Adipocyte Fatty Acid-Binding Protein 4 is Altered in Growth Discordant Dichorionic, but not in Monochorionic Twins

Susanne Schrey-Petersen,[1] Saskia Bäumer,[1] Ulrike Lössner,[2] and Holger Stepan[1]

[1]*Department of Obstetrics, University of Leipzig, 04103 Leipzig, Germany and* [2]*Department of Internal Medicine III, University of Leipzig, 04103 Leipzig, Germany*

ORCiD numbers: 0000-0002-8012-0998 (S. Schrey-Petersen); 0000-0003-1264-7365 (H. Stepan).

Context: The fetal period has a critical and long-lasting impact on the regulation of metabolic processes and a life-long predisposition for obesity and metabolic syndrome. The exact mechanisms are unknown, but epigenetic regulation likely plays a major role. Twins represent an excellent model to study these mechanisms, as they share the same intrauterine environment and similar or even the same genetic information. We examined cord blood levels of adipocyte fatty-acid binding protein 4 (A-FABP or FABP4), a novel adipokine correlated with obesity and metabolic disease in children and adults.

Objective: To examine A-FABP levels in the cord blood of twins with concordant and discordant growth and in singletons with intrauterine growth restriction (IUGR).

Design: Cohort study of 36 twin pairs (25 growth concordant and 11 growth discordant), and 42 singleton pregnancies (28 IUGR and 13 normally grown controls, 1 HELLP).

Outcome Measures: Cord blood A-FABP levels measured by enzyme-linked immunosorbent assay (ELISA).

Results: A-FABP levels were higher in the smaller of growth discordant dichorionic (DC) twins versus their co-twins (109.46 ± 62.80 ng/mL vs. 72.93 ± 36.66 ng/mL, $P = 0.028$). A-FABP was negatively correlated with birth weight and gestational age ($P < 0.001$), but not with birth weight z-score ($P = 0.37$).

Conclusions: Increased A-FABP levels might be associated with an increased metabolic risk in growth-restricted (twins) and prematurely born infants.

Obesity, metabolic syndrome and associated disorders such as cardiovascular disease are one of the major health problems in the world, with dramatically increasing incidence even in (early) childhood. Metabolic syndrome in this context is defined as a cluster of multicausal

(inherited and acquired) metabolic disorders, which increase the risk of future cardiovascular disease, diabetes, stroke, and diseases related to fatty buildups in the artery walls [1]. Since the 1980s, various studies have suggested, that regulation of metabolic processes are shaped not only early in life, but already prenatally. Normal or abnormal growth during the intrauterine period seems to have a critical and long-lasting impact on the modulation of metabolic processes, and the effects can be seen not only during the immediate postnatal period, but far into adulthood [2]. An increased risk for obesity, metabolic, and cardiovascular disorders seems to exist at both ends of the birth weight scale—both in those born far too small and in those born far too large[1].

The phenomenon of intrauterine modulation of postnatal processes has been described as "fetal programming," and it is assumed that epigenetic processes, meaning the modulation of gene expression, play a major role, although the specific mechanisms are largely unknown.

Twins represent a special group to study the effects of intrauterine growth or epigenetic regulation, as they share the same intrauterine environment and similar—in case of monozygotic twins even the same—genetic information. Despite this, severe growth discordance between the two fetuses can be found in many twin pregnancies, which offers a unique possibility to study the effects of growth modulation and epigenetic regulation of metabolic processes.

Adipokines are a variety of protein mediators, which are produced primarily by the adipose tissue and play an essential role in the regulation of metabolic, immunologic, and vascular processes and function [3]. They are also involved in the modulation of nutrient uptake, of tissue growth and turnover. Many adipokines, especially those which are proinflammatory (eg, leptin, chemerin) are increased in subjects with obesity and insulin resistance, and are often positively correlated with other parameters of fat mass and distribution, insulin sensitivity, and glucose metabolism. Anti-inflammatory adipokines, such as adiponectin are often decreased or negatively correlated with high fat mass [4, 5]. This indicates that adipokines play an important role in the connection between elevated body weight and insulin sensitivity or metabolic syndrome.

Whereas the expression of various long-known adipokines, such as leptin or adiponectin, has been (extensively) studied in the perinatal period, little is known about the relation between intrauterine growth/ birth weight and the serum expression of so-called novel adipokines, such as adipocyte fatty-acid binding protein 4 (A-FABP, FABP4).

Adipocyte fatty acid binding protein (A-FABP or FABP4, OMIM 600424) has recently been introduced as a novel fat-derived circulating protein [6]. It is a 15 kDA molecule from the group of fatty acid transport-proteins (FABP), which are primarily produced by adipocytes and macrophages [7]. The main function of A-FABP is to facilitate the transport of fatty acids into specific cell organelles [7], but it also plays a role in the transformation of fatty acids into eicosanoids and the stabilization of leukotrienes [7]. A-FABP serum concentrations have been positively correlated with markers of the metabolic syndrome and vascular disease in various cross-sectional and interventional studies [6]. A small set of prospective studies indicates that high A-FABP serum levels at baseline predict the risk for metabolic and vascular morbidity and mortality [6].

Various studies indicate that A-FABP might also be correlated with childhood obesity and early onset of metabolic and cardiovascular disorders [8–12], but A-FABP has so far not been studied in the context of severely impaired intrauterine growth. A recent study has demonstrated, that cord blood A-FABP levels correlated with gestational age and birth weight in normally grown neonates [13]. In a previous study, we could show, that A-FABP was elevated in mothers with preeclampsia and gestational diabetes. [14, 15].

The aim of the present study was to examine the relation between impaired intrauterine growth and the expression of A-FABP in the cord blood, with special attention on the group of growth concordant and discordant twins.

1. Methods and Materials

A. Study Population

We performed a cohort study of 36 twin pairs with concordant (N = 25) or discordant (N = 11) fetal growth born at the University Hospital of Leipzig in the years 2015 and 2016. Discordant growth was defined as a growth discordance of ≥ 20% of the weight of the larger twin. Of the 25 twin pairs with concordant growth, 16 were DC and 9 were monochorionic (MC). Of the 11 twin pairs with discordant growth, 7 were DC and 4 were MC. The individual twin was categorized as "smaller twin" if it was the twin with the lower birth weight within a pair. Patients were selected either before birth, when they presented to our clinic for ultrasound examination, or at time of birth, if patients were not seen at our clinic before. Umbilical cord blood was collected immediately after birth from both vaginal and cesarean deliveries. One twin mother was lost for follow-up due to delivery in a different hospital. The study protocol was reviewed and approved by the Institutional Review Board of the Medical Faculty of the University of Leipzig. Informed written consent was obtained from the pregnant patients. The study adheres to the declaration of Helsinki.

The group of singletons consisted of 42 pregnancies—28 infants were classified as IUGR, as they had a birth weight below the 10th percentile plus signs of severely abnormal placental function (24 with abnormal umbilical Doppler, 3 with normal umbilical but severely abnormal uterine perfusion, and one with borderline abnormal uterine perfusion but with signs of severe [asymmetric] IUGR ([birth weight below the 3rd percentile and abdominal circumference far below the 5th percentile]). One patient was analyzed separately, as the patient developed HELLP syndrome (hemolysis, elevated liver enzymes, low platelet-syndrome) and placental morphology was severely abnormal ("jelly-like"). Birth weight in this infant was in the 33rd percentile. The control group consisted of 13 infants with normal birth weight between the 10th and 90th percentile and absence of any signs of placental dysfunction.

B. Cord Blood Collection and Measurement of Plasma A-FABP

Cord blood samples were taken immediately after birth, and to avoid hemolysis, samples were kept at room temperature for 10 minutes, before they were stored in the refrigerator until collection from the laboratory team within the next 24 hours. They were centrifuged (4000 g at 20°C for 10 minutes), and serum samples were divided into smaller aliquots, which were stored in Eppendorf tubes at -80°C until analysis. At time of analysis, aliquots were thawed at room temperature, vortexed, and centrifuged (2000 rp, for 2 minutes). Nine samples from the twin groups, 8 samples from the IUGR/SGA group, and 6 samples from the control group could not be analyzed due to hemolytic serum. Serum A-FABP was measured using sandwich ELISA [16] according to the manufacturer´s instructions (BioVendor, Brno, South Moravia, Czech Republic). The following performance criteria were provided by the manufacturer. The sensitivity of the assay (or limit of detection) was 0.08 ng/mL. The antibodies in the assay were specific for human A-FABP, but there was observed cross reactivity with samples from monkey, dog, and mouse. The manufacturer performed cross-reactivity testing with other members of the FABP family, and no cross-reactivity was found for liver FABP, intestinal FABP, heart FABP, brain FABP, myelin FABP, testis FABP, ileal FABP, and FABP 12. Data on reproducibility was assessed for A-FABP ELISA as an IVD (in-vitro-Diagnostikum) kit on 6 samples in various labs, and the average CV (coefficient of variability) value was 4.4% (this parameter is not listed in the manual). Further performance data can be found in the manufacturer's manual [16].

C. Measurement of Other Proteins and Adipokines

Besides A-FABP, the following proteins and adipokines were measured accordingly as described above: leptin [17], adiponectin [18], chemerin [19], retinol-binding protein 4 (RBP-4) [20], tissue inhibitor of metalloproteinase 1 (TIMP-1) [21], growth hormone (GH) [22], insulin-like growth factor 1 (IGF-1) [23], zinc-alpha 2 glycoprotein (ZAG) [24], fibroblast growth factor 21 (FGF-21) [25], and progranulin [26]. Whereas leptin and adiponectin have already been studied in the context of metabolic syndrome (also in children), as well as their expression in cord blood and relation to abnormal birth weight, the remaining proteins where chosen as novel adipokines, which have recently been shown to possibly play an important role in association with obesity and other metabolic diseases [27, 28], and have also been studied in gestational diabetes and pre-eclampsia, very little to nothing is known about their expression in cord blood and their relation to abnormal birth weight and intrauterine growth. Most of the adipokines chosen for this study have been previously studied at our center in the context of gestational diabetes and/or preeclampsia [4, 15, 29-37].

D. Clinical Data of Mothers and Neonates

We collected clinical data from our hospital database (VIEWPOINT and SAP). Maternal data included gestational week at first presentation to our clinic, obstetrical anamnesis/previous pregnancies, pre-existing maternal diseases, gestational diabetes, medication, smoking status, body height and weight, body mass index (BMI) before pregnancy and at time of birth, and serum levels of insulin, glucose, and HbA1c. Related to the course of pregnancy, we collected the following data: estimated fetal weight by ultrasound (one or several estimations), abdominal circumference, amniotic fluid status, placental abnormalities, Doppler results of uterine arteries, umbilical arteries, middle cerebral arteries, and of ductus venosus.

E. Statistical Analysis

Results were expressed as mean ± standard deviation. Data from the twin groups was analyzed using the nonparametric Wilcoxon signed-rank test for paired samples. In the singleton cohort, the Mann–Whitney U test was used for the comparison between the IUGR and control groups. Correlation between A-FABP expression and birth weight, gestational age, birth weight z-score, and with other adipokines was calculated by Spearman´s rank correlation test. Significance level was defined as $P = 0.05$. All statistical analysis was performed using SPPS 25 software (IBM, AMonk, USA).

2. Results

A. Patient Characteristics

Among 25 twin pairs with concordant growth, 16 were DC and 9 were MC, of which 9 twin pairs (5 DC and 4 MC) had to be excluded from ELISA analysis, as 8 serum probes were hemolytic and 1 patient delivered in a different hospital.

Of the remaining 11 concordant DC twin pairs, the average gestational age at birth was 35.3 weeks, with an average difference in birth weight of 9.81% [min 2.3; max 15.1]. Of 5 concordant MC twin pairs, the average gestational age at birth was 35.0 weeks, with an average difference in birth weight of 9.34% [min 0.5; max 13.9]. Further clinical data are shown in Table 1.

Among 11 twin pairs with discordant growth, 7 were DC and 4 were MC. Three discordant DC twin pairs had to be excluded from analysis because of hemolytic serum probes. The average gestational age at time of birth was 33.3 weeks in discordant DC and 29.5 weeks in discordant MC twins. The average weight difference of the 4 remaining discordant

Table 1. Clinical Data of Twins

	Concordant DC/DA	Discordant DC/DA	Concordant MC/DA	Discordant MC/DA
Total number (No.)	16	7	9	4
No. of analyzed serum probes	11 (4 hemolyt., 1 lfu)	6 (1 hemolyt)	5 (4 hemolyt)	4
Maternal age at birth in years (mean ± STD)	31.3 ± 3.9 [min 24; max 37]	31 ± 4 [min 27; max 37]	27.6 ± 2.7 [min 25; max 32]	35 ± 2.7 [min 30; max 36]
BMI (mean; min, max) at birth	28.3 [22;37.8]	29.2 [22.2;34.4]	30.9 [23.1;37.7]	34.3 [30.9;39.5]
Parity	I:8, II:2; III:1	I: 5; II: 1	I: 3; II: 1; IV: I	I: 2; III: 2
Gestational age at birth in weeks [mean; min, max]	35.3 [26;39]	33.3 [32;37]	35.0 [33;37]	29.5 [28;30]
Birth weight (gramm) [mean ± SD; min, max]	-	-	-	-
Larger twin	2619 ± 365 [1890;3010]	2343 ± 772 [995;3220]	2160 ± 385 [1790;2650]	1484 ± 370 [980;1870]
Smaller twin	2390 ± 311 [1800;2980]	1459 ± 576 [450; 2090]	2001 ± 288 [1670; 2310]	810 ± 141 [620;995]
Birth weight difference in % [mean; min, max]	9.81 [2.3;15.1]	39.5 [21.1;54.8]	9.34 [0.5; 13.9]	42.6 [22.3; 60.2]
Z-score birth weight [mean]	-	-	-	-
Larger twin	-0.37	0.21	-0.82	0.26
Smaller twin	-0.96	-2.08	-1.18	-1.99
Gender				
Larger twin	7males/4 females	5 males/1 female	5 females	3 males/1 female
Smaller twin	5 males/6 females	4 males/2 females	-	-
Mode of delivery	5 vaginal / 6 CS (3 prim, 3 sec)	6 prim CS	1 vag / 4 CS (2 prim, 2 sec)	4 prim CS

Abbreviations: CS, cesarean section; DC/DA, dichorionic diamniotic twins; MC/DA, monochorionic diamniotic twins.

DC twin pairs was 39.5% [min 21.1; max 54.8], and of 4 discordant MC twins 42.6% [min 22.3; max 60.2]. Further clinical data can be found in Table 1.

Of 42 singleton pregnancies, 28 were classified as IUGR, 13 as controls and one as HELLP. Because of hemolytic serum probes, 8 patients from the IUGR group and 6 from the control group had to be excluded from analysis. Gestational age at birth in the IUGR group was 33.2 ± 4.9 weeks, with an average birth weight of 1446 ± 751 g, and in the control group average gestational age at birth was 32 ± 3 weeks, with an average birth weight of 1897 ± 525 g. IUGR and control group were adjusted for and did not show a significant difference in maternal age, gestational age at birth, and maternal BMI. Further clinical data are shown in Table 2.

B. Comparison of Plasma A-FABP Levels Between Twins

In discordant DC twin pairs, A-FABP [16] levels in umbilical cord serum were significantly higher in the smaller twins than in their larger co-twins (109.46 ng/mL $\pm$ 62.80 ng/mL vs. 72.93 ± 36.66 ng/mL, $P = 0.028$, nonparametric Wilcoxon signed-rank test for paired samples), but this difference could not be shown for growth discordant MC twins, where A-FABP [16] had a (nonsignificant) trend to be higher in the larger twin (94.76 ng/mL $\pm$ 33.92 in smaller twins vs. 130.92 ± 34.16 ng/mL in larger twins, $P = 0.14$). Within both growth concordant twin groups, no significant difference was found between smaller and larger twins. Comparison of A-FABP [16] levels in twins is demonstrated in Fig. 1.

C. A-FABP Levels in IUGR versus Normal Weight Controls

In the IUGR group, the mean AFABP [16] level in cord serum was higher (103.08 ± 80.61 ng/mL) than in the normal control group (49.63 ± 23.75 ng/mL), but this difference was not significant (Mann–Whitney U test, $P = 0.263$). Comparison of A-FABP levels between IUGR infants and controls is demonstrated in Fig. 2.

D. Correlation Between AFABP Levels and Birth Weight, Gestational Age, and Z-Score of Birth Weight

Cord blood A-FABP [16] was highly significant, negatively correlated with birth weight and gestational age (both P-values < 0.001, $r = -0.668$ and $r = -0.691$), but not with z-score of birth

**Table 2. Clinical Data of Singletons**

	IUGR	Control	HELLP
Total No. of patients	28	13	1
No. of analyzed serum probes	20	7	1
	(8 hemolytic)	(6 hemolytic)	
Maternal Age at birth in years [mean ± STD]	28.75 ± 4.9	30.86 ± 4.2	29
Maternal BMI at birth [mean; min; max]	30.8 ± 6.6	28.6 ± 2.6	34.8
Gestational Age at Birth [weeks, mean ± STD; min, max]	33.2 ± 4.9 [26; 41]	32 ± 3 [26; 36]	30
Birth weight (gramm) [Mean ± SD; min, max]	1446 ± 751 [520; 2730]	1897 ± 525 [725; 2510]	1155
Z-score birth weight (mean ± STD)	-2.11 ± 0.50	-0.10 ± 0.72	-0.84
Gender	13 males/7 females	4 males/3 females	1 female
Mode of delivery	5 vaginal, 15 CS (12 primary, 3 secondary)	4 vaginal, 3 CS (2 primary, 1 secondary)	CS (primary)

Abbreviations: CS, cesarean section; HELLP, hemolysis, elevated liver enzymes, low platelet; IUGR, intrauterine growth restriction.
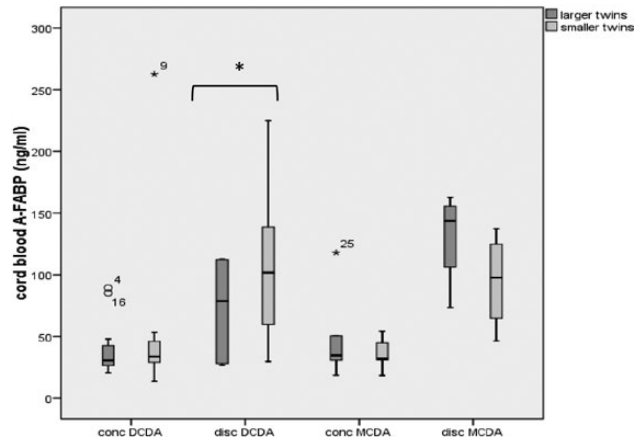
Figure 1. Cord blood levels of A-FABP in twins (ng/mL). A significant difference of cord blood A-FABP levels was found between the smaller and larger of growth discordant DCDA twins, but not between growth discordant MCDA twins (P = 0.14) or between smaller and larger of concordant twins. * Significant difference in mean A-FABP between the larger and the smaller of discordant growth DCDA twins (nonparametric Wilcoxon signed-rank test, P = 0.028); conc = concordant growth (weight difference less than 20% of birth weight of the larger twin); disc = discordant growth (weight difference ≥ 20% of the birth weight of the larger twin); MCDA = monochorionic diamniotic; DCDA = dichorionic diamniotic twins.
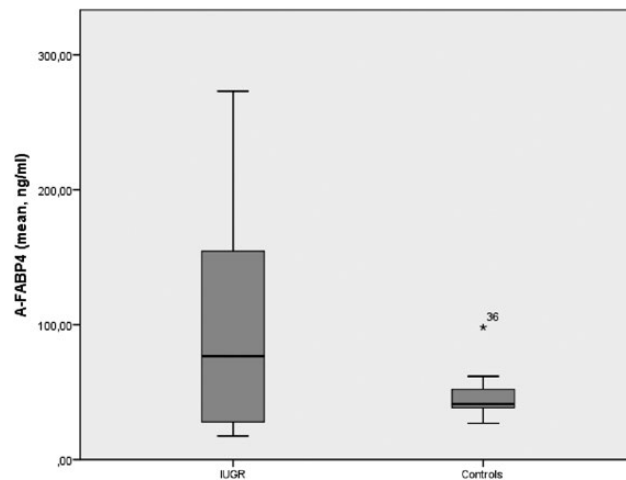

Figure 2. Cord blood levels of A-FABP in singletons (IUGR vs. controls), P = 0.263. No significant difference could be shown between A-FABP levels in cord blood of IUGR infants and normal weight controls (Mann–Whitney U test, P = 0.263).

weight (P = 0.37, r = -1.02). This was the case if all infants were analyzed together as well as in singletons or twins separately, as well as in all DC twins alone. Correlation between A-FABP [16] and birth weight, gestational age, and birth weight z-score is demonstrated in Fig. 3A–C (all infants).

E. Comparison Between A-FABP Levels and Other Adipokines

There was a significant negative correlation between A-FABP [16] and IGF-1 [23] (Spearman's rank correlation test: r = -0.468, P = 0.014) and a positive correlation between A-FABP and FGF-21 [25] (r = 0.551, P = 0.004) and progranulin [26] (r = 0.597, P = 0.001) when all subjects were analyzed together. Further, no significant positive or negative correlation was found between A-FABP [16] and any of the other analyzed
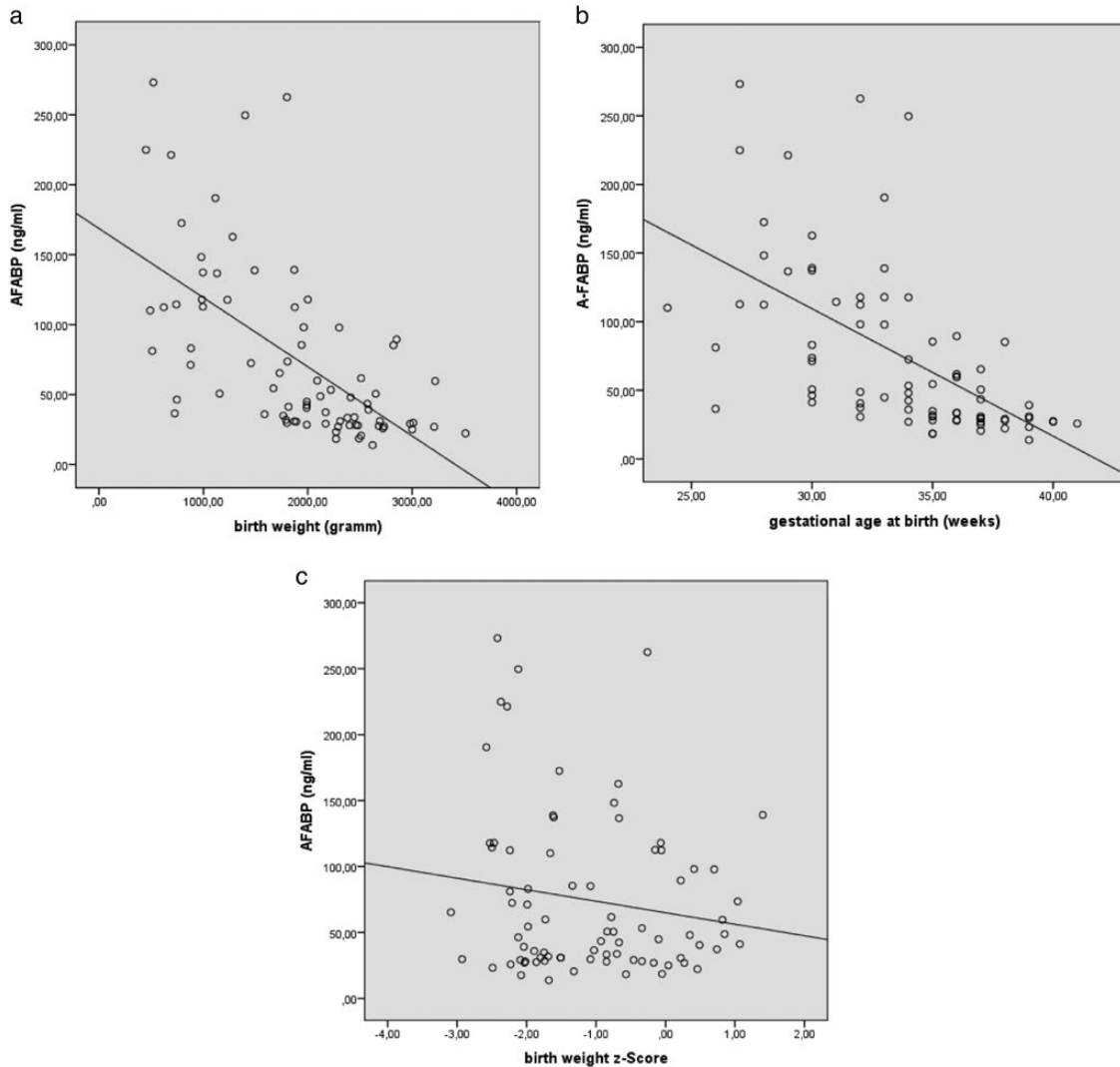
Figure 3. Correlation between cord blood levels of A-FABP versus birth weight, gestational age, and z-score of birth weight (all infants). (A) A-FABP vs. birth weight. (B) A-FABP vs. gestational age at birth. (C) A-FABP vs. z-score of birth weight. There was a strongly significant negative correlation between cord blood A-FABP level and birth weight (Fig. 3A, Spearman's rank correlation test [r = -0.668, P < 0.001] and gestational age [r = -0.691, P < 0.001], but no significant correlation between A-FABP level and z-score of birth weight [Fig 3C, P = 0.37, r = -1.02]).

adipokines. In the discordant DC twin group, significant differences between the smaller and the larger twin were also found for IGF-1 [23] (higher in larger twins, P = 0.028), progranulin [26] (higher in larger twins, P = 0.046), and for ZAG [24] (higher in smaller twins, P = 0.028).

3. Discussion

In adults, increased levels of A-FABP have been found in patients with obesity [38], metabolic syndrome [39], and NAFLD (non-alcoholic fatty liver disease) [8, 40], and A-FABP has been suggested as a biomarker to predict metabolic syndrome [9], as A-FABP elevation was an independent predictor of metabolic syndrome in a 5-year prospective study [38] and of T2DM (type 2 diabetes mellitus) in a 10-year prospective study [8, 41]. Studies in mice suggest that A-FABP promotes insulin resistance (IR), increased levels of triacylglycerols

(TAG), and expression of proinflammatory genes and foam cell development, hence favoring atheroscleroris independently of weight gain [6, 8]. In a previous study, we could demonstrate that A-FABP was elevated in women with preeclampsia and gestational diabetes mellitus [14, 15], diseases which are known to be associated with an increased risk of diabetes and cardiovascular disease later in life.

There are only a few studies, which have examined the association between A-FABP and childhood obesity and metabolic syndrome, but here as well, increased levels of A-FABP have been demonstrated in children that were obese [8–12] and have been associated with an increased risk of developing metabolic syndrome [11]. In two interventional studies, weight loss was associated with a decrease in A-FABP [9, 10].

Our study is the first to study the expression of the novel adipokine A-FABP in a larger group of growth concordant and discordant twins at time of birth, as well as in a set of severely growth-restricted newborns compared to a gestational-age adjusted group of normally grown controls. We showed that cord blood A-FABP [16] levels were significantly higher in the smaller of discordant DC twins, and that A-FABP was significantly negatively correlated with birth weight and gestational age.

There has been one previous study, which has looked at A-FABP in cord blood [13] in 361 newborns (217 full-term infants and 144 preterm infants between 30.4 to 35.9 weeks). In this study, as well, a significant negative correlation was seen between A-FABP and gestational age, but not with birth weight. In Joung's study, neonates where categorized as small for gestational age (SGA; birth weight < 10th percentile), appropriate for gestational age (AGA, birth weight ≥ 10th and ≤ 90th percentile), and large for gestational age (LGA, 90th percentile), and in opposition to our study, in infants born at term it was the largest children that had the highest A-FABP levels [13]. This association, however, was not seen in preterm infants, which in Joung´s study as well had higher A-FABP levels than term infants. Increased A-FABP levels were also seen in the offspring of mothers with preeclampsia or gestational hypertension [13].

At first sight, these results might be in contrast to ours, as we found significantly higher A-FABP levels in the smaller children (of discordant DC twins). This might, however, be explained by the different kind of "small" children we looked at. In all our growth-restricted singletons, we had signs of severe placental dysfunction/ insufficient nutritional supply, whereas the SGA children in Young´s study were mostly born at term and where not characterized by severely impaired placental function.

As A-FABP in our study was highly negatively correlated with gestational age and birth weight, it can be assumed that (early) gestational age and low (absolute) birth weight in itself have a great influence on A-FABP expression. The lack of correlation between A-FABP and z-score might be explained by the fact that gestational age and absolute birth weight in itself are more important for circulating A-FABP levels than the fact that a neonate is "too small" or "too large" for its gestational age. Therefore, a growth-restricted fetus at, for example, 33 weeks might still have lower absolute A-FABP levels because of higher gestational age and higher (absolute) birth weight than a normally grown fetus at 26 weeks, who has higher A-FABP levels because of a much earlier gestational age and lower (absolute) birth weight. Our results in growth-discordant twins support the hypothesis that being "too small" for gestational age might have an impact on A-FABP levels, as A-FABP was higher in the smaller twins as compared to their larger co-twins.

Increased A-FABP has been suggested as a marker of (metabolic) "stress" or higher metabolic activity correlated with an increased risk of obesity and metabolic syndrome later in life [13]. This is in accordance with the results that have been found in both our and Joung´s study in newborns. An increased risk has been described as both ends of the birth weight scale, and it can be hypothesized that this is reflected in the results of these studies, where both those born very large, as well as those born very small, very early, or with maternal disease (eg, preeclampsia, gestational hypertension) have increased A-FABP levels. A difficult question to answer is why MC twins (in our study) did show a completely different pattern of A-FABP expression than DC twins, with a tendency of higher levels in the larger twin,

although the number of twin pairs in this group was very small [4]. In 3 of these 4 pairs, A-FABP levels were higher in the larger twin as compared to its co-twin. This could possibly be explained by the special architecture of the MC twin placenta with vascular anastomosis between the placental area of each twin, which might cause an exchange of adipokines between the twins.

A-FABP has also been examined in mothers with gestational diabetes mellitus (GDM) and their offspring [42]. In this study, A-FABP levels were higher in mothers with GDM as compared to controls, but lower A-FABP levels were found in cord blood of children from diabetic mothers than in controls. A-FABP levels in controls were also higher in cord blood than in maternal serum, which is highly suggestive that A-FABP in cord blood stemmed from the fetus and not the mother [42]. Interestingly, in this study, A-FABP levels in cord blood of GDM offspring were positively correlated with markers of adiposity, which supports the hypothesis that A-FABP is a marker of increased metabolic risk also in these infants. From a weak, but significant negative correlation between A-FABP and glucose levels in cord blood of controls, but not of GDM offspring, Ortega-Senovilla et al concluded that this might indicate a role of A-FABP in facilitating glucose utilization in fetuses, but that this mechanism might not operate correctly in gestational diabetes [42]. From our study, we cannot clearly conclude if increased A-FABP levels are correlated with a facilitation of glucose utilization, but this could possibly also be the case in IUGR fetuses and in smaller (relatively growth restricted) twins.

In summary, in our study, we could confirm that increased A-FABP was correlated with lower birth weight and lower gestational age, and that A-FABP was significantly increased in the smaller of discordant DC twins as compared to their co-twins. If A-FABP is seen as a marker of an increased metabolic risk, than this could reflect an increased risk of obesity and metabolic syndrome in infants born very early or very small or in the smaller of discordant growth DC twins. Follow-up studies at a later time in childhood are needed to see if higher A-FABP levels at birth are actually correlated with an increased risk of obesity and metabolic syndrome during (early) childhood or later.

A. Strength of the Work

Most studies that look at children born small for gestational age in association with metabolic disorders define growth restriction just by birth weight in relation to gestational age and do not look deeper into any prenatal aspects such as placental (dys)function. We, in contrast, carefully looked at the antenatal course as well and selected fetuses/infants, which were not only small for gestational age, but where we also found signs of severe placental dysfunction, which defines truly abnormal intrauterine growth on the basis of impaired nutritional supply.

Also, whereas the number of singletons was rather small in our study, we collected a comparably high number of growth concordant and discordant twins, which, as described above, offer a unique model to study (epigenetic) effects of abnormal intrauterine growth and environment.

B. Weaknesses of the Study

Especially in the very small infants and in twins, acquisition of cord blood samples was difficult, which often caused hemolysis, and a relatively high number of probes (10 out of 36 in twins, and 14 out of 27 in singletons) could not be analyzed. This decreased the power for many of our questions. Maternal serum probes were not analyzed—thus it cannot be excluded that higher A-FABP levels stemmed from mothers. However, if higher AFABP levels stemmed from mothers, then they should be similar/elevated in both twins of one pair. The singleton groups in our study are very small—with only 20 IUGR and 7 controls. Higher A-FABP levels in IUGR need to be validated in a larger group of singletons.

Acknowledgments

Additional Information

Correspondence: Susanne Schrey-Petersen, MD, University of Leipzig, Faculty of Medicine, Department of Obstetrics, Liebigstrasse 20A, 04103 Leipzig, Germany. E-mail: Susanne.schrey@medizin.uni-leipzig.de.

Disclosure Summary: The authors declare no conflict of interest.

Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

References and Notes

1. American Heart Association. About Metabolic Syndrome. https://www.heart.org/en/health-topics/metabolic-syndrome/about-metabolic-syndrome. Accessed February 25, 2020.
2. Barker DJ. The fetal and infant origins of adult disease. *Brit. Med. J.* 1990;**301**(6761):1111.
3. Bluher M. Adipose tissue--an endocrine organ. *Der Internist.* 2014;**55**(6):687–697; quiz 698.
4. Farooqi IS, O'Rahilly S. Genetic factors in human obesity. *Obes Rev.* 2007;**8**(Suppl 1):37–40.
5. Blüher M. Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes.* 2009;**117**(6):241–250.
6. Kralisch S, Fasshauer M. Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? *Diabetologia.* 2013;**56**(1):10–21.
7. Furuhashi M, Saitoh S, Shimamoto K, Miura T. Fatty acid-binding protein 4 (FABP4): pathophysiological insights and potent clinical biomarker of metabolic and cardiovascular diseases. *Clin Med Insights Cardiol.* 2014;**8**(Suppl 3):23–33.
8. Barraco GM, Luciano R, Semeraro M, Prieto-Hontoria PL, Manco M. Recently discovered adipokines and cardio-metabolic comorbidities in childhood obesity. *Int J Mol Sci.* 2014;**15**(11):19760–19776.
9. Krzystek-Korpacka M, Patryn E, Bednarz-Misa I, et al. Circulating adipocyte fatty acid-binding protein, juvenile obesity, and metabolic syndrome. *J Pediatr Endocrinol Metab.* 2011;**24**(11-12):921–928.
10. Corripio R, Gónzalez-Clemente JM, Pérez-Sánchez J, et al. Weight loss in prepubertal obese children is associated with a decrease in adipocyte fatty-acid-binding protein without changes in lipocalin-2: a 2-year longitudinal study. *Eur J Endocrinol.* 2010;**163**(6):887–893.
11. Choi KM, Yannakoulia M, Park MS, et al. Serum adipocyte fatty acid-binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: a 3-y prospective cohort study. *Am J Clin Nutr.* 2011;**93**(1):19–26.
12. Yun KE, Kim SM, Choi KM, Park HS. Association between adipocyte fatty acid-binding protein levels and childhood obesity in Korean children. *Metabolism.* 2009;**58**(6):798–802.
13. Joung KE, Cataltepe SU, Michael Z, Christou H, Mantzoros CS. Cord blood adipocyte fatty acid-binding protein levels correlate with gestational age and birth weight in neonates. *J Clin Endocrinol Metab.* 2017;**102**(5):1606–1613.
14. Fasshauer M, Seeger J, Waldeyer T, et al. Serum levels of the adipokine adipocyte fatty acid-binding protein are increased in preeclampsia. *Am J Hypertens.* 2008;**21**(5):582–586.
15. Kralisch S, Stepan H, Kratzsch J, et al. Serum levels of adipocyte fatty acid binding protein are increased in gestational diabetes mellitus. *Eur J Endocrinol.* 2009;**160**(1):33–38.
16. RRID:AB_2813774, https://antibodyregistry.org/search.php?q=AB_2813774. RRID:AB_2813774, https://antibodyregistry.org/search.php?q=AB_2813774.
17. RRID:AB_2813737, https://antibodyregistry.org/search.php?q=AB_2813737.
18. RRID:AB_2813736, https://antibodyregistry.org/search.php?q=AB_2813736.
19. RRID:AB_2813775, https://antibodyregistry.org/search.php?q=AB_2813775.
20. RRID: AB_2813876, https://antibodyregistry.org/search?q=AB_2813876.
21. RRID: AB_2813877, https://antibodyregistry.org/search?q=AB_2813877.
22. RRID:AB_2813778, https://antibodyregistry.org/search.php?q=AB_2813778.

23. RRID:AB_2813780, https://antibodyregistry.org/search.php?q=AB_2813780.

24. RRID:AB_2813779, https://antibodyregistry.org/search.php?q=AB_2813779.

25. RRID: AB_2783729, https://antibodyregistry.org/search.php?q=AB_2783729.

26. RRID: AB_2813875, https://antibodyregistry.org/search?q=AB_2813875.

27. Fasshauer M, Blüher M. Adipokines in health and disease. *Trends Pharmacol Sci.* 2015;**36**(7):461–470.

28. Ebert T, Gebhardt C, Scholz M, et al. Relationship between 12 Adipocytokines and distinct components of the metabolic syndrome. *J Clin Endocrinol Metab.* 2018;**103**(3):1015–1023.

29. Fasshauer M, Blüher M, Stumvoll M, Tönessen P, Faber R, Stepan H. Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. *Clin Endocrinol (Oxf).* 2007;**66**(3):434–439.

30. Fasshauer M, Waldeyer T, Seeger J, et al. Circulating high-molecular-weight adiponectin is upregulated in preeclampsia and is related to insulin sensitivity and renal function. *Eur J Endocrinol.* 2008;**158**(2):197–201.

31. Miehle K, Stepan H, Fasshauer M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clin Endocrinol (Oxf).* 2012;**76**(1):2–11.

32. Pfau D, Stepan H, Kratzsch J, et al. Circulating levels of the adipokine chemerin in gestational diabetes mellitus. *Horm Res Paediatr.* 2010;**74**(1):56–61.

33. Stepan H, Philipp A, Roth I, et al. Serum levels of the adipokine chemerin are increased in preeclampsia during and 6 months after pregnancy. *Regul Pept.* 2011;**168**(1-3):69–72.

34. Stein S, Stepan H, Kratzsch J, et al. Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. *Metabol.* 2010;**59**(1):33–37.

35. Stepan H, Kley K, Hindricks J, et al. Serum levels of the adipokine fibroblast growth factor-21 are increased in preeclampsia. *Cytokine.* 2013;**62**(2):322–326.

36. Stepan H, Philipp A, Roth I, et al. Serum levels of the adipokine zinc-α2-glycoprotein are increased in preeclampsia. *J Endocrinol Invest.* 2012;**35**(6):562–565.

37. Stepan H, Ebert T, Schrey S, et al. Preliminary report: serum levels of retinol-binding protein 4 in preeclampsia. *Metab.* 2009;**58**(3):275–277.

38. Xu A, Wang Y, Xu JY, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem.* 2006;**52**(3):405–413.

39. Cabré A, Lázaro I, Girona J, et al. Fatty acid binding protein 4 is increased in metabolic syndrome and with thiazolidinedione treatment in diabetic patients. *Atherosclerosis.* 2007;**195**(1):e150–e158.

40. Milner KL, van der Poorten D, Xu A, et al. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. *Hepatology.* 2009;**49**(6):1926–1934.

41. Tso AW, Xu A, Sham PC, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care.* 2007;**30**(10):2667–2672.

42. Ortega-Senovilla H, Schaefer-Graf U, Meitzner K, et al. Gestational diabetes mellitus causes changes in the concentrations of adipocyte fatty acid-binding protein and other adipocytokines in cord blood. *Diabetes Care.* 2011;**34**(9):2061–2066.