WHR	0.9	0.1	1.0	0.0	0.022
BP systolic (mmHg)	125	11	130	12	NS
BP diastolic (mmHg)	78	8	76	10	NS
B. Chemical phenotype					
TSH (μIU/mL)	2.7	1.2	3.0	1.3	NS
Cortisol 8.00 (μg/dL)	17.4	6.2	19.7	6.1	NS
Cortisol 24.00 (μg/dL)	3.4	3.9	1.6	1.0	<0.001
T chol (mg/dL)	171	35	178	31	NS
HDL chol (mg/dL)	50	10	46	12	NS
TG (mg/dL)	134	60	182	76	0.004
GLU 0′ (mg/dL)	90	9	92	10	NS
GLU 120′ (mg/dL)	112	20	129	21	0.001
INS 0′ (μIU/mL)	16.7	9.2	35.1	24.1	0.002
INS 120′ (μIU/mL)	91.6	67.9	163.6	114.2	0.011
INS/GLU 0′	0.2	0.1	0.4	0.3	0.003
INS/GLU 0′ (% > 0.3)	10/64, 15.6%		10/21, 47.6%		0.004
HOMA-IR	3.7	2.2	8.1	5.6	0.002
ALT/GPT (U/L)	24	9	54	29	<0.001
AST/SGOT (U/L)	25	8	36	13	0.002
GGTP (U/L)	22	10	33	12	0.006

Values are means and S.D. Significance by Student *t* test.
ALT/GPT, alanine aminotransferases; AST/SGOT, aspartate aminotransferases; BMI, body mass index; BP, blood pressure; f, female; GGTP, gamma-glutamyl transpeptidase; GLU, glucose; HDL-chol, HDL-cholesterol; hSDS, height standard deviation score; INS, insulin; L0, non-liver disease patients; L1, liver disease patients (ALT+ or sonographic liver steatosis or both); m, male; NS, not significant; T chol, total cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone; WHR, waist-to-hip ratio.

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Table 2 Comparison of clinical (A) and chemical (B) phenotype of non-liver disease patients (L0) and patients with liver disease features: $L1^{A}$ (ALT+).

Ratio*	L0 Mean ± SD. (*n* = 64)	$L1^{A}$ Mean ± SD. (*n* = 5)	$L1^{L}$ Mean ± SD. (*n* = 10)	$L1^{LA}$ Mean ± SD. (*n* = 6)	*P* value	All
A. Clinical phenotype						
Sex (f/m)	36/28	2/3	4/6	1/5		43/42
Age (years)	14.4 ± 2.3	15.4 ± 1.5	13.0 ± 2.3	14.6 ± 2.6	NS	14.3 ± 2.3
Weight (kg)	87.0 ± 20.0	110.3 ± 21.6	81.0 ± 11.9	92.3 ± 26.5	NS	88.1 ± 20.4
Height (cm)	164.0 ± 11.4	171.2 ± 5.9	162.6 ± 7.7	167.6 ± 18.4	NS	164.5 ± 11.4
hSDS	0.4 ± 1.4	0.4 ± 0.5	1.2 ± 1.2	0.5 ± 2.6	NS	0.5 ± 1.5
BMI (kg/m^2)	32.0 ± 5.2	37.5 ± 5.9	30.6 ± 3.8	32.4 ± 5.5	NS	32.2 ± 5.2
BMI z score IOTF	2.7 ± 0.5	3.1 ± 0.5	2.7 ± 0.5	2.8 ± 0.4	NS	2.7 ± 0.5
Waist (cm)	100.6 ± 11.5	115.8 ± 16.3	98.6 ± 7.1	98.6 ± 10.1	NS	101.0 ± 11.5
WHR	0.93 ± 0.1	0.98 ± 0.00	0.97 ± 0.0	1.00 ± 0.03	NS	0.95 ± 0.09
BP systolic (mmHg)	125 ± 11	136 ± 15	129 ± 11	124 ± 5	NS	126 ± 11
BP diastolic (mmHg)	78 ± 8	80 ± 10	76 ± 11	74 ± 5	NS	77 ± 8
B. Chemical phenotype						
TSH (μIU/mL)	2.7 ± 1.2	3.3 ± 1.0	3.0 ± 1.6	2.8 ± 1.1	NS	2.8 ± 1.2
Cortisol 8.00 (μg/dL)	17.4 ± 6.1	22.3 ± 3.2	19.3 ± 4.0	18.2 ± 10.0	NS	18.0 ± 6.2
Cortisol 24.00 (μg/dL)	3.4 ± 3.9	1.7 ± 1.2	1.5 ± 1.2	1.4 ± 0.2	NS	2.9 ± 3.5
T chol (mg/dL)	171 ± 35	182 ± 32	158 ± 18	208 ± 23	0.029 $L1^{LA} > L1^{L}$	172 ± 34
HDL-chol (mg/dL)	50 ± 10	49 ± 19	43 ± 9	49 ± 10	NS	49 ± 10
TG (mg/dL)	134 ± 60	176 ± 86	184 ± 67	184 ± 94	0.044 $L1^{LA}$ and $L1^{L} > L0$	146 ± 67
GLU 0′ (mg/dL)	90 ± 9	89 ± 9	90 ± 7	97 ± 13	NS	90 ± 9
GLU 120′ (mg/dL)	112 ± 20	126 ± 5	130 ± 22	129 ± 30	0.017 $L1^{L} > L0$	116 ± 21
INS 0′ (μIU/mL)	16.7 ± 9.2	23.1 ± 8.7	39.1 ± 29.9	38.5 ± 21.6	0.001 $L1^{L}$, $L1^{LA} > L0$	21.3 ± 16.3
INS 120′ (μIU/mL)	91.6 ± 67.9	139.6 ± 85.4	186.1 ± 140.5	146.0 ± 93.9	0.033 $L1^{L} > L0$	109.6 ± 87.0
INS/GLU	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.4 ± 0.2	0.001 $L1^{L}$, $L1^{LA} > L0$	0.2 ± 0.2
INS/GLU (*n*, % > 0.3)	10/64, 15.6%	1/5, 20%	6/10, 60%	3/6, 50%	0.014 $L1^{L}$, $L1^{LA} > L0$	20/85, 23.5%
HOMA-IR	3.7 ± 2.2	5.1 ± 2.2	8.8 ± 6.7	9.4 ± 5.5	0.001 $L1^{LA}$, $L1^{L} > L0$	4.8 ± 3.9
ALT/GPT (U/L)	24 ± 9	77 ± 33	34 ± 9	69 ± 28	<0.0001 $L1^{A}$, $L1^{LA} > L1^{L} > L0$	31 ± 21
AST/SGOT (U/L)	25 ± 8	47 ± 14	27 ± 4	43 ± 15	0.0001 $L1^{A}$, $L1^{LA} > L1^{L}$, L0	28 ± 11
GGTP (U/L)	22 ± 10	39 ± 10	26 ± 12	35 ± 12	0.003 $L1^{A}$, $L1^{LA} > L0$, $L1^{L}$	25 ± 12

$L1^{L}$ (sonographic liver steatosis) and $L1^{LA}$ (both ALT+ and sonographic liver steatosis). Values are means and SD. Significance by ANOVA.
ALT/GPT, alanine aminotransferases; AST/SGOT, aspartate aminotransferases; BMI, body mass index; BP, blood pressure; f, female; GGTP, gamma-glutamyl transpeptidase; GLU, glucose; HDL-chol, HDL-cholesterol; hSDS, height standard deviation score; INS, insulin; L0, non-liver disease patients; L1, liver disease patients; $L1^{A}$, patients L1 with ALT+; $L1^{AL}$, patients with ALT+ and sonographic liver steatosis; $L1^{L}$, patients L1 with sonographic liver steatosis; m, male; NS, not significant; T chol, total cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone; WHR, waist to hip ratio.

obtained from each patient over age 16 years, a parent or a legal guardian, after full explanation of the purpose and nature of all procedures.

Statistical analysis and visualization of metabolomic data

Steroid metabolites quantities were z-transformed based on sex- and age-adjusted normal reference groups, as described elsewhere (11). Per each of 31 z-transformed steroid metabolites and per each one of the groups L0, L1 and subgroups $L1^{A}$, $L1^{L}$, $L1^{AL}$, the median was computed. The standard R-function 'matplot' (https://www.R-project.org/) (14) was used to depict the steroidal signatures of each subgroup as the difference between the above medians versus the median of L0 group (11).

Clinical and chemical data as well as steroid metabolites' concentrations ratios of patients in each

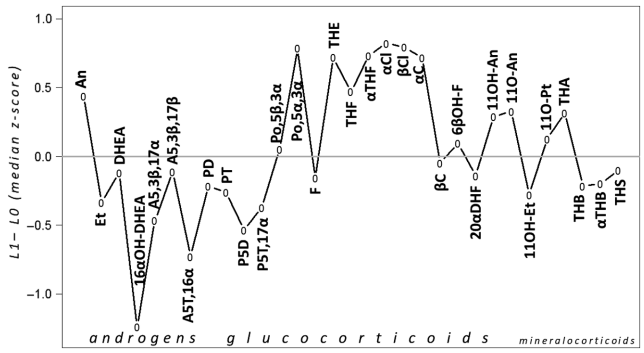

Figure 1
Steroidal signature of liver disease in childhood obesity: differences between z-transformed concentrations of steroid metabolites (androgens, glucocorticoids and mineralocorticoids) in liver diseases (L1) and with non-liver disease features (L0) patients.

group were analyzed, and Student *t* test, *t*-test with separate variance estimation, ANOVA or Kruskal–Wallis ANOVA where appropriate were utilized to assess the difference between groups. *P* value <0.05 was considered statistically significant.

Results

Clinical phenotype

Out of 85 obese children, a liver disease was diagnosed in 21 (21/85, 24.7%; L1); in five patients by elevated ALT activity ($L1^{A}$), in ten by sonographic liver steatosis ($L1^{L}$) and in six by both markers ($L1^{AL}$). The clinical phenotype is presented in Tables 1A and 2A.

The mean age, BMI, BMI z score, hSDS, waist and hip circumference as well as blood pressure values were not significantly different between patients of groups L0 and L1 and among L0, $L1^{A}$, $L1^{L}$, $L1^{AL}$ subgroups. There were relatively more males in L1 group than in L0 (14/21, 67% vs 28/64, 44%, Table 1A).

Chemical phenotype

At the biochemical level, patients of L1 comparing to L0 group presented higher concentration of fasting triglycerides and insulin, postprandial glucose and insulin. Both indices of insulin resistance, insulin/glucose ratio (INS/GLU) and HOMA-IR, GGTP, AST/SGOT activity were significantly higher in L1 group. Comparison of three liver-affected subgroups $L1^{A}$, $L1^{L}$, $L1^{AL}$ and L0 (ANOVA) confirmed significantly the highest level of T cholesterol and triglycerides in $L1^{AL}$ patients. Fasting INS and postprandial GLU levels were higher in $L1^{L}$ and $L1^{AL}$ patients than in $L1^{A}$ and L0, postprandial INS was the highest in $L1^{L}$ group. INS/GLU ratio and HOMA-IR values were also the highest in both groups with liver steatosis features in the ultrasonography – $L1^{L}$ and $L1^{AL}$. The highest GGTP and AST/SGOT activities were observed in $L1^{A}$ group (Table 2B).

Steroidal signature of liver disease

Obese patients of the L0 group presented higher midnight plasma cortisol concentration ($P<0.001$) than the L1 group (Table 1B). Comparing the z-transformed values of steroid metabolites, significantly higher tetrahydrocortisone (THE) concentration were found in L1 group ($P=0.046$).

'Steroidal signature' of liver disease is presented as the difference between z-transformed concentrations of steroid metabolites in L0 and L1 patients (Fig. 1). Liver-affected patients have shown significantly enhanced 5α-reductase and 21-hydroxylase activity and lower activity of 11βHSD1 than L0 subjects (Table 3).

'Steroid metabolomic disease signature' of $L1^{A}$, $L1^{L}$, $L1^{AL}$ are presented in Fig. 2A, B and C. Liver-affected patients $L1^{L}$ presented significantly enhanced activity of 21-hydroxylase and those with elevated ALT ($L1^{A}$ and $L1^{AL}$) showed enhanced 5α-reductase activity (Table 4).

Table 3 Ratio of steroid metabolites (enzyme activity): differences between patients with non-liver diseases (L0) and with liver disease features (L1) patients.

Ratio*	Mean L0 (*n* = 64)	S.D. L0	Mean L1 (*n* = 21)	S.D. L1	*P* value
An/Et (5α reductase)	2.1	0.9	2.5	0.9	0.041
5αTHF/THF (5α reductase)	1.3	0.6	1.3	0.5	NS
5αTHB/THB (5α reductase)	3.4	1.8	3.3	1.3	NS
(THF + αTHF)/THE (11βHSD1)	0.9	0.3	0.7	0.2	0.019
(THE + THF + αTHF)/P5T-17α (3βHSD)	19.1	15.4	24.2	21.3	NS
(THE + THF + αTHF)/PT (21-OHase)	10.1	3.9	13.6	5.1	0.009
(THE + THF + αTHF)/PO5α3α (21-OHase)	300.9	160.4	320.7	193.3	NS

Values are means and S.D. Significance by *t*-Student test.
*Ratio of steroid metabolites was calculated based on steroid metabolites concentrations.
L0, non-liver disease patients; L1, liver disease patients (ALT+ or sonographic liver steatosis or both); NS, not significant.

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Discussion

Based on our previous definition of 'steroid metabolomic disease signature' by quantitative urinary steroidal GC-MS data (10, 11), here we define the steroidal signature of liver disease in nonsyndromic childhood obesity.

The results emphasize the fact that the clinical picture of obese children with liver disease is not different from that of obese children with no liver disease; they have comparable age, height, weight, BMI, waist and hip circumference and blood pressure. They have comparable serum TSH and 08:00 h cortisol, while their midnight cortisol is lower. Those with liver disease have higher circulating triglycerides, though their lipoproteins are comparable, as previously reported (15). We confirm that obesity and insulin resistance play important roles in the development of NAFLD (16). The insulin sensitivity indices of obese children with liver disease are marked by higher postprandial glucose and insulin, higher insulin/glucose ratio and higher HOMA-IR (17) than those with no liver disease.

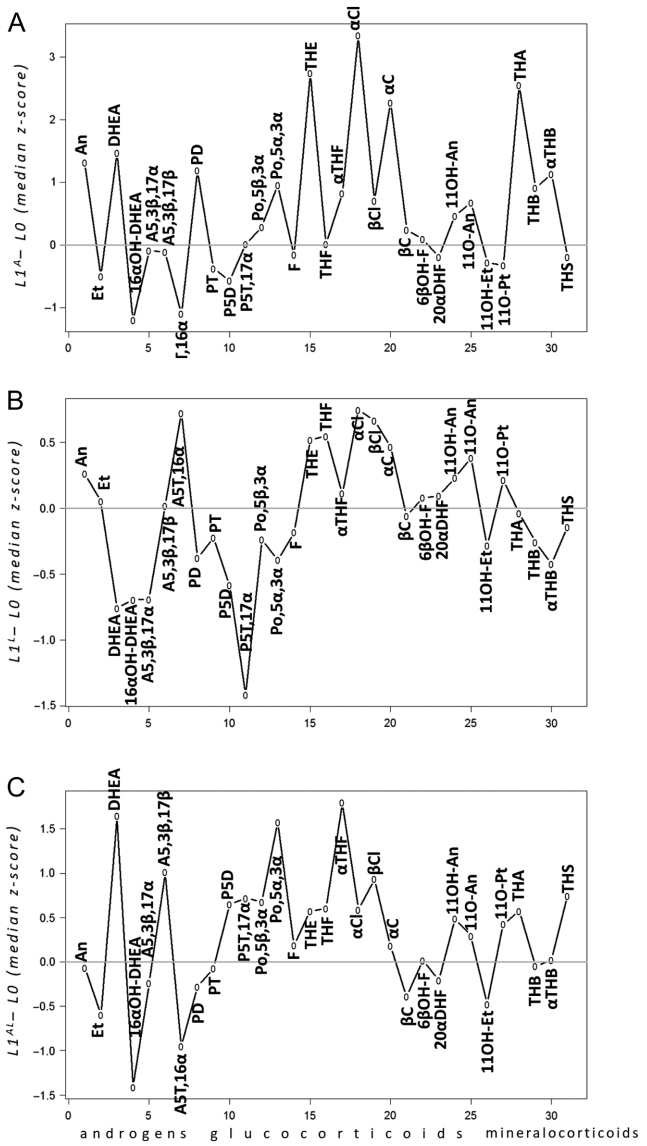

Figure 2
Steroidal signatures of liver disease in childhood obesity: differences between z-transformed concentrations of steroid metabolites (androgens, glucocorticoids and mineralocorticoids) in non-liver disease features (L0) patients and (A) patients with ALT+ ($L1^{A}$), (B) patients with sonographic liver steatosis ($L1^{L}$) and (C) patients with ALT+ and sonographic liver steatosis ($L1^{AL}$).

This complex 'steroidal signature' of liver disease reflects previously published single observations. The steroidal disease signature is marked by low urinary DHEA (18, 19) and its metabolites, higher glucocorticoid metabolites, due to increased glucocorticoid production rate (20), and lower mineralocorticoid metabolites. It is characterized by derangement of the cortisol/cortisone shuttle generated by 11β hydroxysteroid dehydrogenase (HSD) type 1 (20), as is evident from the lower (THF+αTHF)/THE ratio, enhanced 3βHSD activity ((THE+THF+αTHF)/5PT-17α ratio) and enhanced 21-hydroxylase activity ((THE+THF+αTHF)/PT). These findings may suggest lesser hepatic recycling (reduction) of cortisone to cortisol in liver steatosis, which is compensated for by increased adrenal cortisol generation and further metabolic consequences resulting from higher glucocorticoids concentrations – this mechanism results in a model of a vicious circle.

Therefore, it is not surprising that higher tetrahydrocortisone concentration in L1 patients corresponds with unfavorable biochemical profile: higher triglycerides and insulin resistance. The clinical profile, however, defined by BMI z score or waist circumference, is not useful in the prediction of liver disease as well as other obesity complications.

A previous study focused on the measurement of circulating DHEAS and found low DHEAS in NASH patient. The authors assumed that this might have resulted from reduced sulphonation of DHEA (19). Low sulphonation of steroids has also been found in a study in obese children (21). A further important feature of the obesity-associated liver disease signature is the low urinary DHEA excretion rate and its metabolites. It was previously suggested that DHEA treatment reduced hepatic injury in experimental animals by inhibiting several inflammatory mediators such as tumor necrosis factor-α and macrophage mitogen inhibitory factor, and preventing the increase in serum ALT levels (22). Thus, we speculate that DHEA might have a protective effect

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Table 4 Ratio of steroid metabolites (enzyme activity): differences between non-liver disease patients (L0) and patients with liver disease features: $L1^A$ (ALT+).

Ratio	L0 Mean ± SD (*n* = 64)	$L1^A$ Mean ± SD (*n* = 5)	$L1^L$ Mean ± SD (*n* = 10)	$L1^{LA}$ Mean ± SD (*n* = 6)	*P* value	All
An/Et (5α-reductase)	2.1 ± 0.9	2.9 ± 1.1	2.0 ± 0.7	3.1 ± 0.8	0.011 $L1^{LA}$, $L1^A$ > L0, $L1^L$	2.2 ± 0.9
5αTHF/THF (5α-reductase)	1.3 ± 0.6	1.4 ± 0.4	1.1 ± 0.4	1.7 ± 0.5	NS	1.3 ± 0.5
5αTHB/THB (5α-reductase)	3.4 ± 1.8	3.6 ± 0.8	2.7 ± 1.1	4.0 ± 1.6	NS	3.4 ± 1.7
(THF + αTHF)/THE (11βHSD1)	0.9 ± 0.3	0.8 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	NS	0.8 ± 0.3
(THE + THF + αTHF)/P5T-17α (3βHSD)	19.1 ± 15.4	15.1 ± 8.1	27.5 ± 17.1	26.4 ± 33.5	NS	20.4 ± 17.0
(THE + THF + αTHF)/PT (21-OH)	10.1 ± 3.9	12.8 ± 6.0	14.4 ± 4.6	12.7 ± 5.9	0.016 $L1^L$ > L0	11.0 ± 4.5
(THE + THF + αTHF)/PO5α3α (21-OH)	300.9 ± 160.4	228.5 ± 99.0	416.1 ± 230.8	238.4 ± 104.0	NS	305.8 ± 168.1

$L1^L$ (sonographic liver steatosis) and $L1^{LA}$ (both ALT+ and sonographic liver steatosis). Values are means and SD. Significance by ANOVA.
*Ratio of steroid metabolites was calculated based on steroid metabolites concentrations.
L0, non-liver disease patients; L1, liver disease patients; $L1^A$, patients L1 with ALT+; $L1^L$, patients L1 with sonographic liver steatosis; $L1^{AL}$, patients with ALT+ and sonographic liver steatosis; NS, not significant.

against hepatotoxicity. It has been shown that DHEA inhibits 11β-hydroxysteroid dehydrogenase-1 expression in liver and adipose tissues (23) – another component of the steroidal signature. The liver is also the site of greatest activity of 11βHSD (24), and as such responds to liver disease with decreasing activity. Obesity *per se* tends to enhance 11βHSD-1 activity (25), but insulin resistance, a prominent manifestation of the metabolic syndrome in obesity and the group of children presented here, inhibits 11βHSD-1 activity (26). Moreover, insulin resistance and the metabolic syndrome are involved in the development and progression of NAFLD (15).

In conclusion, we present the 'disease signature' of liver disease in childhood obesity. We are aware of the limitations of our study as our results may be biased by observational cross-sectional character of the study and the relatively small number of participants in subgroups with liver dysfunction. Moreover, we did not quantify the ultrasonographic steatosis, other than visually. However, our findings suggest a new approach to the diagnosis and further understanding of the metabolic consequences of liver disease as part of the metabolic syndrome of obesity. They reflect the derangements of steroid metabolism in NAFLD that includes enhanced glucocorticoid production and deranged androgens and mineralocorticoids and suggests a protective effect of DHEA on the liver in childhood obesity. Knowledge of these sequels may provide ways for personalized medicine in obese children with liver disease. Future prospective intervention study is also needed to verify if obtained findings are only reversible consequences of obesity or whether they reflect non-modifiable individual genetic predisposition.

Declaration of interest
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Author contribution statement
Conceptualization: A G, Z H, M S, M F H and S A W. Methodology: Z H, A G, M S, M F H, S A W, Z O and K G. Software: M S and A G. Validation: A G and M S. Formal analysis: M S. Investigation: A G. Resources: A G and S A W. Data curation: A G. Writing – original draft preparation: Z H, A G, M S, M F H and S A W. Writing – review and editing: Z H, A G, M S, M F H, S A W, Z O, K G. Visualization: Z O and K G. Supervision: Z H and A G. Project administration: A G. Funding acquisition: A G.

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