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ORIGINAL ARTICLE

^{1}H-MRS measured ectopic fat in liver and muscle is associated with the metabolic syndrome in Danish girls but not in boys with overweight and obesity

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Summary

Background

The metabolic syndrome (MetS) is a complication to overweight and obesity, which can be observed already in childhood. Ectopic lipid accumulation in muscle and liver has been shown to associate with the development of insulin resistance and dyslipidemia. Thus, the interaction between MetS and ectopic fat may offer clinical relevance.

Objectives

To investigate the prevalence of MetS, or components hereof, and ectopic fat accumulation in liver and skeletal muscle tissue in children, as well as interactions between these.

Methods

Two-hundred-and-sixteen children and adolescents (95 boys) with overweight/obesity were investigated, as well as 47 controls (22 boys) with normal weight. The assessments included anthropometry, fasting blood biochemistry and blood pressure measurements. Liver and muscle lipid contents were assessed by proton magnetic resonance spectroscopy.

Results

We observed an odds ratio in girls with overweight/obesity of 12.2 (95% confidence interval: [3.8; 49.0]) for exhibiting MetS when hepatic steatosis was present, whereas no association was observed in boys with overweight/obesity (odds ratio 0.7 [0.2; 2.7]). The odds ratio of exhibiting MetS in the presence of muscular steatosis was 3.5 [1.4; 9.5] in girls with overweight/obesity and 1.0 [0.2; 5.6] in boys with overweight/obesity. Similar results were seen for girls with overweight/obesity exhibiting concurrent hepatic and muscular steatoses.

Conclusion

Hepatic and muscular steatoses were associated with MetS among girls, but not among boys with overweight/obesity.

Keywords: Ectopic fat, metabolic syndrome X, obesity, paediatrics.

Introduction

Obesity among children and adolescents is a health challenge worldwide (1–3). Childhood obesity is associated with an increased risk of developing a variety of comorbidities (4–8), including the metabolic syndrome (MetS) which is often defined as the presence of at least three of the following components: Abdominal obesity, dyslipidemia, hypertension and impaired glucose tolerance (7,9). The presence of MetS in adults is a known risk

factor for later development of cardiovascular disease and type 2 diabetes mellitus (10).

However, not all children and adolescents with obesity develop MetS (11). The development of MetS is related to the lipid distribution within the body and the proportion of lipids deposited as ectopic fat (12,13), which in turn may be associated with insulin resistance (14,15).

Ectopic fat is the presence of lipids in organs that are not primary physiological sites for lipid storage, including the liver and skeletal muscle tissues. Ectopic fat can already manifest in childhood (16–18), and to a greater extent in children with overweight and obesity than in children with normal weight (4,18–20). Further, some studies indicate that boys with overweight/obesity may have a greater tendency to accumulate fat as ectopic fat in the liver as compared to girls with overweight/obesity (18,21–23).

The relationship between the components of MetS and ectopic fat accumulation has previously been reported in children and adolescents with obesity, showing that hepatic steatosis is associated with higher body mass index (BMI) (18,24), dyslipidemia (14), fasting hyperinsulinemia (23,24) and impaired glucose tolerance (21). Likewise, insulin sensitivity has been shown inversely correlated to intramyocellular lipid content (15). Further, correlations between impaired glucose tolerance and muscle fat content (MFC) have been observed (25).

In a recent study from our group (18), the relationships between ectopic fat and measures of insulin resistance, dyslipidemia and anthropometric data were examined in a group of children and adolescents with overweight and obesity.

In the present study, we wanted to explore these data further by analysing the relationships between MetS in its entirety and ectopic fat in liver and muscle, and to elucidate whether observed sex differences regarding ectopic fat deposition would translate into differentiated risk profiles regarding the development of MetS.

Methods

Study population

The study population consisted of 221 children and adolescents (98 boys) with overweight/obesity enrolled in a tertiary multidisciplinary childhood obesity treatment programme (26) in the period between August 2009 and August 2014. The participants had a magnetic resonance spectroscopy (MRS) assessment of ectopic lipid accumulation in liver and/or skeletal muscle within 60 days of anthropometric measures in the clinic, and estimates of fasting plasma glucose and lipids. The inclusion criteria were a BMI standard deviation score (SDS) above 1.28 for age and sex according to Danish reference charts (27) and an age between 6 and 22 years. The exclusion criteria were inability to lie still for 45 min in the magnetic resonance (MR) scanner or a body weight above 135 kg (thereby exceeding the maximum capacity of the MR scanner).

The control group consisted of 47 children and adolescents with normal weight (22 boys) recruited from local schools in the period between April 2012 and August 2014. The inclusion criteria for the control group were a BMI SDS below 1.2 and an age between 6 and 22 years and the exclusion criteria were identical to those of the study group.

Informed written and oral consents were obtained from parents or participants aged 18 years or older. The study was approved by the Ethics Committee of Region Zealand, Denmark (protocol no. SJ-104) and was conducted in accordance with the Helsinki Declaration.

Definition of the metabolic syndrome

In this study, MetS was defined according to a modified version of the International Diabetes Federation criteria (28). In the International Diabetes Federation's version, age- and sex-matched reference values are used for waist circumference (WC), whereas absolute cut-off values are used for systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma triglycerides, fasting plasma high-density lipoprotein (HDL) cholesterol and fasting plasma glucose (28). We moderated this version by applying cut-off values for SBP and DBP according to age-, height- and sex-matched reference values (29), as we considered these more appropriate in a paediatric population because of changes during growth and development. Further, we used a German reference population for the WC (30,31), as we found this study material to be comparable with our study population. Hence, our definition of MetS was:

- WC above the 90th percentile on age- and sex-matched reference curves (30,31), *and two or more of the following:*
- SBP and/or DBP above or equal to the 95th percentile for age, height and sex (29)
- Fasting plasma triglycerides above or equal to 1.7 mmol/L (150 mg/dL)
- Fasting plasma HDL below 1.03 mmol/L (40 mg/dL)
- Fasting plasma glucose above or equal to 5.6 mmol/L (100 mg/dL)

A MetS score was calculated based on the presence of the above-mentioned components of MetS: Fulfilling the WC criteria was scored 3 points, whereas each of the

other components was scored 1 point. Children with a MetS score of 5 or more had by definition MetS.

Anthropometric measures

Weight was measured to the nearest 0.1 kg on a Tanita® digital medical scale, WB-110 MA (Tanita Corp., Tokyo, Japan), while wearing light indoor clothing with empty pockets and no shoes. Height was measured to the nearest 1 mm with a stadiometer. BMI was calculated as weight divided by height squared (kg/m^2). The BMI SDS was calculated by the LMS method by converting BMI into a normal distribution by sex and age using the median coefficient of variation and a measure of the skewness (32), based on the Box–Cox power plot based on Danish BMI charts (27).

WC was measured at the level of the umbilicus to the nearest 1 mm with a non-elastic tape measure. The measurement was performed with the child in standing position, with the arms down and post-exhalation.

Pubertal developmental stages were evaluated according to the methods of Tanner (33,34); children and adolescents with overweight/obesity were clinically evaluated by a paediatrician, whereas the controls self-reported by picture pattern recognition supplemented by a detailed written explanation.

Blood pressure

Blood pressure was measured after 5 min of rest in the supine position. The measurement was repeated three times, and the average of the second and third measurements was used. The blood pressure values were compared to the distribution in an American reference population (29), and blood pressure SDS was calculated according to the guidelines from the European Society of Hypertension (35).

Until 10 June 2011, blood pressure was measured manually on the right arm by an aneroid sphygmomanometer (MaxiStabil3 ®, Speidel & Keller, Welch Allyn inc., New York, USA) using the auscultatory method in a total of 50 cases. After 10 June 2011, in a total of 166 cases and 47 controls, blood pressure was measured with an electronic sphygmomanometer (Omron 705IT ®, Omron Healthcare Co. Ltd., Kyoto, Japan). The device has been validated for use in children and adolescents who are normotensive (36), and no significant difference was found comparing measurements performed by the device to manually measured blood pressure in 197 children (36).

Blood samples

Blood samples were collected after an overnight fast between 7 and 9 am by venipuncture of the antecubital vein. If necessary, a local anaesthetic cream (lidocain/prilocain mixture, EMLA®, AstraZeneca, Sweden) was applied 1 h prior to the venipuncture.

Fasting blood samples of serum triglycerides, serum HDL cholesterol and plasma glucose were stored at room temperature for less than 30 min after sampling before being centrifuged at 4 °C and then immediately analysed on a Siemens Dimension Vista® 1500 analyser (Siemens AG, Munich, Germany). Blood samples for analysis of plasma glucose were collected in fluoride containing tubes.

Magnetic resonance spectroscopy

Hepatic and muscular fat content measurements were obtained by MRS. The MRS scans were performed by trained personnel using an Achieva 3.0 T MR imaging system (Philips Medical Systems, Best, the Netherlands). The cut-off value for liver steatosis was set as a 5% or higher content of macrovesicular fat in the hepatocytes as suggested by Schwimmer *et al.* (37). Further details on the method for measuring liver fat content have previously been described by Bille *et al.* (16).

MFC was measured in the psoas major muscle. The cut-off value for muscular steatosis was defined as a MFC of 5% or more as suggested by Fonvig *et al.* (17). Details on the measurements of MFC have previously been described by Fonvig *et al.* (17,18).

Statistical analysis

In Table 1, the Wilcoxon signed rank test was used for group comparisons in the continuous variables, while Fisher's exact test was used for analysing differences in categorical data. In Tables 2 and 3, multiple linear regression models adjusted for age and pubertal developmental stage were used for the association analyses where the ectopic fat content was used as the independent variable in all analyses except for WC. In analyses of the population stratified by age, differences across groups of continuous variables were evaluated by the analyses of variance (ANOVA) test, and the categorical variables were evaluated by Pearson's Chi-squared test.

Odds ratios (OR) were analysed using multiple logistic regression analyses adjusted for age and pubertal developmental stage. The level of significance was set at $p < 0.05$. *P*-values were not adjusted for multiple hypothesis testing. The statistical analyses were performed in R statistical software, version 3.1.2 (http://www.r-project.org).

Table 1 *Baseline characteristics of the 216 children and adolescents with overweight/obesity and the 47 children and adolescents with normal weight.* Data are given as medians (with ranges), unless otherwise stated. The Wilcoxon signed rank test was used for group comparisons in the continuous variables, while Fisher's exact test was used for analysing differences in categorical data

	Overweight/ Obese ♀	Controls ♀	*P*	Overweight/ Obese ♂	Controls ♂	*P*	*P* Overweight/ Obese ♀ versus ♂	*P* Controls ♀ versus ♂
N	121	25		95	22			
Age, *years*	13.4 (7.8; 20.4)	16.0 (10.9;20.7)	***0.002***	12.6 (8.3;18.0)	14.0 (9.0;18.9)	*0.19*	*0.24*	*0.07*
BMI SDS	2.73 (1.5;4.1)	−0.06 (−1.2;1.2)	***<0.001***	3.2 (1.6;5.8)	0.25 (−1.2;1.0)	***<0.001***	***<0.001***	*0.81*
WC, *cm*	94.0 (59.0;137.0)	68.0 (61.5;78.5)	***<0.001***	98.5 (77.0;145.0)	71.0 (60.0;82.0)	***<0.001***	***0.005***	*0.31*
HDL *mmol/L*	1.1 (0.6;2.1)	1.6 (1.1;2.1)	***<0.001***	1.2 (0.4;2.2)	1.8 (0.8;2.3)	***<0.001***	*0.38*	*0.32*
TG *mmol/L*	1.0 (0.4;4.1)	0.7 (0.4;1.9)	***0.003***	0.9 (0.2;3.9)	0.5 (0.3;0.9)	***<0.001***	*0.13*	***0.006***
fPG *mmol/L*	4.9 (3.4;6.3)	5.0 (4.3;6.0)	*0.20*	5.2 (4.1;6.2)	5.2 (4.1;5.7)	*0.14*	***<0.001***	*0.81*
SBP SDS	2.3 (−2.2;5.6)	1.7 (0.1;4.4)	0.06	2.1 (−0.4;5.3)	2.5 (0.3;4.8)	*0.66*	*0.33*	*0.16*
DBP SDS	1.0 (−1.5;3.4)	0.4 (−0.1;1.5)	***0.002***	0.7 (−0.8;2.5)	0.7 (−0.7;1.5)	*0.78*	***0.003***	*0.74*
Tanner stage								
P	4.0 (1.0;5.0)	5.0 (1.0;5.0)	*1.00*	2.0 (1.0;5.0)	2 (1;5)	*0.50*	***<0.001***	***0.32***
B, ♀	4.0 (1.0;5.0)	4.5 (1.0;5.0)	*0.44*				***<0.001***	***0.25***
G, ♂				2.0 (1.0;5.0)	4.0 (1.0;5.0)	*0.24*		
LFC, %	3.0 (0.5;32.8)	1.5 (1.0;6.0)	***<0.001***	3.0 (1.0;75.9)	1.5 (1.0;3.0)	***<0.001***	***0.002***	*0.55*
Hepatic steatosis fraction, %	23 (28/121)	4 (1/25)	***0.03***	38 (36/95)	0 (0/22)	***<0.001***	***0.02***	*1.00*
MFC*, %	7.3 (0.6;46.7)	2.6 (0.4;8.7)	***<0.001***	7.3 (1.0;36.8)	1.0 (0.5;8.3)	***<0.001***	*0.34*	*0.32*
Muscular steatosis fraction*, %	67 (74/110)	22 (5/23)	***<0.001***	66 (51/77)	10 (2/21)	***<0.001***	*1.00*	*0.42*

*$n_{obese\ girls} = 110$, $n_{lean\ girls} = 23$, $n_{obese\ boys} = 77$, $n_{lean\ boys} = 21$.
B, breast stage; *BMI,* body mass index; *DBP,* diastolic blood pressure; *fPG,* fasting plasma glucose; *G,* genital stage; *HDL,* high density lipoprotein; *LFC*, liver fat content; *MetS,* metabolic syndrome; *MFC,* muscular fat content; *P,* pubic hair; *SBP,* systolic blood pressure; *SDS,* standard deviation score; *TG,* triglycerides; *WC,* waist circumference; Bold, $p < 0.05$.

Table 2 *Multiple linear regression analyses of components of the metabolic syndrome and hepatic fat in the 121 girls and 95 boys with overweight/obesity.* Estimates ± standard deviation (Est ± SD), correlations coefficients (R^2) of the relationships between metabolic syndrome (MetS) score or MetS components and liver fat content. The analyses were adjusted for age and pubertal developmental stage. The MetS score was calculated based on the number of components fulfilled, where fulfilling the WC criteria was scored 3 points, and each of the other components was scored 1 point

	Girls			Boys		
	Est ± SD	*P*	R^2	Est ± SD	*P*	R^2
MetS score	0.08 ± 0.02	***0.001***	0.15	−0.00 ± 0.01	*0.49*	0.35
WC *cm*	0.18 ± 0.04	***<0.001***	0.18	0.36 ± 0.16	***0.03***	0.26
TG, *mmol/L*	0.03 ± 0.01	***0.01***	0.07	0.00 ± 0.01	*0.78*	0.20
HDL, *mmol/L*	−0.01 ± 0.00	***0.05***	0.06	−0.00 ± 0.00	*0.10*	0.24
fPG, *mmol/L*	0.02 ± 0.01	***0.008***	0.09	−0.00 ± 0.00	*0.71*	0.11
SBP SDS	−0.01 ± 0.02	*0.76*	0.27	−0.00 ± 0.00	*0.64*	0.49
DBP SDS	0.01 ± 0.01	*0.68*	0.17	−0.00 ± 0.001	*0.36*	0.26

BMI, body mass index; *DBP,* diastolic blood pressure; *fPG,* fasting plasma glucose; *HDL,* high density lipoprotein; *SBP,* systolic blood pressure; *SDS,* standard deviation score; *TG,* triglycerides; *WC,* waist circumference. Bold, $p < 0.05$.

Results

Five patients were excluded from the study because of a body weight above 135 kg. The eligible 216 children and adolescents (95 boys) with overweight/obesity had a median BMI SDS of 2.91 (range: 1.49; 5.80) and a median age of 12.9 years (7.8; 20.4), and the 47 (22 boys) children and adolescents with normal weight had a median BMI SDS of 0.00 (−1.23; 1.18) and a median age of 14.8 (9.0; 20.7). Baseline characteristics of the children and adolescents with overweight/obesity and the control children are shown in Table 1.

The girls and boys with overweight/obesity had a higher prevalence of MetS compared to the controls (girls: 40% vs. 0%, $p < 0.001$; boys: 41% vs. 0% $p < 0.001$), a higher prevalence of hepatic steatosis (girls:

Table 3 *Multiple linear regression analyses of components of the metabolic syndrome and muscular fat in the 110 girls and 77 boys with overweight/obesity.* Estimates ± standard deviation (Est ± SD), correlations coefficients (R^2) of the relationships between metabolic syndrome (MetS) score or MetS components and muscle fat content. The analyses were adjusted for age and pubertal developmental stage. The MetS score was calculated based on the number of components fulfilled, where fulfilling the WC criteria was scored 3 points, and each of the other components was scored 1 point

	Girls			Boys		
	Est ± SD	*P*	R^2	Est ± SD	*P*	R^2
MetS score	0.04 ± 0.02	***0.046***	0.10	0.01 ± 0.012	*0.60*	0.34
WC, *cm*	0.09 ± 0.05	*0.10*	0.09	0.02 ± 0.08	*0.80*	0.06
TG, *mmol/L*	0.01 ± 0.01	*0.20*	0.03	−0.01 ± 0.02	*0.56*	0.28
HDL, *mmol/L*	−0.01 ± 0.01	*0.10*	0.06	−0.00 ± 0.01	*0.66*	0.26
fPG, *mmol/L*	0.01 ± 0.01	*0.21*	0.04	−0.01 ± 0.01	*0.34*	0.10
SBP SDS	−0.02 ± 0.02	*0.18*	0.28	0.00 ± 0.03	*0.93*	0.39
DBP SDS	0.00 ± 0.01	*0.75*	0.20	−0.00 ± 0.02	*0.74*	0.10

BMI, body mass index; *DBP,* diastolic blood pressure; *fPG,* fasting plasma glucose; *HDL,* high density lipoprotein; *SBP,* systolic blood pressure; *SDS,* standard deviation score; *TG,* triglycerides; *WC,* waist circumference. Bold, $p < 0.05$.

23% vs. 4%, $p = 0.03$; boys: 38% vs. 0% $p < 0.001$) and of muscular steatosis (girls: 61% vs. 22 %, $p < 0.001$; boys: 66% vs. 10%, $p < 0.001$) (Table 1). Muscle fat measurement was available in 187 children and adolescents (77 boys) with overweight/obesity, and 44 of the controls (21 boys).

Tables 2 and 3 show the results of the multiple regression analyses including the associations between each of the components of MetS, as well as the MetS score, and hepatic fat content (Table 2) respectively muscular fat content (Table 3) in the group of children and adolescents with overweight/obesity. The analyses were adjusted for age and pubertal development.

In order to evaluate the influence of age on the relationship between MetS and ectopic fat accumulation, the part of the population with overweight/obesity were stratified by age, resulting in a group of children (7.8–13.0 years old, $n = 111$), a group of adolescents (13.0–18.0 years old, $n = 101$) and a group of adults (18.1–20.4 years old, $n = 4$). When comparing the groups regarding the presence of MetS and components hereof and hepatic or muscular steatosis, the groups were significantly different in MetS score, with the children having a lower score than the adolescents and adults ($p = 0.002$), mean WC (children 91.7 cm, adolescents 103.2 cm, adults 126 cm, $p < 0.001$), mean HDL cholesterol (children 1.23 mmol/L, adolescents 1.12 mmol/L, adults 1.27 mmol/L, $p = 0.019$), mean SBP *z*-score (children 1.67, adolescents 2.85, adults 4.25, $p < 0.001$), mean DBP *z*-score (children 0.71, adolescents 1.1, adults 2.24, $p < 0.001$) and presence of MetS (children 29.7%, adolescents 52.5%, adults 50%, $p = 0.003$).

Regression analyses were performed in the groups of children and adolescents only, as the adult group was too small in number. In the girls in the children group, we observed a positive association between liver fat and WC ($p < 0.001$), whereas for the girls in the adolescent group, we observed a positive association between liver fat and MetS score ($p = 0.01$), triglycerides ($p = 0.003$), fasting plasma glucose ($p < 0.001$) and SBP ($p = 0.045$). In the boys in the children group, we observed no significant associations between liver fat and MetS score or components of MetS. In the boys in the adolescent group, we observed a positive association between liver fat and WC ($p = 0.04$).

In younger girls, we observed a positive association between muscle fat and MetS score ($p = 0.049$), WC ($p = 0.026$) and DBP ($p = 0.018$), whereas in the older girls we observed a positive association between fasting plasma glucose ($p = 0.03$) and SBP ($p = 0.02$). In boys, there were no significant associations between muscle fat and MetS score or components of MetS in neither age group.

The presence of hepatic steatosis in girls with overweight/obesity was associated with an increased risk of having MetS with an OR of 12.2 (95% confidence interval (CI): [3.8; 49.0], $p < 0.001$). In boys with overweight/obesity, no significant association was found between hepatic steatosis and the risk of having MetS (OR 0.7, 95% CI: [0.2; 2.7], $p = 0.61$) (Table 4).

Muscular steatosis was associated with an increased risk of having MetS in girls with overweight/obesity (OR 3.5; 95% CI: [1.4; 9.5]; $p = 0.009$), but not in boys with overweight/obesity (OR 1.0; 95% CI: [0.2; 5.6]; $p = 0.96$) (Table 4).

Finally, concomitant hepatic and muscular steatosis was significantly associated with an increased OR of having MetS in girls with overweight/obesity (OR 31.4; 95% CI: [6.4; 205.9]; $p < 0.001$), but not in boys with overweight/obesity (OR 0.8; 95% CI: [0.1; 9.7]; $p = 0.88$) (Table 4).

Table 4 *Logistic regression analyses of having MetS when either hepatic steatosis (n = 216, 95 boys), muscular steatosis (n = 187, 77 boys) or both (as compared to no steatosis) are present in the girls and boys with overweight/obesity*. The analyses are adjusted for age and pubertal developmental stage. The results are given as odds ratios (OR) with 95% confidence intervals ([CI95%])

	Girls		Boys	
	OR [CI95%]	*P*	OR [CI95%]	*P*
Hepatic steatosis	12.2 [3.8; 49.0]	***<0.001***	0.7 [0.2; 2.7]	0.61
Muscular steatosis	3.5 [1.4; 9.5]	***0.009***	1.0 [0.2; 5.6]	0.96
Both	31.4 [6.4; 205.9]	***<0.001***	0.8 [0.1; 9.7]	0.88

Bold, $p < 0.05$.

When stratifying the analyses of Table 4 for age, we observed comparable results, except that in the girls in the children group we did not observe a significant association between muscular steatosis and MetS ($p = 0.065$).

Discussion

In the present study, we found a higher prevalence of MetS, liver steatosis and muscular steatosis in girls and boys with overweight/obesity when compared to children with normal weight. The high prevalence rates of MetS, liver steatosis and muscular steatosis in the children and adolescents with overweight/obesity were expected considering obesity as a known risk factor of development of both MetS and ectopic fat accumulation (5,7,19).

The comparable prevalence rates of MetS between the sexes in our study are in contrast to the recently reported European IDEFICS study (38), which included 18,169 children (2,606 with overweight or obesity). In that study, four different definitions of MetS were applied, and three of the definitions showed that MetS prevalence rates were higher among girls with obesity as compared to boys with obesity. However, in the IDEFICS study slightly other definitions of MetS than the one applied in this study were applied, which may explain the different findings in the prevalence of MetS. In a study by Cook *et al.*, several of the most commonly used definitions of MetS were applied in analyses of a group of adolescents with obesity, and the prevalence rates varied from 12.4% to 44.2% (11), suggesting that the results are dependent on the applied MetS definition. To date, there is no international consensus on the preferable definition of MetS in children and adolescents (11,38).

The difference in prevalence rates of hepatic steatosis between girls and boys with overweight/obesity found in this study is in line with previous studies (21–23). The higher prevalence of muscular steatosis in children with overweight/obesity as compared to the controls is also similar to previous findings (39).

Interestingly, the MetS score and some components of the MetS were associated to the liver fat content in the girls with overweight/obesity, whereas in the boys with overweight/obesity, only WC showed an association with liver fat content. Similarly, the association between hepatic steatosis and MetS was only significant in girls with overweight/obesity, while not in boys with overweight/obesity.

These findings are in line with some of the previously reported associations between hepatic steatosis and MetS in children with overweight/obesity (8,14), although in those studies the potential influence from sex differences was not examined.

In a community-based cohort of 1,170 adolescents, Ayonrinde *et al.* (40) showed a higher prevalence of non-alcoholic fatty liver disease (NAFLD) in boys as compared to the girls with obesity, which support findings from the present study, whereas MetS was more prevalent in boys as compared to girls with NAFLD, which is in contrast to the results of the present study.

Unfortunately, that study (40) did not evaluate the MetS prevalence stratified by obesity. These results may differ from the present study because of the community-based – and not an obesity-based – cohort or because liver steatosis was assessed by ultrasound and not MRS, which has previously been shown to be less accurate than MRS in diagnosing hepatic steatosis (41).

Other studies have shown associations between hepatic steatosis and MetS in boys, but not in girls (42,43). However, in these studies the diagnosis of NAFLD was based on elevated levels of alanine aminotransferase (ALT), which have shown to correlate poorly with the actual liver fat content as compared to measures obtained by ultrasound, MRS or even liver biopsies (14,40,44,45). In a study of 134 adolescents by Rehm *et al.* (45), the relationship between ALT concentrations and magnetic resonance imaging-diagnosed hepatic steatosis was analysed. They found that, with an ALT cut-off value at 65 U/L, the sensitivity for predicting hepatic steatosis was only 9% (20 out of 22 with hepatic steatosis did not have ALT concentrations above 65 U/L) and the specificity was 100%. Therefore, we find it difficult to compare our results to the results in those studies (42,43), as we would have underestimated the prevalence of hepatic steatosis if diagnosed by ALT concentrations. Finally, a study by Cicero *et al.* (46) showed associations between indices of hepatic steatosis and MetS in both men and

women; however, these indices were also based on ALT levels, which as argued limits comparability between the studies.

Knowledge is sparse in regards to the association between MetS and muscular steatosis in children and adolescents with overweight/obesity. We found that MetS score associated to MFC in girls with overweight/obesity and that the risk of MetS was greater in girls with overweight/obesity and exhibiting muscular steatosis, whereas no such association was seen in the boys with overweight/obesity. In a study by Cali and Caprio (5) of 89 children and adolescents with overweight/obesity, an increased prevalence of MetS was associated with increasing amount of visceral fat, whereas no association was found between increasing visceral fat and increasing intramyocellular fat content. These findings imply a lack of association between intramyocellular fat content and MetS, as in contrast to the present study. In the study by Cali and Caprio (5), the study population was not stratified by sex, which might have masked an association between MetS and muscular steatosis in girls as seen in the present study, and further, different MetS definitions were applied which also impedes comparison between the two studies.

Altogether, the present study shows, that the risk of accumulating ectopic fat in boys with overweight/obesity, whether being in the liver, the muscle, or both, is not associated with the presence of MetS, as in opposition to what was found in girls with overweight/obesity, despite a higher prevalence of hepatic steatosis among the boys. This may indicate that boys may have a risk of accumulating liver fat independent from their risk of developing MetS.

A possible explanation for the findings might be a smaller capacity to store fat as subcutaneous adipose tissue in boys compared to girls and/or a greater tendency to store fat as visceral fat, as a result of differences in circulating levels of sex steroids and glucocorticoids. In adults, it has been shown that men have relatively higher amounts of visceral adipose tissue, as compared to premenopausal women (47), whereas this difference declines when comparing to postmenopausal women (48). Similarly, prepubertal children have comparable amounts of visceral adipose tissue (49), whereas peri-/postpubertal boys have more visceral adipose tissue than peri-/postpubertal girls (50), as well as hepatic steatosis (22). Also, it has been shown that increased amounts of visceral adipose tissue are strongly associated to accumulation of hepatic fat (51). Thus, differences in concentrations of sex steroids and glucocorticoids could be a likely explanation for the differences between the sexes regarding the associations of ectopic fat and MetS.

The difference in pubertal development between the girls and boys with overweight/obesity makes it difficult to compare the two sexes as pubertal development may influence the accumulation of hepatic fat (22). However, we have tried to account for this by adjusting our analyses for pubertal development. Another limitation is the fact that the children with overweight/obesity at the point of examination in the present study had recently been enrolled in an obesity treatment programme, which may have altered their lifestyle, and thereby influenced their biochemical variables. The group of control children was relatively small as compared to the group of children and adolescents with overweight/obesity. Finally, all statistical analyses were performed without adjusting for multiple comparisons, which increases the risk of type I errors.

A strength of the present study is the use of MRS in the quantification of ectopic fat, as this is considered a reliable method with high sensitivity and specificity for evaluating fat content in various tissues (41). Ultrasound is another often used method of measuring hepatic fat; however, in a study by Mehta *et al.* (41), which compared the efficacy of ultrasound and MRS at measuring hepatic fat content in 50 adults, 44% of the individuals with absent hepatic fat measured by ultrasound had in fact hepatic fat accumulation measured by MRS.

A further strength is the inclusion of a relatively large group of children and adolescents enabling us to analyse relationships between girls and boys in terms of development of MetS and distribution of ectopic fat accumulation.

In conclusion, we found that hepatic and muscular steatoses, both individually and simultaneously present, are associated with MetS in girls but not in boys with overweight/obesity. These results persisted even after stratifying the population by age. This knowledge may contribute to a better understanding of the mechanisms underlying the development of MetS in childhood and its relation to ectopic lipid accumulation, an understanding that potentially could improve the treatment of MetS and steatoses in children and adolescents with overweight/obesity in daily clinical practice.

Conflicts of Interest Statement

All of the authors declare no conflicts of interest.

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Author contributions

The author contributions were as follows: JH, HST, OP, TH, CEF and CT designed research. AN, EC, CB, CEF and CT conducted the data collection. CEF analysed data. AN, CEF, CB, CT and JH wrote the paper draft. AN generated the tables. All authors contributed to interpretation of data and critical revision of the draft, and approved the final manuscript.

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