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Adipocyte fatty acid–binding protein in obese children before and after weight loss

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Abstract

Adipocyte fatty acid–binding protein (A-FABP) has been reported to be increased in obese adults and to be related to metabolic syndrome. Because studies concerning A-FABP in weight loss are limited and studies in obese children are missing, we analyzed A-FABP in obese children before and after weight loss. Fasting serum A-FABP, leptin, insulin, glucose, triglycerides, low-and high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and tumor necrosis factor α concentrations as markers of the metabolic syndrome, and weight status (body mass index and percentage body fat based on skinfold measurements) were determined in 30 obese children (median age, 11.9 years) before and after participating in a 1-year obesity intervention. Furthermore, A-FABP levels were measured in 10 nonobese children of similar age, sex, and pubertal stage. Obese children had significantly ($P < .001$) higher A-FABP concentrations compared with nonobese children. In backward multivariate linear regression analysis, A-FABP correlated significantly ($P < .05$) with percentage body fat and leptin, but not with any of the markers of the metabolic syndrome. Changes of A-FABP concentrations correlated significantly with changes of percentage body fat ($r = 0.53$, $P = .001$) and leptin ($r = 0.55$, $P < .001$), but not with any changes of parameters of the metabolic syndrome. Substantial weight loss in 10 children led to a significant ($P < .05$) decrease in A-FABP levels in contrast to the 20 children without change of weight status. In cross-sectional as well as longitudinal analyses, A-FABP levels were related to weight status and leptin levels. Further longitudinal studies are necessary to study the relationship between A-FABP concentrations and parameters of the metabolic syndrome.

1. Introduction

Obesity, characterized by excess accumulation of adipose tissue, is the most common risk factor for metabolic syndrome, a cluster of abnormalities including dyslipidemia, insulin resistance, hypertension, and atherosclerosis [1]. Although the molecular pathways that link obesity with such a wide spectrum of metabolic and cardiovascular defects are poorly understood, recent studies indicated a central role of adipose tissue in the development of this syndrome [2,3]. Accumulating evidence suggests that adipose tissue is not simply an inert energy storage depot but also functions as a major endocrine organ, producing and releasing a variety of bioactive adipokines into the bloodstream [4,5]. These adipose tissue–derived bioactive molecules, through their local and systemic actions, are postulated to regulate energy metabolism, insulin sensitivity, inflammation, and vascular responses [4,5].

Adipocyte-specific fatty acid–binding protein (A-FABP) belongs to the fatty acid–binding proteins accounting for approximately 6% of total cellular proteins [6]. This protein may be an important regulator of systemic insulin sensitivity and lipid and glucose metabolism [6,7]. Mice deficient in A-FABP are protected from development of hyperinsulinemia, hyperglycemia, and insulin resistance in the context of both dietary and genetic obesity [8,9]. Adipocytes obtained from A-FABP–null mice had markedly reduced efficiency of lipolysis in vivo and in vitro [10,11] and exhibited a 2- to 3-fold decrease in fatty acid release, suggesting that A-FABP mediates efflux of fatty acids in normal physiology [12]. Furthermore, the acute insulin secretory response to β-adrenergic stimulation was profoundly suppressed in A-FABP(−/−) mice compared

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with their wild-type littermates [11], suggesting that this protein modulates systemic insulin sensitivity through its actions on other distal target tissues. Recently, Furuhashi et al [13] demonstrated that orally available small molecule inhibitors of fatty acid–binding protein were effective in treating diabetes and atherosclerosis in mice.

Adipocyte fatty acid–binding protein is present in adipocytes and macrophages [14,15], which bear striking similarities to adipocytes in biology and function [16]. The expression of A-FABP can be suppressed by cholesterol-lowering statin drugs [17]. Adipocyte fatty acid–binding protein modulates inflammatory cytokine production and cholesterol ester accumulation [18]. In apolipoprotein E–deficient mice, ablation of the A-FABP gene conferred remarkable protection against atherosclerosis [19,20]. Taken together, these animal studies suggest that A-FABP, by integrating metabolic and inflammatory pathways, may be a link between various components of metabolic syndrome, systemic chronic inflammation, and obesity. Nevertheless, the clinical relevance of these findings remains to be confirmed in humans.

One study in obese adults postulated a strong relationship between weight status, markers of the metabolic syndrome, and A-FABP [6]. However, because this study was cross-sectional, these data have to be interpreted with caution while longitudinal data are required but still missing. Moreover, because atherosclerosis and the feature of metabolic syndrome begin in childhood [21], studies on population and individual differences in the early onset and progression of possible initiating risk factors through childhood are important. One further advantage of examining children is that no potential confusion exists with coronary disease or active tobacco smoking [22,23]. However, to our best knowledge, no studies concerning A-FABP have yet been performed in childhood.

Therefore, the aim of this study was to study A-FABP levels in obese and nonobese children to demonstrate whether A-FABP concentrations are already altered in obese children. Furthermore, we analyzed the changes of A-FABP in obese children before and after participating in a 1-year obesity intervention. We hypothesized that A-FABP concentrations are increased in obese children and normalize in weight loss. We also analyzed exploratively the relationship between A-FABP and parameters of the metabolic syndrome such as insulin, glucose, lipids, blood pressure, and insulin resistance and markers of inflammation (high-sensitivity C-reactive protein [hsCRP], tumor necrosis factor α [TNF-α]).

2. Methods

We examined anthropometric markers and fasting serum A-FABP, leptin, glucose, insulin, triglycerides, high- (HDL) and low-density lipoprotein (LDL) cholesterol, hsCRP, and TNF-α in 30 obese white children aged 8 to 15 years (median age, 11.9 years; interquartile range [IQR], 9.9-13.1 years; 40% male; 43% prepubertal) before and after participating in a 1-year outpatient obesity intervention program. In addition, A-FABP concentrations were determined in 10 lean healthy white children of similar age (median, 11.9 years; IQR, 9.9-13.1 years), sex (40% male), and pubertal stage (50% prepubertal). Children with endocrine disorders, premature adrenarche, or syndromal obesity were excluded from the study. All participants were nonsmokers without any regular medication. Subjects with intercurrent infections and/or febrile subjects were rescheduled and examined when they were not ill to exclude artificially elevated hsCRP levels. Obesity was defined according to the International Task Force of Obesity using population-specific data [24,25].

All obese children participated in the 1-year obesity intervention program *Obeldicks*, which has been described in detail elsewhere [26,27]. Briefly, this outpatient intervention program for obese children was based on physical exercise, nutrition education, and behavior therapy including individual psychological care of the child and his or her family. The nutritional course was based on a fat- and sugar-reduced diet as compared with the everyday nutrition of German children: the diet contained 30% fat, 15% proteins, and 55% carbohydrates including 5% sugar.

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale. Because body mass index (BMI) is not normally distributed in childhood, we used the LMS method to calculate standard deviation score (SDS)-BMI as a measurement for the degree of overweight. This method summarizes the data in terms of 3 smooth age-specific curves termed L (λ), M (μ), and S (σ) based on German population-specific data [25,28]. The M and S curves correspond to the median and coefficient of variation (CV) BMI for German children at each age and sex, whereas the L curve allows for the substantial age-dependent skewness in the distribution of BMI [25,28]. The assumption behind the LMS method is that, after Box-Cox power transformation, the data at each age are distributed normally [28]. Substantial weight loss in the course of the 1-year intervention was defined by a reduction in SDS-BMI $\geq$0.5 because with a reduction of <0.5 SDS-BMI, no improvement of any markers of the metabolic syndromes could be measured in obese children [29,30].

The triceps and subscapularis skinfold thickness was measured in duplicate using a caliper and averaged to calculate the percentage of body fat using a skinfold thickness equation with the following formulas [31]: boys: body fat percentage = 0.783 × (subscapularis skinfold thickness + triceps skinfold thickness in mm) + 1.6; girls: body fat percentage = 0.546 × (subscapularis skinfold thickness + triceps skinfold thickness in mm) + 9.7.

The pubic hair stage was determined according to Marshall and Tanner. Pubertal developmental stage was categorized into 2 groups (prepubertal: boys with pubic hair stage I and gonadal stage I, girls with pubic hair stage I and breast stage I; pubertal:

boys with pubic hair stage ≥II or gonadal stage ≥II, girls with pubic hair stage ≥II or breast stage ≥II).

Blood sampling was performed in the fasting status at 8:00 AM. Insulin concentrations were measured by microparticle-enhanced immunometric assay (Abbott, Wiesbaden, Germany). Glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). High-sensitivity C-reactive protein concentrations were measured by means of a particle-enhanced immunonephelometric assay using a BN II analyzer (Dade Behring, Marburg, Germany). The sensitivity of this assay was 0.18 mg/L. Tumor necrosis factor α concentrations were determined by an immunometric assay using an Immulite analyzer (DPC Biermann, Bad Nauheim, Germany). The sensitivity of this assay was 1.7 pg/mL. The 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 were measured by a colorimetric assay using a Vitros analyzer (Ortho Clinical Diagnostics). Intra- and interassay CVs were <5% in all methods. Homeostasis model assessment (HOMA) was used to detect the degree of insulin resistance [32]: The resistance can be assessed from the fasting glucose and insulin concentrations by the following formula: resistance (HOMA) = (insulin [micro-units per liter] × glucose [millimoles per liter])/22.5. Serum A-FABP concentrations were measured by a high-specific enzyme-linked immunoassay (human A-FABP ELISA; BioVendor, Heidelberg, Germany). The sensitivity was 0.1 μg/L. Intra- and interassay CVs were <7%. Leptin was determined by radioimmunoassay (Human Leptin RIA; Mediagnost, Reutlingen, Germany; intraassay CV <5%, interassay CV <8%, sensitivity 0.1 ng/mL).

Statistical analysis was performed using the Winstat software package (R. Fitch Software, Bad Krozingen, Germany). All variables were normally distributed as tested by Kolmogorov-Smirnov test. Student *t* tests for paired and unpaired observations were used. Correlations between A-FABP, weight status (SDS-BMI), blood pressure, hsCRP, TNF-α, lipids, insulin, and insulin resistance index (HOMA) at baseline and correlations between changes of these parameters after 1 year were calculated by Spearman correlation. Changes were expressed as Δ variable calculated by variable at baseline − variable 1 year later. A backward multivariate linear regression analysis was conducted for the dependent variable A-FABP, including age, sex, pubertal stage, weight status (SDS-BMI), leptin, lipids, blood pressure, hsCRP, TNF-α, glucose, and insulin as independent variables, in the 30 obese children. Furthermore, a backward multivariate linear regression analysis was conducted for the

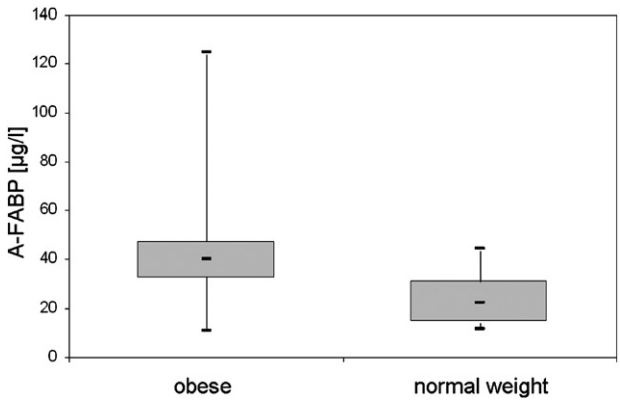

Fig. 1. The A-FABP concentrations in 30 obese and 10 normal-weight children of similar age, sex, and pubertal stage (data as median, IQR and maximum/minimum; $P = .009$).

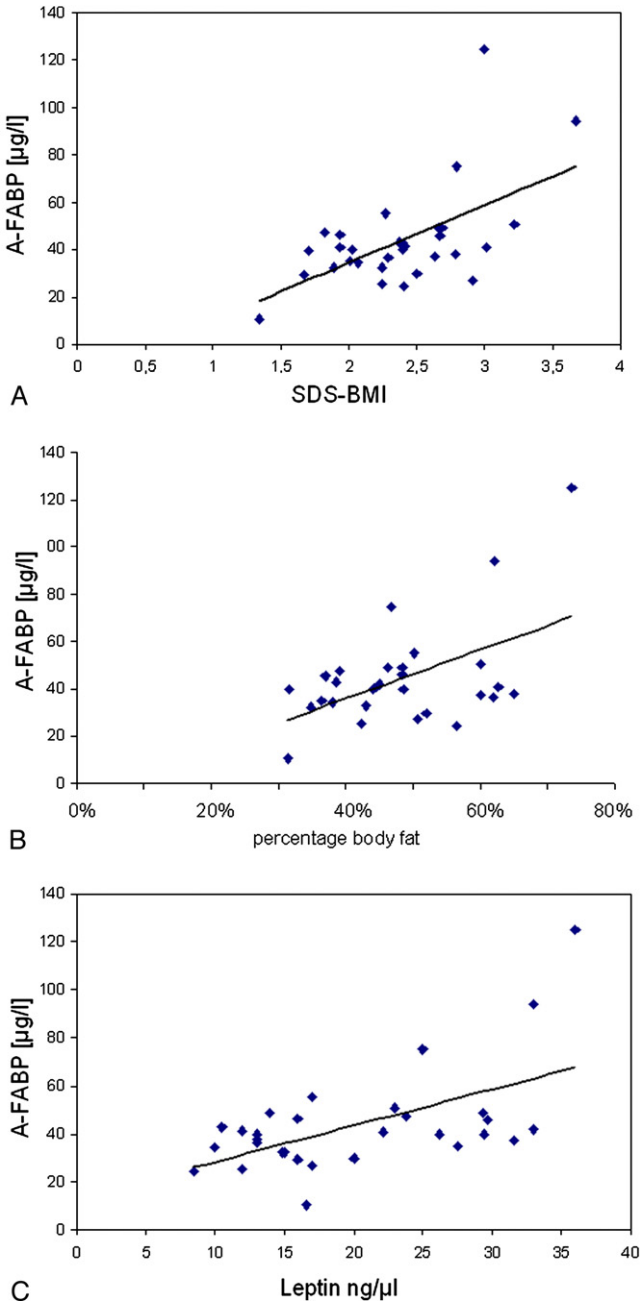

Fig. 2. Relationships between A-FABP concentrations and (A) SDS-BMI ($r = 0.45$, $P = .006$), (B) percentage fat based on skinfold measurements ($r = 0.38$, $P = .020$), and (C) leptin ($r = 0.47$, $P = .004$) in 30 obese children.

dependent variable Δ A-FABP, including age, sex, pubertal stage, Δ SDS-BMI, Δ leptin, Δ lipids, Δ blood pressure, Δ hsCRP, Δ TNF-α, Δ glucose, and Δ insulin as independent variables, in the 30 obese children. Sex and pubertal stage were used as classified variables in these models. A P value < .05 was considered as significant. Data were presented as mean and standard deviation. 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.

3. Results

The 30 obese children demonstrated significantly (P = .009) increased A-FABP concentrations as compared with the 10 lean children (Fig. 1). At baseline, A-FABP correlated significantly to SDS-BMI (r = 0.45, P = .006), percentage body fat (r = 0.38, P = .020), and leptin (r = 0.47, P = .004) (Fig. 2).

Ten children reduced their overweight substantially in the intervention program (Table 1). This substantial weight loss led to a significant decrease of A-FABP and leptin as well as to an improvement of the parameters of metabolic syndrome. In the 20 obese children without substantial weight loss, no significant changes of A-FABP and leptin levels occurred; and the parameters of metabolic syndrome did not change significantly. The changes of A-FABP in the 30 obese children between baseline and 1 year later correlated significantly to changes of SDS-BMI (r = 0.52, P = .001), percentage body fat (r = 0.53, P = .001), and leptin (r = 0.55, P < .001) (Fig. 3).

At baseline, no significant differences were observed in age (P = .225), sex (P = .999), pubertal stage (P = .679), BMI (P = .082), SDS-BMI (P = .826), percentage body fat (P = .679), A-FABP (P = .552), leptin (P = .769), and any markers of the metabolic syndrome or inflammatory markers between the obese children with and without substantial weight loss. One year later, SDS-BMI (P = .001), percentage body fat (P = .006), and leptin (P = .036) and A-FABP (P = .012) concentrations were significantly lower in the children with substantial weight loss as compared with the children without substantial weight loss.

We found no significant correlation between A-FABP and markers of the metabolic syndrome or markers of inflammation both in cross-sectional and longitudinal analyses. In backward multivariate linear regression analysis (r^2 = 0.46), A-FABP correlated significantly to SDS-BMI (coefficient, 17; 95% confidence interval [CI], 5-39; P = .011) and leptin (coefficient 1.1; 95% CI, 0.3-1.9; P = .012), but not age, sex,

Table 1
Changes of weight status, percentage body fat, insulin resistance index (HOMA), insulin, glucose, lipids, hsCRP, TNF-α, leptin, and A-FABP concentrations in 10 obese children with substantial weight loss and 20 children with stable weight status over a 1-year period

	Substantial weight loss		No change of weight status	
n	10		20	
Age (y)	11.2 (8.8-11.9)		11.8 (9.9-12.9)	
Sex	40% male		40% male	
Pubertal stage	50% prepubertal [a]		40% prepubertal [b]	
Change of SDS-BMI	−0.6 (−0.7 to −0.5)		0.0 (−0.1 to +0.2)	
	At baseline	1 y later	At baseline	1 y later
BMI (kg/m^2)	27.2 (24.3-28.5)	25.1 (22.4-26.4) *	29.7 (25.6-34.4)	28.5 (24.7-34.2)
SDS-BMI	2.3 (2.1-2.7)	1.7 (1.2-1.9) **	2.4 (2.0-2.8)	2.2 (2.0-2.9)
Subscapularis ST (mm)	31 (28-35)	22 (20-29) *	32 (26-40)	31 (20-43)
Triceps ST (mm)	32 (25-35)	23 (20-27) *	32 (26-40)	32 (23-36)
Percentage body fat (%)	46 (42-52)	34 (32-44) **	48 (37-60)	46 (33-53)
Triglycerides (mg/dL)	91 (78-129)	76 (54-126)	90 (61-130)	88 (64-115)
LDL cholesterol (mg/dL)	116 (94-137)	83 (75-104) *	108 (95-127)	104 (82-122)
HDL cholesterol (mg/dL)	43 (40-50)	50 (40-58) *	47 (42-56)	52 (44-63)
Systolic BP (mm Hg)	113 (110-126)	107 (101-111) *	120 (111-129)	121 (111-126)
Diastolic BP (mm Hg)	62 (50-64)	55 (51-71)	59 (51-81)	64 (51-71)
Insulin (mU/L)	16 (12-27)	11 (8-19) *	16 (12-22)	15 (8-25)
Glucose (mg/dL)	88 (84-89)	86 (82-91)	86 (81-89)	87 (77-91)
HOMA	3.4 (2.6-5.8)	2.3 (1.7-3.9) **	3.6 (2.3-4.7)	3.3 (1.7-5.0)
hsCRP (mg/L)	1.2 (0.6-1.5)	0.4 (0.2-0.9) **	1.6 (1.0-3.5)	1.1 (0.5-1.4)
TNF-α (pg/mL)	6.8 (4.9-8.8)	6.8 (5.7-8.4)	5.8 (4.8-6.8)	6.2 (4.7-7.9)
Leptin (ng/mL)	19.6 (13.5-27.0)	15.0 (5.9-20.7) **	16.8 (13.0-32.9)	17.8 (14.8-28.5)
A-FABP (μg/L)	41 (31-49)	29 (20-37) **	39 (33-46)	46 (20-59)

Data are presented as median and IQR. ST indicates skinfold thickness; BP: blood pressure.
[a] One child entered into puberty during the intervention.
[b] Two children entered into puberty during the intervention.
* P < .05 baseline vs 1 year later.
** P < .01 baseline vs 1 year later.

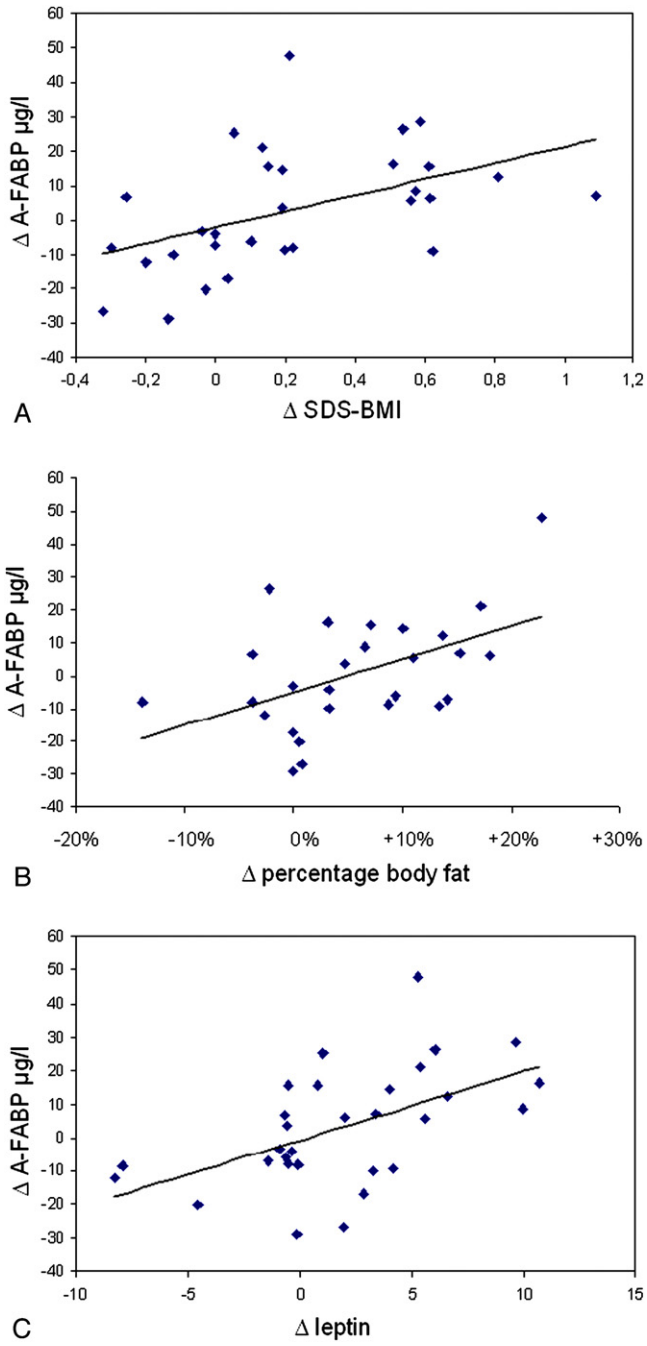

Fig. 3. Relationships between changes of A-FABP concentrations and (A) changes of SDS-BMI (r = 0.52, P = .001), (B) changes of percentage fat based on skinfold measurements (r = 0.53, P = .001), and (C) changes of leptin (r = 0.55, P < .001) in 30 children between baseline and 1 year later after participating in an obesity intervention program (Δ: variable at baseline − variable 1 year later).

pubertal stage, or any factor of the metabolic syndrome or inflammatory markers (lipids, blood pressure, hsCRP, TNF-α, glucose, insulin, HOMA). If percentage body fat instead of SDS-BMI was included in the backward multivariate linear regression analysis (r^2 = 0.61), A-FABP correlated to percentage body fat (coefficient, 227; 95% CI, 216-338; P = .006) and leptin (coefficient, 1.1; 95% CI, 0.3-1.9; P < .001), but not to any factor of the metabolic syndrome or inflammatory markers. In backward multivariate linear regression analysis (r^2 = 0.29), the changes of A-FABP correlated significantly to changes of leptin (coefficient, 2.0; 95% CI, 0.8-3.2; P = .002), but not to changes of any factor of the metabolic syndrome or inflammatory markers independently if SDS-BMI or percentage body fat was included in the model.

The A-FABP levels at baseline of the girls (median, 40 μg/L; IQR, 34-49 μg/L) did not significantly (P = .836) differ from the A-FABP concentrations of the boys (median, 39 μg/L; IQR, 27-44 μg/L). Boys and girls did not differ in respect of age (P = .426), pubertal stage (P = .760), and SDS-BMI (P = .867). The A-FABP levels of the prepubertal children (median, 39 μg/L; IQR, 32-45 μg/L) did not significantly (P = .225) differ from the A-FABP concentrations of the pubertal children (median, 40 μg/L; IQR, 32-52 μg/L). Prepubertal and pubertal children did not differ in respect of sex (P = .550) and SDS-BMI (P = .310).

4. Discussion

This is the first study analyzing cross-sectional and longitudinal relationships between A-FABP and leptin concentrations, components of the metabolic syndrome, and inflammatory markers in childhood. We were able to demonstrate that obese children had significantly higher A-FABP levels as compared with lean children. The A-FABP levels decreased significantly in obese children who achieved a substantial reduction after 1 year in overweight in contrast to obese children without substantial weight loss. In concordance with this finding, a decreased expression of A-FABP in human subcutaneous adipose tissue was reported in weight loss [33]. Therefore, the increase of A-FABP in obesity seems to be a consequence of obesity. The positive association between serum A-FABP concentrations and indicators of adiposity (BMI and fat percentage) in children and adults [6] suggests that adipose tissue, which is composed of adipocytes and macrophages, is probably the major contributor of serum A-FABP. In concordance, A-FABP was significantly related to the adipocyte-derived hormone leptin both in cross-sectional and longitudinal analysis.

The physiologic functions of circulating A-FABP remain to be determined. Cytoplasmic A-FABP has been proposed to be involved in the intracellular trafficking and targeting of fatty acids inside cells [33]. Like most FABPs, A-FABP can bind with a variety of hydrophobic lipid ligands known to influence systemic inflammation [7,34-36]. Accumulating evidence from animal experiments suggests that A-FABP is a central regulator of systemic insulin sensitivity, lipid metabolism, and inflammation [7,14], although its relevance in humans remains to be proven. One study reported a relationship between dyslipidemia, HOMA, blood pressure, and A-FABP concentrations in obese adults [6]. Moreover,

Xu et al [37] demonstrated recently that A-FABP was predictive of the metabolic syndrome in a 5-year prospective study in adults. Our cross-sectional and longitudinal analyses in obese children detected no relationship between A-FABP concentrations and any parameters of the metabolic syndrome or inflammatory markers. These differences may be explained in part by analyzing different ethnicities and different age groups. Conversely, our study sample was only moderate. The lack of significance may be caused by lack of power. Further larger longitudinal studies are necessary.

This study has a few additional potential limitations. First, BMI percentiles and skinfold measurements were used to classify overweight. Although BMI and skinfold measurements are a good measure for overweight, one needs to be aware of their limitations as an indirect measure of fat mass. Secondly, HOMA model is only an assessment of insulin resistance; and clamp studies are the criterion standard to analyze insulin resistance. Because the HOMA model correlated to clamp studies, it is a suitable method to study insulin resistance in field studies [38].

In summary, the fasting A-FABP levels were increased in obese children. This increase tended to normalize after weight loss, demonstrating the reversibility. The A-FABP concentrations were significantly related to leptin, but we found no correlation to any parameter of the metabolic syndrome or inflammatory marker in cross-sectional as well as longitudinal analyses. Further prospective research is required to determine the physiologic role of A-FABP in humans.

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