Fetuin-A and Its Relation to Metabolic Syndrome and Fatty Liver Disease in Obese Children Before and After Weight Loss

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Context: There are very limited data available concerning the relationships between fetuin-A, weight status, nonalcoholic fatty liver disease (NAFLD), and features of the metabolic syndrome (MetS) in obese humans, and especially in children.

Objective: Our objective was to study the longitudinal relationships between fetuin-A, NAFLD, and MetS in obese children.

Design: This was a 1-yr longitudinal follow-up study.

Setting: This study was performed in primary care.

Patients: A total of 36 obese and 14 lean children was included in the study.

Intervention: An outpatient 1-yr intervention program based on exercise, behavior, and nutrition therapy was performed.

Main Outcome Measures: Changes of weight status (SD score-body mass index), waist circumference, fetuin-A, blood pressure, lipids, transaminases, insulin resistance index homeostasis model assessment (HOMA), and prevalence of NAFLD (defined by liver ultrasound) were calculated.

Results: The 12 obese children with NAFLD had significantly higher fetuin-A levels (0.35 $\pm$ 0.07 g/liter) than the 24 obese children without NAFLD (0.29 $\pm$ 0.06 g/liter) and the 14 normal weight children (0.29 $\pm$ 0.05 g/liter). Fetuin-A levels were independent of age, pubertal stage, and gender. Fetuin-A correlated significantly to systolic ($r = 0.50$) and diastolic blood pressure ($r = 0.41$), insulin resistance index HOMA ($r = 0.28$), and high-density lipoprotein-cholesterol ($r = -0.31$). Changes of fetuin-A correlated significantly to changes of insulin resistance index HOMA ($r = 0.34$), systolic ($r = 0.31$) and diastolic blood pressure ($r = 0.37$), and waist circumferences ($r = 0.36$). Substantial weight loss in 21 children led to a significant decrease of fetuin-A and the prevalence of NAFLD in contrast to the 15 children without substantial weight loss.

Conclusions: Fetuin-A levels were higher in children with NAFLD, and were related to insulin resistance and to features of the MetS in both cross-sectional and longitudinal analyses. Therefore, fetuin-A might be a new promising link between obesity and its comorbidities. **(*J Clin Endocrinol Metab* 93: 4479–4485, 2008)**

Obesity is the most common risk factor for nonalcoholic fatty liver disease (NAFLD) and the metabolic syndrome (MetS), a cluster of increased waist circumference, dyslipidemia, impaired glucose metabolism, hypertension, and atherosclerosis (1, 2). Insulin resistance is thought to be one of the primary underlying abnormalities leading to both NAFLD and MetS (2,

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; LMS, least median squares; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; SDS, SD score.

3). Fetuin-A (former name for the human protein: α2-Heremans-Schmid glycoprotein) is an abundant serum protein, which was recently proposed as a link between obesity, fatty liver, insulin resistance, and MetS (4–6).

Animal studies have shown that human fetuin-A, a protein secreted by the liver and found in high concentrations in serum, inhibits insulin receptor tyrosine kinase activity in muscles and in the liver (7, 8). Fetuin-A knockout mice have enhanced glucose clearance and insulin sensitivity, resistance to weight gain, and lower serum free fatty acid and triglyceride levels (8). The human fetuin-A gene resides on chromosome 3q27, which has been mapped as a type 2 diabetes susceptibility locus (9) and is linked to a quantitative trait locus for MetS (10). Recently, polymorphisms in the gene-encoding human fetuin-A were found to be not only associated with type 2 diabetes (11), but also to affect insulin action in adipocytes (12). Furthermore, fetuin-A exerted pro-adipogenic properties (13).

However, the link between fetuin-A, obesity, insulin resistance, NAFLD, and MetS in humans is less clear. Some previous studies in adults have reported significant associations between fetuin-A, NAFLD, and insulin resistance (4–6). However, most of the studies were cross-sectional and, therefore, susceptible to many confounders. Longitudinal studies are preferable to clarify these metabolic relationships. Because NAFLD and MetS often begin in childhood or young adulthood (14), studies in this age group are important. One further advantage of examining children is that the risk of potential confusion with adult-onset complications such as coronary disease, medications such as birth control pills, active tobacco smoking, alcohol use, *etc.*, is diminished.

Given the limited data concerning fetuin-A in obesity and especially in obese children, we studied fetuin-A levels and their changes in obese children, as well as their correlation to the presence of NAFLD, insulin resistance, and further markers of the MetS in the course of 1 yr in a lifestyle intervention.

Subjects and Methods

Written informed consent was obtained from all children and their parents. The study was approved by the local ethics committee of the University of Witten/Herdecke in Germany.

We examined anthropometrical markers, fasting serum fetuin-A, transaminases, and as parameters of MetS waist circumference, glucose, insulin, blood pressure, triglycerides, high-density lipoprotein (HDL)- and low-density lipoprotein (LDL)-cholesterol concentrations in 36 obese Caucasian children. Furthermore, ultrasound measurements were performed in obese children to detect NAFLD. In addition, fetuin-A was determined in 14 lean healthy Caucasian children of similar age, gender, and pubertal stage. The obese children were studied before and after participating in the 1-yr lifestyle intervention "Obeldicks," which has been described in detail elsewhere (15). Briefly, this outpatient intervention program for obese children is based on physical exercise, nutrition education, and behavior therapy, including the individual psychological care of the child and his or her family. The nutritional course is based on a fat and sugar-reduced diet compared with the everyday nutrition of German children.

None of the children in the cohort of the current study suffered from endocrine disorders, premature adrenarche, or syndromal obesity. Obesity was defined according to the definition of the International Task Force of Obesity using population-specific data (16, 17).

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. Because the distribution of body mass index (BMI) is not comparable in children and adults, not even among the various childhood age groups, we used the LMS method to calculate SD score (SDS) BMI as a measurement for the degree of overweight. The LMS method was chosen because it summarizes the data in terms of three smooth age-specific curves called L (λ), M (μ), and S (σ), based on German population-specific data (17, 18). The M and S curves correspond to the median and coefficients of variation (CVs) of BMI for German children at each age and gender, whereas the L curve allows for the substantial age-dependent skewness in the distribution of BMI. The assumption underlying the LMS method is that after Box-Cox power transformation, the data at each age are normally distributed (18).

The pubertal developmental stage was determined according to Marshall and Tanner (41, 42), and categorized into two groups (prepubertal: boys with pubic hair and gonadal stage I, and girls with pubic hair stage and breast stage I; and pubertal: boys with pubic hair or gonadal stage II or higher, and pubertal girls with pubic hair stage or breast stage II or higher).

Blood pressure was measured according to the guidelines of the National High Blood Pressure Education Program (19). Systolic and diastolic blood pressure was measured twice at the right arm after a 10-min rest in the supine position using a calibrated sphygmomanometer and averaged. The cuff size of the sphygmomanometer used, based on the length and circumference of the upper arm, was as large as possible without having the elbow skin crease obstruct the stethoscope (19).

Blood sampling was performed in the fasting state at 0800 h. All serum probes were frozen opaque at −81 C and thawed only once. Serum fetuin-A concentrations were measured by an ELISA kit (BioVender Laboratory Medicine, Brno, Czech Republic). The antibodies are highly specific for the human fetuin-A protein. The assay uses a two-side sandwich technique with two selected polyclonal antibodies that bind to different epitopes of human fetuin-A. The intraassay and interassay CVs were less than 10%. Insulin concentrations were measured by microparticle-enhanced immunometric assay (Abbott, Wiesbaden, Germany). Glucose levels were determined by a colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). HDL- and LDL-cholesterol concentrations were measured by an enzymatic test (HDL-C-Plus and LDL-C-Plus; Roche Diagnostics, Mannheim, Germany), and triglyceride concentrations by a colorimetric assay using a Vitros analyzer (Ortho Clinical Diagnostics). Serum aspartate aminotransferase and alanine aminotransferase concentrations were measured using commercially available test kits (ALTL-, ASTPL-Cobas Integra 400; Roche Diagnostics). Intraassay and interassay CVs were less than 5% in all these methods. Homeostasis model assessment (HOMA) was calculated by the formula: resistance (HOMA) = [insulin (mU/liter) × glucose (mmol/liter)]/22.5 (20).

NAFLD was diagnosed by standardized criteria based on liver ultrasound and transaminases measurements, as well as the absence of alcohol abuse according to the American Gastroenterological Association Medical Position Statement (21). The liver ultrasound procedures were read by a single blinded radiologist, and quantification of fatty liver was performed according to the criteria of Saverymuttu *et al.* (22). Differential diagnoses were excluded in all children with suspected NAFLD by measuring serum creatinkinase, antinuclear antibodies, liver autoantibodies (smooth muscle antibodies, autoantibodies against liver and kidney microsomal antigens, and soluble liver antigens), copper, ceruloplasmin, 24-h urinary copper, α1-antitrypsin, and Epstein-Barr virus, hepatitis A virus, hepatitis B virus, and hepatitis C virus serologies according to international recommendations (2).

Using the LMS calculation method described previously, substantial weight loss in the course of 1 yr was defined as a reduction of SDS-BMI more than or equal to 0.5 because with a reduction of less than 0.5 SDS-BMI, no improvement of insulin resistance and cardiovascular risk factors can be measured in obese children (23).

Statistical analyses were performed using the Winstat software package (Microsoft Corp., Redmond, WA). All variables were normally dis-

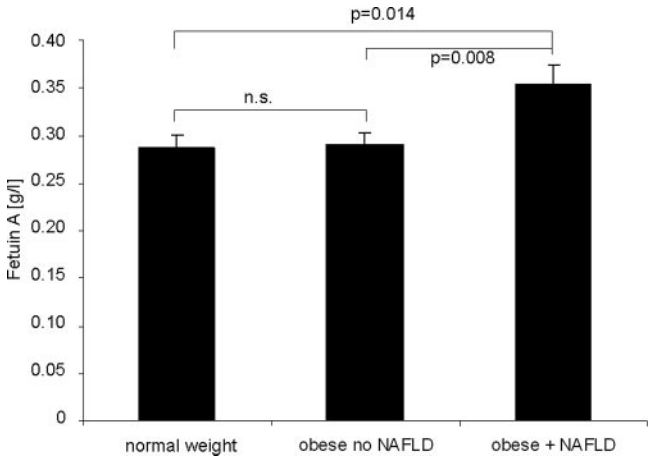

FIG. 1. Fetuin-A concentrations in 14 normal weight children, 24 obese children without NAFLD, and 12 obese children with NAFLD (data are shown as mean and SEM). n.s., Nonsignificant.

tributed tested by the Kolmogorov-Smirnov test. The Student's *t* test for paired and unpaired observations, χ^2, and ANOVA for multiple comparisons were used as appropriate. Correlations between fetuin-A, lipids, insulin, and insulin resistance index HOMA at baseline, as well as correlations between changes of weight status, fetuin-A, lipids, transaminases, and HOMA in the course of 1 yr were calculated by Pearson's correlation. Partial regressions analyses adjusted to SDS-BMI were also performed. Changes were expressed as the δ-variable calculated by the variable at baseline minus the variable measured 1 yr later. A *P* value less than 0.05 was considered significant. Data are presented as mean and SD.

Results

At baseline we found significantly ($P = 0.011$ derived from an ANOVA adjusted for age, gender, and pubertal stage) higher fetuin-A concentrations in the 12 obese children with NAFLD compared with the 24 obese children without NAFLD and the 14 lean children (Fig. 1). The obese children with and without NAFLD and the normal weight children did not differ significantly in respect to age, gender, or pubertal status (Table 1). However, there was a tendency of male predominance in obese children with NAFLD. The degree of overweight (SDS-BMI) did not differ significantly between the obese children with and without NAFLD.

In the 36 obese children, baseline fetuin-A levels correlated significantly ($P < 0.05$) to waist circumference (r = 0.45), systolic blood pressure (r = 0.50), diastolic blood pressure (r = 0.41), insulin resistance index HOMA (r = 0.28), HDL-cholesterol (r = −0.31), but not to LDL-cholesterol, triglycerides, transaminases, and age in partial regression adjusted to SDS-BMI.

Changes of fetuin-A concentrations in the course of 1 yr correlated significantly ($P < 0.05$) to changes of insulin resistance index HOMA (r = 0.34), systolic blood pressure (r = 0.31), diastolic blood pressure (r = 0.37), waist circumferences (r = 0.36), and in tendency ($P = 0.08$) to changes of SDS-BMI (r = 0.23; β-coefficient 0.05 adjusted for age, gender, and pubertal stage; Fig. 2), but not to changes of transaminases, HDL-cholesterol, or triglycerides.

Fetuin-A concentrations decreased significantly ($P = 0.028$) in the 21 obese children with substantial weight loss, whereas fetuin-A levels did not change significantly in the 15 patients without change of weight status (Fig. 3). In addition, in all obese children with NAFLD and substantial weight loss, fetuin A concentrations decreased (0.39 ± 0.08 to > 0.31 ± 0.04 g/liter). The prevalence of NAFLD decreased significantly ($P = 0.027$) in the obese children with substantial weight loss in contrast to the children without substantial weight loss (Table 2). All six children with NAFLD at baseline and without substantial weight loss also demonstrated NAFLD at the end of intervention. From the six children with NAFLD at baseline and substantial weight loss, only one child (17%) showed NAFLD at the end of intervention.

The changes of weight status, waist circumference, blood pressure, HOMA, insulin, glucose, lipids, and transaminases in the course of 1 yr in the 21 obese children with substantial weight loss and in the 15 obese children without substantial weight loss are shown in Table 2. Substantial weight loss led to a significant decrease of waist circumference, blood pressure, insulin resistance index HOMA, insulin, and LDL-cholesterol concentrations. In the obese children without substantial weight loss, there were no significant changes. The number of children entering puberty during the lifestyle intervention period did not differ significantly between the children with and without substantial weight loss (5 *vs.* 6%, respectively).

At baseline we found no significant differences in age, gender, pubertal stage, SDS-BMI, and the prevalence of NAFLD between the obese children with and without substantial weight loss. Furthermore, glucose, insulin, insulin resistance index HOMA, lipids, transaminases, and fetuin-A concentrations did not differ

TABLE 1. Age, gender, pubertal stage, weight status, and fetuin-A concentrations in obese with and without NAFLD and normal weight children

	No. 1 Obese with NAFLD	No. 2 Obese without NAFLD	No. 3 Normal weight	*P* value No. 1 *vs.* No. 2	*P* value No. 1 *vs.* No. 3	*P* value No. 2 *vs.* No. 3
No.	12	24	14			
Age (yr)	11.2 ± 2.8	10.2 ± 2.0	10.3 ± 2.9	0.251	0.255	0.899
% Male	67	33	36	0.053	0.851	0.115
% Prepubertal	50	54	50	0.821	0.812	0.999
BMI (kg/m^2)	29.6 ± 4.0	27.0 ± 4.4	16.8 ± 2.1	0.093	<0.001	<0.001
SDS-BMI	2.5 ± 0.3	2.4 ± 0.4	−0.1 ± 0.8	0.292	<0.001	<0.001
Fetuin-A (g/liter)	0.35 ± 0.07	0.29 ± 0.06	0.29 ± 0.05	0.009	0.014	0.880

Data are shown as mean ± SD, unless stated otherwise.

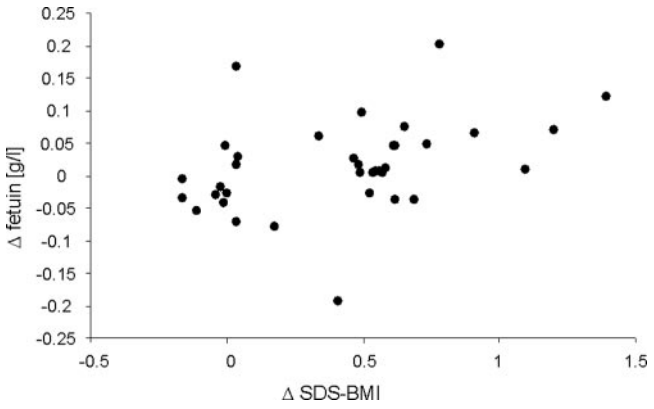

FIG. 2. Relationship between changes of fetuin-A and changes of SDS-BMI in the course of 1 yr (Δ defined as parameter at baseline − parameter 1 yr later).

significantly at baseline between the children with and without substantial weight loss. One year later, blood pressure, triglycerides, insulin, insulin resistance index HOMA, transaminases, and fetuin-A concentrations were significantly lower in the children with substantial weight loss compared with the children without substantial weight loss.

At baseline we did not find a significant difference between the fetuin-A levels of prepubertal children (mean 0.30 ± 0.06 g/liter) and pubertal children (mean 0.31 ± 0.08 g/liter). The fetuin-A levels of boys (mean 0.31 ± 0.07 g/liter) did not differ significantly from those of girls (mean 0.30 ± 0.07 g/liter).

Discussion

To the best of our knowledge, this is the first study analyzing both the cross-sectional and longitudinal relationships between fetuin-A, obesity, NAFLD, insulin resistance, and other markers of the MetS in childhood. We were able to show that obese children with NAFLD demonstrated higher fetuin-A concentrations than obese children without NAFLD and healthy controls in concordance with a study in adults (4). We also found that the fetuin-A levels of the children we studied were similar to those of adults

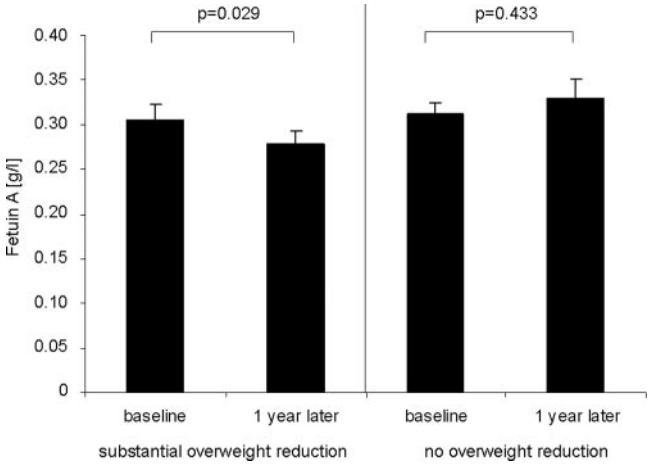

FIG. 3. Fetuin-A concentrations in 21 obese children with substantial weight loss and 15 obese children without change of weight status in the course of 1 yr (data are shown as mean and SEM).

(4, 5, 24–26). Fetuin-A concentrations were independent of weight status, pubertal stage, age, and gender. Studies in adults also demonstrated no gender differences (6, 27). Fetuin-A correlated to many features of the MetS such as blood pressure, waist circumference, and HDL- cholesterol accordingly to studies in adults (4, 5). Most importantly, these correlations were also found in longitudinal analyses. Furthermore, fetuin-A levels decreased significantly and in a parallel manner to the decrease of insulin resistance index HOMA and the prevalence of NAFLD in obese children who reduced their overweight substantially (reduction of SDS-BMI ≥0.5) in contrast to obese children without substantial weight loss in the course of 1 yr.

The increased liver fat in obesity has been proposed as the link between obesity and increased fetuin-A concentrations (4). We found the highest fetuin-A concentrations in obese children with NAFLD and a decrease of fetuin-A in a parallel manner to a decrease of the prevalence of NAFLD in obese children with substantial weight loss. In concordance, Stefan *et al.* (4) found in a cross-sectional analysis that fetuin-A plasma concentrations were elevated in subjects with high liver fat. While analyzing the longitudinal data, they found that a decrease in liver fat was accompanied by a decrease in fetuin-A plasma levels. These findings suggest that fetuin-A is a link between NAFLD and obesity. Interestingly, fetuin-A in humans is exclusively expressed in the liver except for the tongue and placenta (28). Stefan *et al.* (4) found that fetuin-A expression was significantly elevated in mice with fatty liver. Thus, it is tempting to speculate that fat accumulation in the liver may result in an increased secretion of fetuin-A. However, it is noteworthy that we do not have proof that in humans with fat accumulation in the liver, fetuin-A mRNA expression is up-regulated. Furthermore, we do not know why some obese individuals suffer from NAFLD with associated elevated fetuin-A concentrations while other obese individuals did not develop NAFLD and demonstrated fetuin-A levels similar to healthy controls.

NAFLD is frequently associated with insulin resistance and the features of MetS (2). The cross-sectional and longitudinal significant relationships between serum fetuin-A levels, features of the MetS, and insulin resistance index HOMA in our study support the hypothesis that fetuin-A is probably involved in the pathogenesis of insulin resistance and MetS in humans. Interestingly, the fetuin-A gene in humans localizes to a site previously linked to the MetS quantitative trait locus (10). Prior research relating fetuin-A to insulin resistance in animal studies suggests that fetuin-A interferes with insulin action at peripheral tissues through its interaction with the insulin receptor (7). Studies to evaluate whether this effect is mediated through tissue-specific actions of the inhibitory effect of fetuin-A on insulin receptor tyrosine kinase or through alternative pathways in humans could yield novel insights into the regulatory mechanisms of dyslipidemia, hypertension, and disturbed glucose metabolism. Unfortunately, there is not much data available on the role of fetuin-A as a regulator of insulin sensitivity in humans. Mori *et al.* (5) did not find a significant association between fetuin-A and insulin resistance in type 2 diabetic subjects. In contrast, other studies demonstrated a relationship between fetuin-A and insulin resistance in adults without type 2 diabetes (4–6, 29). Furthermore,

TABLE 2. Changes of weight status, blood pressure, waist circumference, insulin resistance index HOMA, fasting serum insulin, glucose, lipids, transaminases, and fetuin-A concentrations, as well as NAFLD prevalences in obese children with substantial weight loss and obese children with stable weight status in the course of 1 yr

	Substantial weight loss			No change of weight status		
No.	**21**			**15**		
Age (yr)	10.6 ± 2.8			10.4 ± 2.4		
Gender	43% male			47% male		
Pubertal stage	48% prepubertal			47% prepubertal		
Change of SDS-BMI	−0.7 ± 0.2			0.0 ± 0.2		
	At baseline	**1 yr later**	***P* value[a]**	**At baseline**	**1 yr later**	***P* value[a]**
BMI (kg/m^2)	27.6 ± 3.8	24.5 ± 2.8	0.001	28.2 ± 5.3	28.4 ± 5.5	0.961
SDS-BMI	2.4 ± 0.4	1.7 ± 0.4	<0.001	2.5 ± 0.4	2.5 ± 0.4	0.375
Waist circumference (cm)	94 ± 12	83 ± 12	<0.001	95 ± 17	94 ± 14	0.774
Systolic blood pressure (mm Hg)	121 ± 13	107 ± 5	<0.001	119 ± 14	117 ± 12	0.418
Diastolic blood pressure (mm Hg)	67 ± 13	56 ± 8	<0.001	65 ± 12	65 ± 9	0.952
Triglycerides (mmol/liter)	1.12 ± 0.51	1.05 ± 0.54	0.442	1.29 ± 0.60	1.31 ± 0.67	0.855
LDL-cholesterol (mmol/liter)	2.98 ± 0.78	2.64 ± 0.80	0.009	2.69 ± 0.59	2.49 ± 0.59	0.092
HDL-cholesterol (mmol/liter)	1.35 ± 0.26	1.35 ± 0.28	0.995	1.45 ± 0.36	1.34 ± 0.31	0.271
Insulin (mU/liter)	19 ± 9	12 ± 7	0.020	19 ± 9	21 ± 6	0.581
Glucose (mmol/liter)	4.7 ± 0.4	4.7 ± 0.3	0.814	4.5 ± 0.4	4.6 ± 0.4	0.314
HOMA	3.9 ± 2.8	2.4 ± 1.4	0.031	3.8 ± 2.0	4.2 ± 3.5	0.449
AST (U/liter)	28 ± 8	25 ± 6	0.236	32 ± 9	31 ± 7	0.366
ALT (U/liter)	28 ± 12	25 ± 14	0.632	39 ± 12	37 ± 18	0.320
% NAFLD	29	5	0.038	40	40	0.999
Fetuin-A (g/liter)	0.30 ± 0.08	0.27 ± 0.06	0.029	0.31 ± 0.05	0.33 ± 0.09	0.433

Data are shown as mean and SD, unless stated otherwise. Baseline parameters of the obese children with substantial weight loss and without change of weight status did not differ significantly. ALT, Alanine aminotransferase; AST, aspartate aminotransferase.

[a] Baseline *vs.* 1 yr later.

fetuin-A has been demonstrated as an independent risk factor of type 2 diabetes (30). In addition, in one study maternal fetuin-A serum levels were positively associated with indexes of maternal insulin resistance in pregnant women (31).

Ix *et al.* (6) suggested that the relationship between fetuin-A and the MetS may be a result of fetuin-A induced suppression of adiponectin production. In fact, Hennige *et al.* (24) recently demonstrated that fetuin-A represses adiponectin production in animals and humans. Adiponectin is an adipocytokine, and represents an important determinant of whole body sensitivity and cardiovascular disease (32). In addition, fetuin-A induced low-grade inflammation (24), which is also associated with the MetS and an atherogenic lipid profile (1, 5).

In conjunction with existing animal data, these observations suggest that fetuin-A may promote the development of the MetS and, therefore, an atherogenic profile in humans. In concordance, fetuin-A concentrations were related to vascular stiffness and calcification in children on dialysis (33). However, previous data from select populations such as patients with end-stage renal diseases suggest that low fetuin-A concentrations may predict increased cardiovascular risk (34). Fetuin-A has emerged as a potent inhibitor of vascular calcification in experimental animals (35). Fetuin-A is considered as mineral chaperone mediating the stabilization, safe transport, and clearance in the body of calcium and phosphate as colloidal complexes, thus preventing calcification (36). Conversely, Mori *et al.* (25) recently demonstrated that fetuin-A levels in healthy subjects were positively associated with carotid arterial stiffness, whereas Roos *et al.* (26) found no correlation between fetuin-A levels and artery calcification evaluated by multi-slice computed tomography. Future studies are required to evaluate whether fetuin-A independently predicts incident cardiovascular diseases.

Because fetuin-A knockout mice are resistant to weight gain on a high-fat diet (8), one could speculate that high fetuin-A levels lead to obesity. Conversely, obese children demonstrated similar fetuin-A concentrations compared with normal weight children in our study. Fetuin-A levels decreased after substantial weight loss in our study, demonstrating the reversibility of the increased fetuin-A concentrations in humans, and pointing toward increased fetuin-A levels rather as a consequence than a cause of obesity. The hypothesis that obesity leads to increased fetuin-A levels in some individuals is supported by animal studies. In a rat model of diet-induced obesity, an increase in fetuin-A mRNA expression was observed in the liver (37).

This study has a few potential limitations. First, BMI percentiles were used to classify overweight. Although BMI is a good measure for overweight, one needs to be aware of its limitation as an indirect measure of fat mass. Second, the HOMA model is only an assessment of insulin resistance (38). Clamp studies are actually the gold standard for analyzing insulin resistance. Third, we are not able to differentiate the effect of diet, increased physical exercise, and weight loss on fetuin-A concentrations due to our study protocol. Fourth, fetuin-A has been reported as biomarker for kidney injury (39). Therefore, renal function studies in obese subjects with increased fetuin-A concentrations are necessary. However, the obese children in our study did not dem-

onstrate any clinical sign of renal diseases. Fifth, the day-to-day variation of fetuin-A is unknown in children. In adult patients with chronic kidney diseases, fetuin-A levels demonstrated a large within-subject variation (40). Sixth, we found no correlation between transaminases and fetuin-A concentrations. However, the levels of transaminases have been a poor marker of the severity of NAFLD (2). Finally, the diagnosis of NAFLD was not confirmed by liver biopsy. Liver biopsies in children are difficult to perform, also for ethical reasons, because no specific therapy follows histological diagnosis of NAFLD apart from recommending reduction of overweight, which is generally advised to all obese children.

In summary, fetuin-A concentrations were higher in obese children with NAFLD compared with lean children and obese children without NAFLD, suggesting a relationship between fetuin-A and NAFLD. Fetuin-A levels were independent of age, pubertal stage, and gender. Because fetuin-A was significantly related to insulin resistance and other features of the MetS such as increased waist circumference, increased blood pressure, and decreased HDL-cholesterol levels both in cross-sectional and longitudinal analyses, these findings support the hypothesis of a functional relevant relationship between fetuin-A and MetS in obesity. Further prospective research is necessary to clarify the role of fetuin-A in the pathogenesis of insulin resistance, especially in obese humans, as well as related molecular pathways leading to the development of MetS and NAFLD or other diseases, such as impaired kidney function, calcification, and vascular stiffness.

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This study is registered at clinicaltrials.gov (NCT00435734).

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