Gender-Specific Differences in Adipose Distribution and Adipocytokines Influence Adolescent Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is a predominantly adult-diagnosed disorder. Knowledge regarding the epidemiology, phenotype, and metabolic risk factors, during adolescence is limited. We sought to determine the prevalence, phenotype, and predictors of NAFLD in 1170 community-based adolescents in the Western Australian Pregnancy Cohort (Raine) Study (the Raine Cohort) who underwent a cross-sectional assessment that included questionnaires, anthropometry, cardiovascular examinations, blood tests, and abdominal ultrasound examinations. Among the 1170 adolescents assessed, the prevalence of NAFLD was 12.8%. Females compared with males had a significantly higher prevalence of NAFLD (16.3% versus 10.1%, $P = 0.004$) and central obesity (33.2% versus 9.9%, $P < 0.05$). The severity of hepatic steatosis was associated with the body mass index, waist circumference, subcutaneous adipose tissue thickness (SAT), serum leptin level, homeostasis model assessment for insulin resistance score ($P < 0.001$ for all), and serum alanine aminotransferase level ($P < 0.005$) in both genders, but it was associated with increasing visceral adipose tissue thickness (VAT; $P < 0.001$) and decreasing serum adiponectin levels ($P < 0.05$) in males alone. Males and females with NAFLD had similar amounts of SAT ($P > 0.05$); however, in comparison with females with NAFLD, males with NAFLD had greater VAT, a more severe metabolic phenotype with higher glucose levels and systolic blood pressure and lower adiponectin and high-density lipoprotein cholesterol levels ($P < 0.001$ for all), and greater measures of liver injury (alanine aminotransferase and aspartate aminotransferase, $P < 0.001$ for all). Similarly, metabolic syndrome was more common in males than females with NAFLD (24% versus 8%, $P = 0.01$). Suprailiac skinfold thickness predicted NAFLD independently of the body mass index, insulin resistance, and VAT. *Conclusion:* Gender differences in adolescent NAFLD are related to differences in adipose distribution and adipocytokines. The male phenotype of NAFLD is associated with more adverse metabolic features and greater visceral adiposity than the female phenotype despite the lower prevalence of NAFLD. (HEPATOLOGY 2011;53:800-809)

Nonalcoholic fatty liver disease (NAFLD) is a predominantly adult-diagnosed liver disorder. Predisposing factors for NAFLD may originate during early life. In adults, NAFLD increases the risk of liver cirrhosis and forecasts an increased risk of type 2 diabetes mellitus (type 2 diabetes) and atherosclerotic cardiovascular disease (CVD) and possibly reduced life expectancy.[1,2] Liver fibrosis, including cirrhosis, has

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; GGT, gamma-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; IL-6, interleukin-6; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SAT, subcutaneous adipose tissue thickness; SBP, systolic blood pressure; SFT, skinfold thickness; SVAR, subcutaneous to visceral adipose tissue ratio; VAT, visceral adipose tissue thickness.

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been reported in children with NAFLD.[3,4] Therefore, a diagnosis of NAFLD in childhood or adolescence heralds potentially serious liver-related or metabolically related outcomes in adulthood.

NAFLD is a global public health problem. The prevalence of NAFLD is increased in first-degree relatives of individuals with NAFLD, particularly if they are obese,[5,6] and this suggests a potential cascade of population risk. Unfortunately, NAFLD is often unrecognized, particularly in children and adolescents.[7] Serum alanine aminotransferase (ALT) levels are frequently used to define NAFLD[8-10]; however, ALT is relatively insensitive and nonspecific for NAFLD.[11,12] The population prevalence of metabolic risk factors for NAFLD in children and adolescents is increasing.[13] The prevalence of NAFLD increases with age: at least 2.6% in children who are 4 to 12 years old,[14] 17.3% in adolescents who are 15 to 19 years old,[15] and up to 34% in adults.[16] Although adolescent and childhood NAFLD phenotypes share adiposity, dyslipidemia, and insulin resistance with adult NAFLD, limited phenotypic and metabolic characterization has prevented an in-depth analysis of these relationships.[12,13]

NAFLD is associated with metabolic syndrome.[17-20] An Australian population–based adolescent cohort study found an association between the highest ALT and gamma-glutamyl transpeptidase (GGT) levels and high-risk metabolic characteristics in 14-year olds.[21] To gain insight into the unique pathogenic mechanisms of childhood NAFLD and the population risk for NAFLD-related progressive liver disease, type 2 diabetes, and CVD, we determined the prevalence, anthropometric, cardiovascular, and metabolic phenotype, and predictors of NAFLD in the same cohort of adolescents at 17 years of age.

Patients and Methods

Seventeen-Year Survey

The study population comprised adolescents with a mean age of 17 years (standard deviation = 0.25 years) who were participating in the Western Australian Pregnancy Cohort (Raine) Study (the Raine Cohort). The background and methods of the Raine Cohort Study have previously been described.[22] The Raine Cohort is a prospective cohort of pregnancy, childhood, and now adolescence (http://www.rainestudy.org.au) and is representative of the broader Western Australian population.[23]

Eighty-three percent of the original Raine Cohort participants have been retained. All active members of the Raine Cohort were invited to participate in the 17-year Raine follow-up survey, which was a cross-sectional study conducted between July 2006 and June 2009. One thousand seven hundred seventy-one adolescents participated in the 17-year survey; 66% underwent a liver ultrasound evaluation, 71% underwent a physical assessment, and 72% underwent a blood examination. Institutional ethics committee approval was obtained from the human research ethics committee of Princess Margaret Hospital for Children. Signed, informed parental consent and adolescent assent were obtained before participation in the study.

Assessment

The 17-year survey comprised detailed health questionnaires and anthropometric, abdominal ultrasound (liver, subcutaneous, and visceral fat), cardiovascular, and biochemical assessments. Information on alcohol intake over the previous year was documented by self-reporting and by the completion of a semiquantitative food frequency questionnaire developed by the Commonwealth Scientific and Industrial Research Organisation (Adelaide, Australia).[24] Medications and a comprehensive medical history (including a history of fatty liver or diabetes) were documented to exclude secondary causes of NAFLD and concomitant liver disease. Anthropometric measurements [weight, height, waist circumference, hip circumference, and skinfold thickness (SFT)] and cardiovascular assessments [resting pulse rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP)] were conducted by trained examiners. Resting blood pressure readings were obtained with an oscillometric sphygmomanometer (a Dinamap 8100 vital signs monitor, a Dinamap XL vital signs monitor, or a Dinamap ProCare 100) set to automatically record readings every 2 minutes with subjects supine. The average of the second and third readings was calculated. SFT measurements were obtained from the anterior abdominal wall, triceps, subscapular, and suprailiac skinfolds with a skinfold caliper (Holtain Tanner/

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Whitehouse skinfold caliper, Holtain, Crosswell, United Kingdom). The body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m^2). Obesity was defined according to age- and gender-adjusted BMI criteria described by Cole et al.[25] and recommended by the International Obesity Task Force. Waist-to-hip and waist-to-height ratios were derived by the division of the waist circumference (cm) by the hip circumference (cm) and height (cm), respectively. Metabolic syndrome was defined according to age- and gender-specific criteria of the International Diabetes Federation,[26] which defines central obesity as a waist circumference greater than 80 cm in females and greater than 94 cm in males, a raised triglyceride level as greater than 1.7 mmol/L, a reduced high-density lipoprotein cholesterol (HDL-C) level less than 1.03 mmol/L in males and less than 1.29 mmol/L in females, a raised SBP as greater than 130 mm Hg, a raised DBP as greater than 85 mm Hg, and a raised fasting glucose level as greater than 5.6 mmol/L. The Tanner stage of puberty was described by individual participants matching against charts with pictures and descriptions of pubertal changes.

Ultrasonography

Trained ultrasonographers performed liver ultrasound with a Siemens Antares ultrasound machine with a CH 6-2 curved array probe (Sequoia, Siemens Medical Solutions, Mountain View, CA) according to the protocol described by Hamaguchi and colleagues,[27] which provides 92% sensitivity and 100% specificity for the histological diagnosis of fatty liver. Abdominal (subcutaneous and visceral) adipose thickness measurements were performed with previously described criteria[28,29] that correlate closely with compartmental adipose areas and cardiovascular and metabolic risk factors.[29,30] The visceral adipose tissue thickness (VAT) was measured as the distance between the anterior wall of the aorta and the internal face of the rectus abdominis muscle perpendicular to the aorta. The subcutaneous adipose tissue thickness (SAT) was measured as the thickness of the fat tissue between the skin-fat interface and the linea alba; SAT compression was avoided. A single specialist radiologist (P.S.) who was blinded to the clinical and laboratory characteristics of the subjects interpreted the ultrasound images. Scores of 0 to 3, 0 to 2, and 0 to 1 were determined from captured images for liver echotexture (bright liver and hepatorenal echo contrast), deep attenuation (diaphragm visibility), and vessel blurring (intrahepatic vessel visibility), respectively. The diagnosis of fatty liver required a total score of at least 2, which included an echotexture score of at least 1. Hepatic fatty infiltration (steatosis) severity was classified by the total fatty liver score as 0 to 1 (no fatty liver), 2 to 3 (mild fatty liver), or 4 to 6 (moderate to severe fatty liver). The intraclass correlation coefficient was 0.93 for SAT [95% confidence interval (CI) = 0.93-0.93] and 0.94 for VAT (95% CI = 0.94-0.95), whereas the intraobserver reliability (κ statistic) for fatty liver was 0.78 (95% CI = 0.73-0.88). Adolescents with sonographic fatty liver and a self-reported weekly alcohol intake of less than 140 g for males and 70 g for females over the previous 12 months were classified as having NAFLD. Testing for hepatitis B or C virus infections was not performed because notification rates for hepatitis B and C virus infections were on average less than 24/100,000 and 23/100,000, respectively, for Western Australian teenagers between the ages of 15 and 19 years over the study period (personal communication, Western Australian Notifiable Infectious Diseases Database, Epidemiology and Surveillance Program, Communicable Disease Control Directorate, Department of Health of Western Australia, July 27, 2010).

Biochemistry

Laboratory assessments were performed with venous blood samples taken from an antecubital vein after an overnight fast. Serum glucose, insulin, ALT, aspartate aminotransferase (AST), GGT, triglyceride, total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), ferritin, transferrin saturation, highly sensitive C-reactive protein (CRP), adiponectin, and leptin levels were assayed. All laboratory assays were performed at an accredited central laboratory (Pathwest Laboratories, Perth, Western Australia, Australia). Serum ALT levels greater than 40 U/L in males and greater than 30 U/L in females were considered elevated in accordance with the reference laboratory. The homeostasis model assessment for insulin resistance (HOMA-IR) score was calculated as follows:

$$\text{HOMA-IRscore} = [\text{Fasting insulin } (\mu\text{U/mL}) \times \text{Fasting glucose (mmol/L)}]/22.5$$

Statistical Analysis

Continuous descriptive data are presented as means and standard deviations for normally distributed data and as medians and interquartile ranges (IQRs) for nonnormally distributed data. Categorical variables are reported as percentages. The main outcome variables were the presence or absence of NAFLD and the severity of sonographic fatty liver. Thus, the analysis was limited to adolescents who had undergone abdominal ultrasound examinations; they are henceforth called the

cohort. Because of significant gender differences in NAFLD prevalence, demographic, anthropometric, and biochemical data for males and females were analyzed separately. Differences in continuous variables between adolescents with or without NAFLD and between steatosis severities or genders were computed with an independent *t* test or one-way analysis of variance with the Bonferroni adjustment for normally distributed variables and with nonparametric analysis (the Mann-Whitney U test or Kruskall-Wallis test) for nonnormally distributed data. Differences between categorical variables were determined with the Pearson chi-square test or Fisher's exact test. Adiponectin levels below the 10th percentile were considered reduced. All *P* values were reported as two-sided and were interpreted at the 5% level of significance. Multiple logistic regression analysis was used to calculate the odds of NAFLD prevalence from individual baseline characteristics. Data were analyzed with SPSS, version 15.0 (SPSS, Chicago, IL).

Results

Prevalence of NAFLD

Among the 1170 adolescents (51% male) assessed by ultrasound, the prevalence of fatty liver was 15.6% (182/1170). After the exclusion of 32 adolescents (27 females and 5 males) who consumed excessive quantities of alcohol, the prevalence of NAFLD was 12.8% (150/1170). NAFLD was more prevalent in females than males (16.3% versus 10.1%, $P = 0.004$). The prevalence of NAFLD by steatosis severity in females versus males was 2.2% versus 3.1% for moderate to severe steatosis, 14.1% versus 7.0% for mild steatosis, and 83.7% versus 89.9% for no steatosis ($P < 0.001$). The prevalence of NAFLD increased with BMI: 4%, 15%, and 65% for normal-weight, overweight, and obese males, respectively, and 10%, 29%, and 57% for normal-weight, overweight, and obese females, respectively.

None of the participants had a previous diagnosis of NAFLD or type 2 diabetes or a history of insulin therapy. Most participants (98% of males and 99% of females) were pubertal or postpubertal according to the Tanner scale. The Tanner stage of puberty was not associated with the presence or absence of NAFLD ($P > 0.05$).

Adipose Distribution in the Cohort

According to International Diabetes Federation criteria, the prevalence of central obesity determined by waist circumference was 21.4%. More females than males had central obesity (33.2% versus 9.9%, $P < 0.05$). There was also a gender difference in abdominal fat distribution. Females had greater mean SAT than males (20.8 ± 11.1 versus 14.6 ± 10.5 mm, $P < 0.001$); however, males had greater mean VAT than females (35.2 ± 10.7 versus 29.8 ± 8.9 mm, $P < 0.001$). The subcutaneous to visceral adipose tissue ratio (SVAR) was higher in females versus males (0.72 ± 0.52 versus 0.42 ± 0.41, $P < 0.001$). Waist circumference was more positively correlated with subcutaneous adiposity (suprailiac SFT or SAT: $r = 0.80$ or 0.78 in males and $r = 0.66$ or 0.71 in females, $P < 0.001$ for both) than visceral adiposity ($r = 0.26$ and 0.09 for males and females, respectively, $P < 0.001$); that is, waist circumference reflected SAT more than VAT.

Despite significant gender differences in central obesity, SAT, and VAT, there were similar proportions of overweight and obese individuals between genders when BMI categories were used (77.0%, 14%, and 9% of normal-weight, overweight, and obese females, respectively, and 77.3%, 14.2%, and 8.5% of normal-weight, overweight, and obese males, respectively).

Anthropometry, Adipose Distribution, and Presence and Severity of Steatosis

Males and females with NAFLD had greater adiposity [including body weight, BMI, waist circumference, waist/hip ratio, waist/height ratio, and subcutaneous adiposity (SAT and SFT)] than those without NAFLD ($P < 0.005$). Apart from the waist/hip ratio in females, adiposity measures were associated with increasing severity of steatosis ($P < 0.05$; Fig. 1 and Table 1).

Males with NAFLD had significantly higher weights, BMIs, waist circumferences, and waist/hip ratios than females with NAFLD ($P < 0.05$); however, subcutaneous adiposity was similar in males and females with NAFLD. In contrast, males with NAFLD had significantly greater VAT than females with NAFLD (40.8 versus 31.1 mm, $P < 0.001$). VAT was associated with steatosis severity in males only ($P < 0.001$; Fig. 1 and Table 1). The prevalence of moderate to severe steatosis was higher with elevated VAT versus nonelevated VAT in males (9.3% versus 0.4%, $P < 0.05$) but not in females (2.5% versus 1.7%, $P = 0.18$). Correspondingly, SVAR was higher in females with NAFLD versus males with NAFLD (median = 0.70 and IQR = 0.52-1.18 for females, median = 0.53 and IQR = 0.31-0.96 for males, $P = 0.02$).

Relationship Between NAFLD and Metabolic Risk Factors

Metabolic syndrome was more prevalent in males than females with NAFLD (24% versus 8%, $P = 0.001$). The severity of the components of the metabolic syndrome worsened with increasing severity of steatosis, particularly in males (Table 1 and Fig. 2).

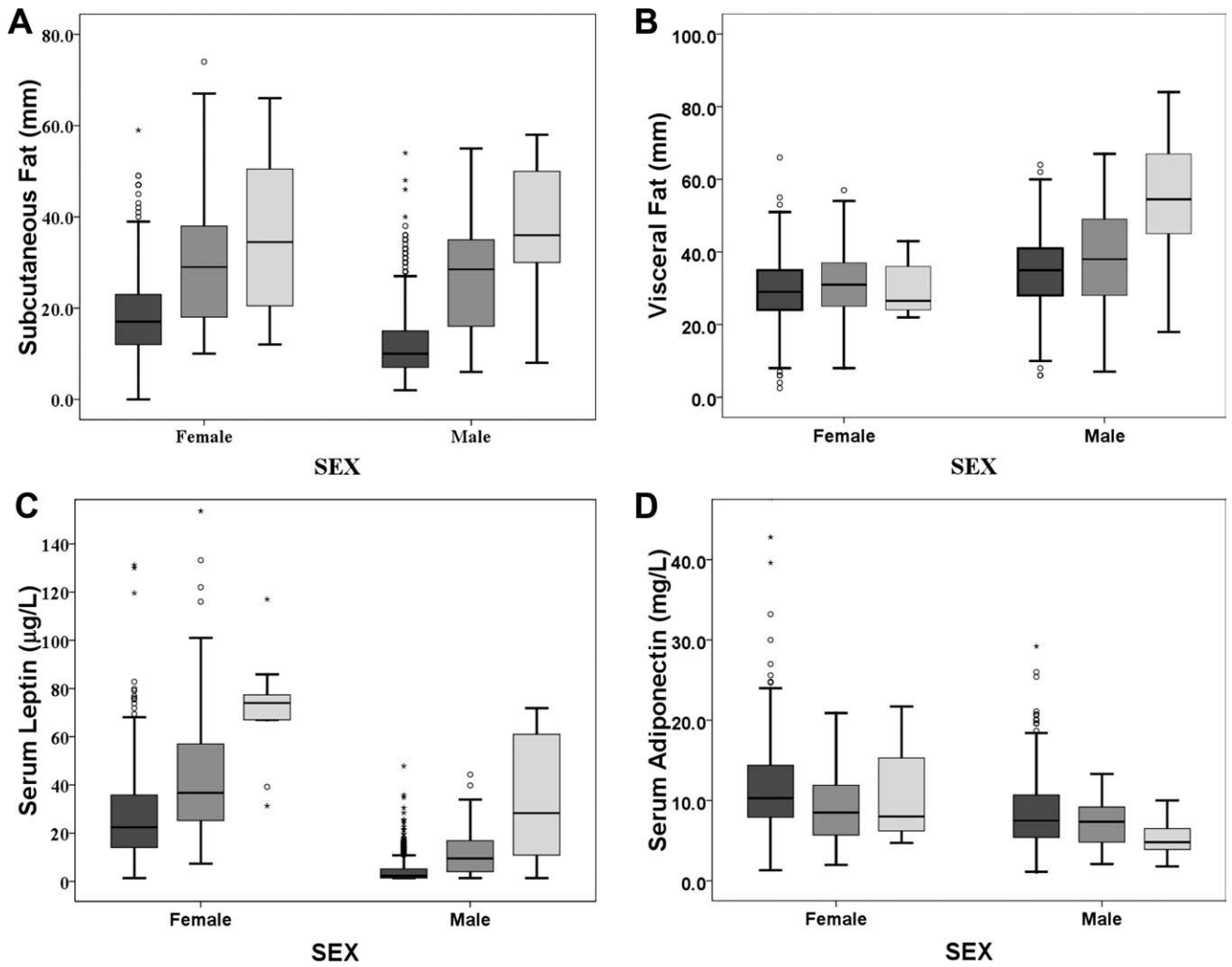

Fig. 1. Adipose distribution and adipocytokine levels in adolescents with NAFLD differ according to gender. Males have greater VAT and lower adiponectin levels than females. Females have greater SAT and higher leptin levels than males. The black bars represent subjects without NAFLD, the dark gray bars represent subjects with mild hepatic steatosis, and the light gray bars represent subjects with moderate to severe hepatic steatosis. The box plots display median values and 25th and 75th percentiles; the whiskers represent the 5th and 95th percentiles.

Cardiovascular Parameters and NAFLD

Males with NAFLD had higher SBPs and resting pulse rates than males without NAFLD; however, this relationship was not evident in females. Also, males with NAFLD had significantly higher SBPs than females with NAFLD ($P < 0.05$), and in males, SBP was associated with increasing severity of NAFLD ($P < 0.05$; Table 1 and Fig. 2).

Markers of Liver Injury and Inflammation

Adolescents with NAFLD had higher measures of liver injury (ALT, AST, and GGT) and inflammation (CRP) than those without NAFLD. Males with NAFLD had significantly higher serum ALT, AST, GGT, and ferritin levels than females with NAFLD. Serum ALT and GGT levels increased with increasing steatosis severity ($P < 0.05$) in both genders (Table 1).

Insulin Resistance and Lipids

Insulin resistance (HOMA-IR and fasting insulin) and lipid abnormalities (fasting triglycerides and HDL-C) were more severe in adolescents with NAFLD versus those without NAFLD. Males (but not females) with NAFLD had higher fasting glucose levels than those without NAFLD (in males, 5.0 versus 4.8 mmol/L, $P < 0.05$; in females, 4.6 versus 4.6 mmol/L, $P > 0.05$). Males with NAFLD had significantly lower HDL-C levels than females with NAFLD (Table 1). Serum insulin levels and HOMA-IR scores increased and HDL-C levels decreased with increasing steatosis severity ($P < 0.05$) in both genders.

Adipocytokines

Leptin. Females in the cohort had higher levels of leptin in comparison with males in the presence or

Table 1. Association of Baseline Anthropometric, Cardiovascular, and Biochemical Characteristics of Males and Females in the Cohort With the Severity of Steatosis

Characteristic		Males				Females			
		(A) NAFLD With Moderate to Severe Steatosis (n = 18)	(B) NAFLD With Mild Steatosis (n = 42)	(C) Non-NAFLD (n = 527)	*P* Value	(A) NAFLD With Moderate to Severe Steatosis (n = 12)	(B) NAFLD With Mild Steatosis (n = 78)	(C) Non-NAFLD (n = 461)	*P* Value
Adiposity	Weight (kg)	103.9 (21.7)‡,¶	87.5 (15.9)‡,§	69.4 (11.0)‡,§	<0.001	84.2 (21.2)‡	72.3 (16.8)†	60.8 (9.8)‡	<0.001
	Waist (cm)	105.8 (18.2)‡,¶	93.5 (14.0)‡,‖	78.3 (7.8)‡,§	<0.001	90.4 (16.7)‡	84.9 (14.5)	75.5 (9.1)‡	<0.001
	BMI (kg/m^2)	32.2 (6.3)‡	27.3 (5.0)‡	21.8 (3.1)‡	<0.001	30.6 (7.3)‡	26.0 (5.5)‡	22.1 (3.3)‡	<0.001
	Waist/hip ratio	0.94 (0.10)‡,‖	0.88 (0.06)‡,§	0.83 (0.05)‡,§	<0.001	0.82 (0.06)	0.82 (0.07)	0.78 (0.06)‡	<0.001
	Waist/height ratio	0.59 (0.10)‡	0.52 (0.08)‡	0.44 (0.4)‡,§	<0.001	0.55 (0.1)‡	0.51 (0.1)	0.46 (0.1)‡	<0.001
	SAT (mm)	38.5 (14.4)‡	27.1 (14.2)‡	12.5 (7.7)‡,§	<0.001	35.7 (17.4)‡	29.7 (14.3)	18.6 (8.6)‡	<0.001
	VAT (mm)	53.8 (22.1)‡,¶	38.1 (14.6)†,¶	34.7 (9.9)§	<0.001	29.8 (7.9)	31.3 (10.6)	29.5 (8.7)	0.94
	SVAR	0.5 (0.3-1.0)‡	0.5 (0.5-1.0)	0.3 (0.2-0.5)‡,§	<0.001	0.8 (0.6-1.0)‡	0.7 (0.5-1.2)	0.5 (0.4-0.80)‡	0.001
	Abdominal SFT (mm)	32.3 (10.1)‡	29.0 (11.5)	15.5 (8.5)‡,§	<0.001	33.4 (9.0)‡	29.1 (7.7)	23.4 (7.3)‡	<0.001
	Triceps SFT (mm)	24.6 (11.0)‡	18.7 (9.9)†	10.0 (4.3)‡,§	<0.001	27.4 (9.0)‡	22.7 (6.7)	18.2 (5.4)‡	<0.001
	Subscapular SFT (mm)	26.2 (11.4)‡	21.3 (10.1)*	11.0 (4.7)‡,§	<0.001	25.9 (9.7)‡	20.4 (8.1)*	14.7 (5.4)‡	<0.001
	Suprailiac SFT (mm)	29.3 (10.2)‡	25.2 (11.2)	11.4 (6.9)‡,§	<0.001	31.8 (8.9)‡	23.8 (8.9)†	17.0 (6.7)‡	<0.001
Cardiovascular	SBP (mm Hg)	130 (111)‡,§	122 (9)§	119 (10)§	<0.001	108 (6.3)	111 (9.7)	109 (10)	0.21
	DBP (mm Hg)	62 (8)	60 (6)	59 (7)	0.17	60 (5.8)	60 (6.4)	60 (6.5)	0.84
	Pulse per minute	70 (14)†	65 (12)	63 (10)§	0.003	68 (5)	69 (11)	67 (10)	0.24
Biochemistry	ALT (U/L)	51.3 (25.7)‡	32.6 (19.9)‡,§	22.0 (9.9)‡,§	<0.001	30.9 (30.4)‡	19.3 (9.8)†	18.1 (10.1)	0.002
	AST (U/L)	34.6 (20.7)†	29.6 (10.4)§	27.0 (8.3)§	0.001	24.7 (10.1)	21.2 (5.0)	22.0 ((5.1)	0.14
	GGT (U/L)	24.1 (11.3)‡	22.7 (14.4)§	15.4 (7.3)‡,§	<0.001	19.9 (14.9)†	13.5 (5.5)*	13.0 (6.7)	0.01
	Triglycerides (mmol/L)	1.5 (0.8)†	1.1 (0.5)	1.0 (0.6)	0.004	0.91 (0.62)	1.2 (0.58)	1.0 (0.5)*	0.03
	HDL-C (mmol/L)	1.01 (0.18)‡	1.11 (0.19)§	1.21 (0.25)*,§	<0.001	1.25 (0.47)	1.32 (0.28)	1.43 (0.31)*	0.009
	LDL-C (mmol/L)	2.26 (1.05)	2.35 (0.64)	2.23 (0.64)§	0.54	2.0 (0.53)	2.6 (0.74)*	2.4 (0.6)	0.02
	Glucose (mmol/L)	5.2 (0.6)	4.9 (0.4)§	4.8 (0.7)§	0.07	4.9 (0.5)	4.6 (0.4)	4.6 (0.4)	0.24
	Insulin (mU/L)	14.3 (10.3-22.7)‡	8.1 (6.0-14.0)†	6.8 (4.4-9.9)*,¶	<0.001	16.0 (10.6-33.5)‡	10.0 (7.0-14.7)‡	7.5 (5.1-10.7)	<0.001
	CRP (mg/L)	1.5 (0.9-3.3)‡	0.8 (0.3-1.8)*	0.4 (0.2-0.8)‡,§	<0.001	2.8 (0.9-7.6)†	1.1 (0.4-7.6)	0.7 (0.3-1.9)†	0.003
	HOMA-IR	3.3 (2.3-5.4)‡	1.8 (1.3-3.0)†	1.4 (0.9-2.1)†	<0.001	3.8 (2.0-7.0)‡	2.1 (1.3-3.0)*	1.5 (1.0-2.2)‡	<0.001
	Adiponectin (mg/L)	5.2 (2.2)*,‖	7.2 (2.9)¶	8.4 (5.1)§	0.01	10.3 (5.8)	9.1 (4.5)	11.8 (6.4)	0.003
	Leptin (μg/L)	28.4 (10.7-61.4)‡,§	9.5 (3.9-17.3)†,§	2.3 (1.4-5.2)‡,§	<0.001	74.0 (53.1-81.7)‡	36.7 (23.9-57.7)†	22.4 (13.9-35.9)‡	<0.001
	Ferritin (μg/L)	76.2 (47.0)	71.5 (45.4)§	62.2 (34.6)§	0.1	40.6 (27.6)	39.8 (27.9)	35.7 (36.1)	0.26

The results are presented as means and standard deviations or medians and IQRs. The *P* values cited in column A compare the NAFLD patients with moderate to severe steatosis to the non-NAFLD group, the *P* values cited in column B compare the NAFLD patients with moderate to severe steatosis to the NAFLD patients with mild steatosis, and the *P* values cited in column C compare the NAFLD patients with mild steatosis to the non-NAFLD group. Males with any degree of steatosis had significantly higher weights, waist circumferences, waist/hip ratios, VAT, and SBP and lower SVARs, HOMA-IR scores, and serum HDL-C, adiponectin, and leptin levels in comparison with females with steatosis.

*$P < 0.05$ (for those comparisons with statistically significant trends).

†$P < 0.01$ (for those comparisons with statistically significant trends).

‡$P < 0.001$ (for those comparisons with statistically significant trends).

§$P < 0.001$ (for males versus comparable females).

‖$P < 0.01$ (for males versus comparable females).

¶$P < 0.05$ (for males versus comparable females).

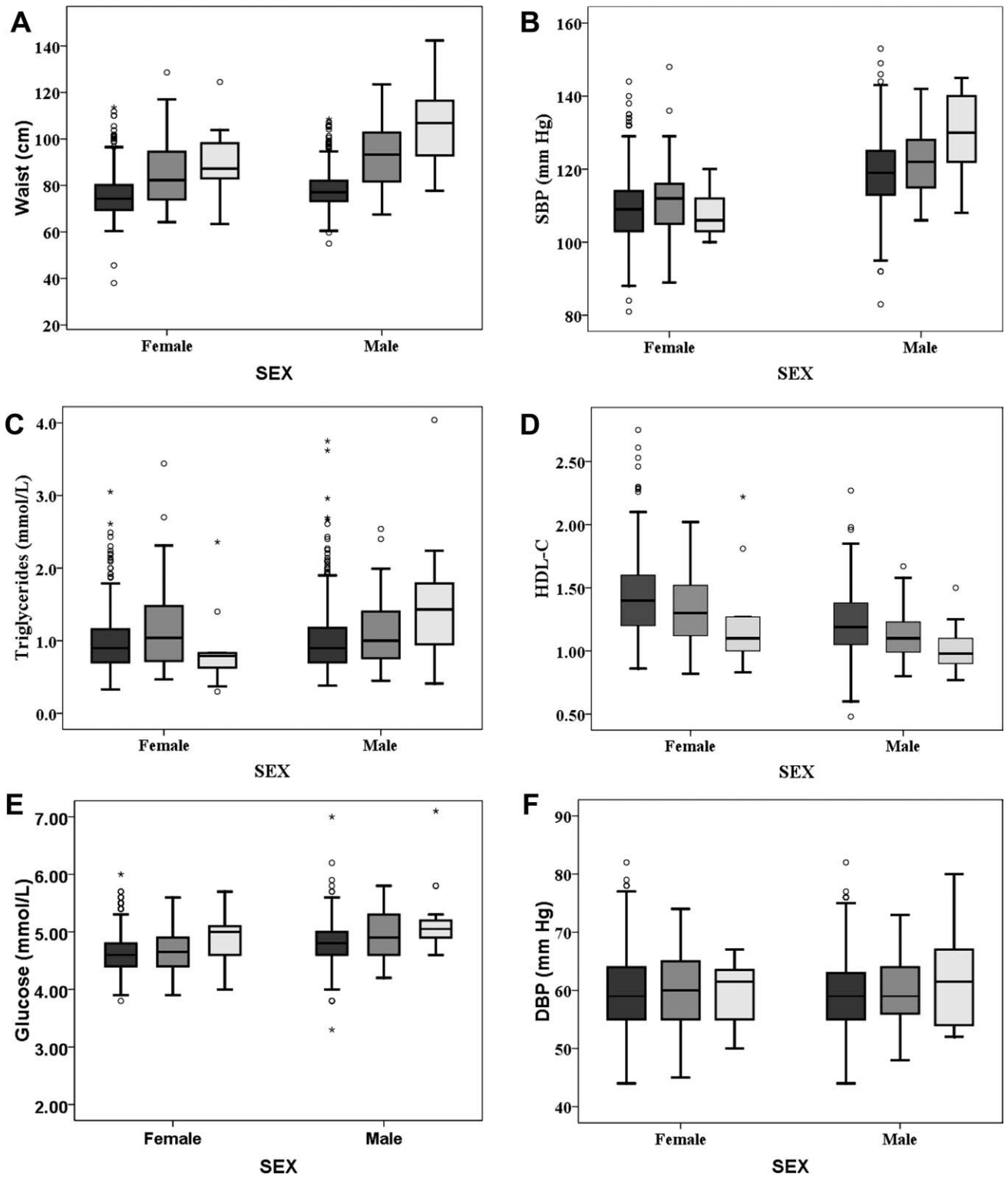

Fig. 2. Association between the components of metabolic syndrome and the severity of ultrasound-diagnosed hepatic steatosis in female and male patients with NAFLD. The black bars represent subjects without NAFLD, the dark gray bars represent subjects with mild hepatic steatosis, and the light gray bars represent subjects with moderate to severe hepatic steatosis. The box plots display median values and 25th and 75th percentiles; the whiskers represent the 5th and 95th percentiles.

absence of NAFLD (Table 1). Serum leptin levels were higher in males and females with NAFLD versus those without NAFLD ($P < 0.05$), and this was independent of BMI or waist circumference in males. Leptin levels were associated with steatosis severity in males and females. Leptin levels were also more strongly

correlated with subcutaneous adiposity ($r = 0.72$ in males and $r = 0.58$ in females, $P < 0.001$ for suprailiac SFT) than visceral adiposity ($r = 0.28$ in males and $r = 0.16$ in females, $P < 0.005$ for VAT).

Adiponectin. Males had lower adiponectin levels than females in the presence or absence of NAFLD (Table 1 and Fig. 1). Males and females with NAFLD had lower serum adiponectin levels than those without NAFLD, and this was independent of BMI and waist circumference in males. Serum adiponectin levels were inversely associated with steatosis severity in males ($P < 0.01$) but not females ($P = 0.4$; Fig. 1). Serum adiponectin was negatively correlated with suprailiac SFT in males and females ($r = -0.14$, $P < 0.05$) and with VAT in males only ($r = 0.12$, $P = 0.01$).

Independent Predictors of NAFLD

Multiple regression analysis, which included gender, VAT, HOMA-IR, serum leptin, adiponectin, and HDL-C, found that suprailiac SFT (odds ratio = 1.14, $P < 0.001$, 95% CI = 1.11-1.17) and serum ALT (odds ratio = 1.02, $P = 0.01$, 95% CI = 1.01-1.04) were significant and independent predictors of NAFLD in the cohort at the age of 17 years. Gender was not independently associated with NAFLD despite NAFLD being more common in females. Gender-specific analysis found that independent predictors of NAFLD at the age of 17 years in males were suprailiac SFT (odds ratio = 1.14, $P < 0.001$, 95% CI = 1.10-1.19) and serum ALT (odds ratio = 1.04, $P = 0.002$, 95% CI = 1.01-1.06), whereas in females, suprailiac SFT alone was significant (odds ratio = 1.13, $P < 0.001$, 95% CI = 1.10-1.17). Although waist circumference and BMI were independent predictors of NAFLD in males (odds ratio = 1.13, $P < 0.001$, and odds ratio = 1.36, $P < 0.001$, respectively) and females (odds ratios = 1.08, $P < 0.001$, and odds ratio = 1.24, $P < 0.001$, respectively), neither remained significant with adjustments for suprailiac SFT.

Discussion

NAFLD was common (13%) in this population-based study of adolescents. There were marked gender differences in the prevalence, anthropometric and metabolic phenotype, adipose distribution, degree of liver injury, and adipocytokine levels in adolescents with NAFLD that may have important implications for the future risk of progressive NAFLD and the development of metabolic syndrome, type 2 diabetes, and CVD.

NAFLD was approximately 1.5 times more prevalent in females than males; this contrasts with other reports that have found NAFLD to be more prevalent in males.[16-18,20] The difference can be explained by the relatively large proportion of females with central obesity, the population-based nature of our study, and the nonrequirement for raised ALT levels for participant recruitment. Using a raised ALT level as an inclusion criterion would have introduced a gender recruitment bias because ALT levels are higher in males than females.[10] Furthermore, 90% of males and 91% of females with fatty liver in our cohort had normal ALT levels, and this indicates that the majority of the subjects would have been missed if we had relied upon ALT.

The gender differences in NAFLD prevalence were associated with significant differences in adipose distribution and metabolic parameters, including adipocytokine levels. Males with NAFLD had the same SAT but had greater VAT than females with NAFLD, and this resulted in a significantly lower SVAR in males (Table 1). Correspondingly, male NAFLD was associated with higher levels of ALT, a more severe metabolic phenotype with higher SBP, a greater risk of metabolic syndrome, and lower adiponectin levels than female NAFLD despite similar insulin resistance rates and similar rates of abdominal obesity. These findings may be explained by functional differences in visceral adipose tissue versus subcutaneous adipose tissue. Visceral adipose tissue is infiltrated with inflammatory cells and releases inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α. Visceral adipose tissue is more insulin-resistant and has greater lipolysis rates and release of potentially hepatotoxic free fatty acids.[31-33] Visceral fat has been demonstrated to be associated with advanced nonalcoholic steatohepatitis (NASH) and fibrosis after adjustments for insulin resistance,[34] and this suggests that male adolescents are at higher risk of developing liver disease than female adolescents. Interestingly, males are overrepresented in histological series of pediatric NASH patients, and male gender has been associated with portal fibrosis in pediatric NAFLD.[15,35,36] Gender has not been a reproducible risk factor for advanced NASH in adults; however, this may be due to the grouping of premenopausal and postmenopausal women, which may obscure any sex hormonal effect. The relationship between hepatic fibrosis and regional fat distribution in adults differs according to gender and, in females, menopausal status, so central adiposity is associated with a higher risk of fibrosis in men and postmenopausal women than in premenopausal women.[37]

Interactions between sex hormones, adipocytokines, insulin resistance, and adipose distribution may explain the differences in the gender distribution of NAFLD. In comparison with visceral adipose tissue, subcutaneous

adipose tissue adipocytes have a higher density of estrogen receptors and leptin release. Greater leptin and estrogen production in females may protect against visceral adipose tissue accumulation and NASH, possibly by inhibiting IL-6.[31] Visceral adipose tissue adipocytes exhibit greater insulin resistance, expression of androgen receptors, and adiponectin and IL-6 release than subcutaneous adipose tissue adipocytes.[31-33,38]

Subcutaneous and visceral compartmentalization of adipose tissue is influenced by age and gender. Visceral adipose tissue accumulates more rapidly with age and weight gain in males and postmenopausal females than in younger females.[39,40] Approximately 90% of abdominal fat in children is subcutaneous adipose tissue, with intra-abdominal adipose tissue representing less than 10% of total abdominal adiposity.[41] With advancing age, visceral adipose tissue increases and accounts for 10% to 20% of total fat in men but a smaller proportion (up to 8%) in women.[33] Because the liver receives portal venous blood containing free fatty acids and cytokines secreted by visceral adipocytes, which may contribute to insulin resistance,[31] an increased prevalence and severity of NAFLD could be expected, particularly in males, with increasing age, abdominal obesity, VAT, and androgenic activity in adults.[42]

Our observation that SAT was similar in males and females with NAFLD and was predictive of NAFLD independently of BMI, VAT, and HOMA-IR implies a role for compartmental fat distribution, including subcutaneous fat, in the development of NAFLD. We found subcutaneous fat to be associated with the severity of hepatic steatosis. Conventionally, visceral adiposity is considered to be more important than subcutaneous adiposity with respect to the risk of NAFLD.[43-45] However, many studies have predominantly focused on adults and overweight and obese populations or have not stratified their analyses by gender. Furthermore, ultrasound-measured abdominal SAT (but not VAT) was the best predictor of insulin resistance in a study of childhood obesity,[46] and SFT has been shown to be more sensitive for obesity than BMI.[47] Similarly, SAT predicts insulin resistance independently of VAT in adults.[48] We observed suprailiac SFT to be the strongest predictor of a diagnosis of adolescent NAFLD. Although subcutaneous adiposity predicts adolescent NAFLD, it is plausible that greater VAT reflects an increased risk for NASH (orchestrated by hypoadiponectinemia) as part of an adverse metabolic risk profile.

In conclusion, NAFLD is relatively common in adolescents. The gender difference in the prevalence and severity of NAFLD is related to differences in the prevalence of central obesity, adipose distribution, adipocytokines, and sex hormones. SAT may play an important role in determining the development of hepatic steatosis, with VAT being related to the severity of liver injury and metabolic disturbance. In our study, males had greater VAT, greater steatosis severity, lower adiponectin levels, and more metabolic risk factors than females, whereas females had a higher prevalence of NAFLD with greater SAT, higher leptin levels, and fewer metabolic risk factors than males. The high prevalence of metabolic risk factors, including insulin resistance, abdominal obesity, and metabolic syndrome, in adolescents with NAFLD underscores the potential risk for long-term hepatic lipotoxicity, liver disease, type 2 diabetes, and CVD. Suprailiac SFT, a subcutaneous adiposity measure, is a stronger predictor of adolescent NAFLD than VAT, BMI, waist circumference, HOMA-IR, or serum ALT. Future studies examining the development of NAFLD should incorporate gender-stratified analyses adjusted for subcutaneous adiposity. It is likely that the pathogenesis of NAFLD in males and females differs in terms of genetic and hormonal influences on compartmental adipose deposition, including the liver.

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