RESEARCH

Correlation of metabolic characteristics with maternal, fetal and placental asprosin in human pregnancy

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Abstract

Objective: Asprosin is a recently discovered hormone associated with obesity and diabetes mellitus. Little is known about asprosin's role during pregnancy, but a contribution of asprosin to pregnancy complications resulting from maternal obesity and gestational diabetes mellitus (GDM) is conceivable. We assessed the potential effects of obesity, GDM and other clinical parameters on maternal and fetal umbilical plasma asprosin concentrations and placental asprosin expression.
Design: The Cologne-Placenta Cohort Study comprises 247 female patients, from whom blood and placentas were collected at the University Hospital Cologne.
Methods: We studied the maternal and fetal umbilical plasma and placentas of pregnant women with an elective, primary section. Sandwich ELISA measurements of maternal and fetal umbilical plasma and immunohistochemical stainings of placental tissue were performed to determine the asprosin levels. Also, the relation between asprosin levels and clinical blood parameters was studied.
Results: There was a strong correlation between the maternal and fetal plasma asprosin levels and both increased with GDM in normal-weight and obese women. Asprosin immunoreactivity was measured in cultivated placental cells and placental tissue. BMI and GDM were not but pre-pregnancy exercise and smoking were correlated with maternal and/or fetal asprosin levels. Placental asprosin levels were associated with maternal but not with fetal plasma asprosin levels and with BMI but not with GDM. Placental asprosin was related to maternal insulin levels and increased upon insulin treatment in GDM patients.
Conclusions: Asprosin could potentially act as a biomarker and contribute to the clinical manifestation of pregnancy complications associated with maternal obesity.

Key Words
- obesity
- GDM
- asprosin
- insulin
- placenta
- smoking
- exercise

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Introduction

Asprosin, the C-terminal cleavage product of profibrillin 1, was recently described as fasting-induced pluripotent adipokine (1). Circulating asprosin targets the liver, where it exerts a glucogenic effect (1), and the hypothalamus, where it stimulates appetite-modulating neurons in the arcuate (2). Clinical studies showed that circulating asprosin levels

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positively correlated with obesity and type 2 diabetes (3, 4). Pre-clinical studies addressing the therapeutic potential of asprosin inhibition demonstrated that anti-asprosin monoclonal antibodies can reduce blood glucose, appetite, and body weight in rodents (5).

During pregnancy, adipokines and/or their receptors are expressed in the placenta (6). Certain adipokines, such as leptin, adiponectin or resistin are increasingly found in blood and placenta as pregnancy progresses (7). High concentrations of adipokines have also been detected in the umbilical blood suggesting an important role in fetal development and metabolism (8). Additionally, adipokines are thought to promote the proliferation and invasiveness of placental trophoblast cells and affect the placental angiogenic processes (9). Thus, adipokines might affect the pregnancy outcome and fetal growth by interfering with placental development (10). In obese pregnancies, elevated maternal adipokine levels have been accused of causing adverse effects in the offspring by affecting placental function (11) and programming offspring's metabolism (12).

Even though asprosin blood levels have been shown to be increased in obese individuals, it has not yet been tested whether asprosin is elevated in obese pregnant women and their fetuses. So far, clinical data are inconsistent regarding the circulating concentrations and their correlation with BMI and GDM in pregnancy (13, 14). However, it has been recently shown that asprosin is expressed in human placenta and elevated in the plasma of pregnant women complicated with GDM and their fetal umbilical blood (14). Since there is an urgent need for a reliable and sensitive method to determine plasma asprosin levels (15), our laboratory took the initiative to develop a functional and dependable asprosin ELISA (16).

Here, we set out to test whether asprosin plasma concentrations are elevated in obese mothers and the umbilical blood of their offspring in our Cologne-Placenta Cohort (CPC) Study. Furthermore, we tested the relation between (1) maternal and (2) fetal plasma asprosin concentrations and (3) asprosin protein expression and distribution in the placenta as indicators of maternal gestational disease.

Materials and methods

Study design and recruitment

Placental tissue and blood plasma samples were taken from the CPC Study (2014–2021 at the University Hospital Cologne). The study was approved by the Ethics Commission of Cologne University's Faculty of Medicine (ethics votum 14-244). The study aimed to analyze the intrauterine programming with regard to maternal diseases like obesity, gestational diabetes and placental dysfunction. The inclusion criteria for pregnant patients of the Department of Obstetrics and Gynecology (University Hospital of Cologne) were singleton pregnancy with delivery mode via cesarian section. Exclusion criteria were multiple pregnancies, maternal serious diseases, infections before pregnancy (e.g. HIV or hepatitis B) and pregnancy-associated diseases (e.g. gestosis and amniotic infectious disease). Consent has been obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. For immunohistochemical (IHC) analyses, patients were split regarding their BMI into normal weight (BMI <25; normal weight (NW)), overweight (BMI = 25–30; overweight (OW)) and obese (BMI >30; obese (OB)) pregnant women. The classification of gestational diabetes mellitus (GDM) was based on the oral glucose tolerance test 50 and 75 or the clinical maternity log. The total patient collective is 247 patients. Plasma samples of only a subset of 77 patients were analyzed by ELISA because of limited sample quantity, and placenta samples from a subset of 30 patients were randomly chosen for histological analyses (see Fig. 1).

Patient sample preparations

Placental tissue was obtained from the placenta directly after the cesarian section. Placental tissue was punched out centrally between the placental border and the insertion of the umbilical cord. It was incubated in 4% PFA for 24 h, followed by 70% isopropanol for 24 h and paraffinated by a tissue embedding machine (Leica ASP300). Paraffinated specimens were cut into 3-µm slices. Maternal-fastened blood was collected on the day of delivery, and fetal umbilical blood was taken from the umbilical cord right after delivery. Reaction tubes for EDTA plasma were used to collect the blood and were centrifuged for 10 min at 3000 ***g*** to gain plasma. Supernatants were kept at 80°C until usage.

Asprosin sandwich ELISA

The asprosin sandwich ELISA was produced in our laboratory, intensively tested and validated by surface plasmone resonance and interaction studies (16). The detection sensitivity ranged at <65 pg/mL. For the procedure, the capture antibody (lab-made pc-asp anti-asprosin antibody; $K_D = 0.29 \pm 0.30$ nM) was coated on 96-well ELISA plates at 3 µg/mL in 100 µL coating buffer (50

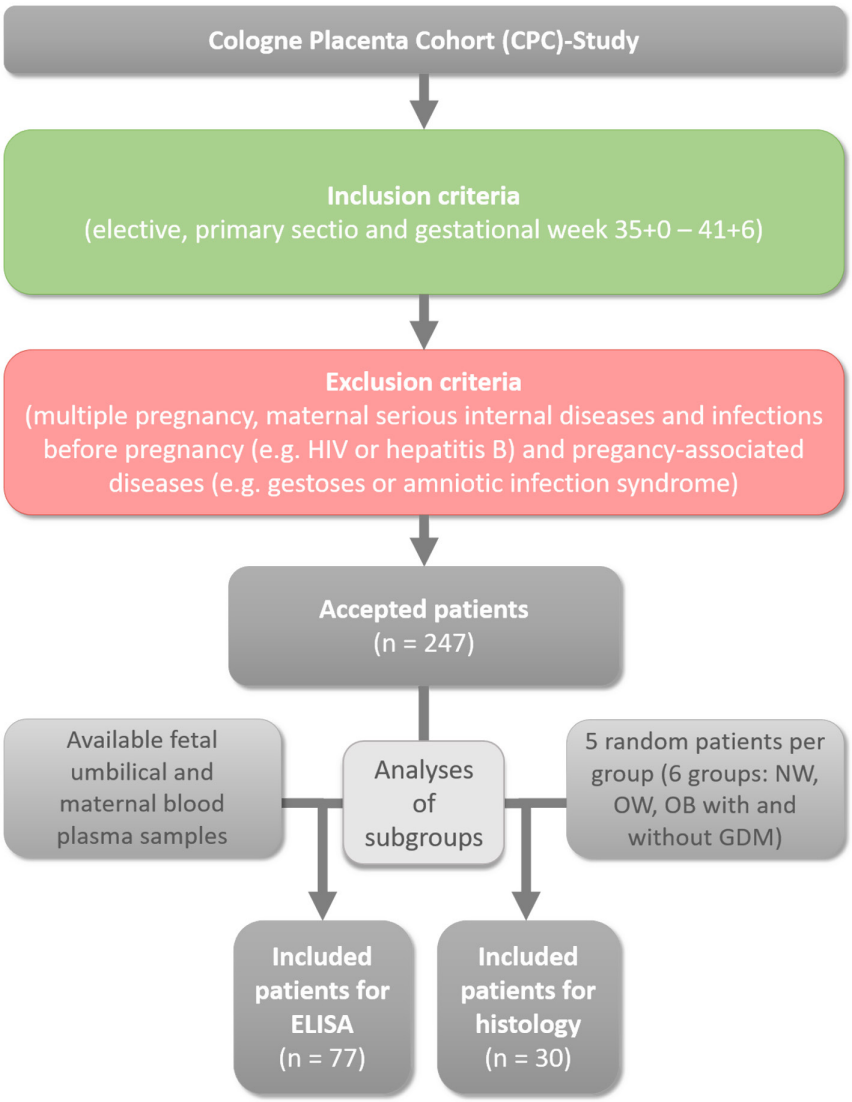

Figure 1
Overview of the study design. GDM, gestational diabetes mellitus; NW, normal weight; OB, obese; OW, overweight.

mM carbonate/bicarbonate buffer, pH 9.6) and incubated overnight at 4°C. After washing with PBST, the plate was incubated with 5% milk/PBS for 2 h for blocking. Asprosin standard and plasma samples were diluted in PBS. After 2h incubation of the samples at room temperature (RT), the plate was washed with PBST again. The detection antibody (mAB anti-asprosin antibody AG-20B-0073-C100 (AdipoGen, Liestal, Switzerland); $K_D = 22 \pm 2$ nM) was diluted 1:2000 in 2.5% milk/PBS and added to the plate for 1 h at RT. After washing the secondary antibody (polyclonal rabbit anti-mouse IgG-HRP conjugate P026002-2 (Agilent Dako) was added 1:2000 in 2.5% milk/PBS for 30 min at RT. The plate was washed again and 1-Step™ Ultra TMB-ELISA Substrate Solution (Thermo Scientific) was added and incubated for 15–20 min until the color developed. The reaction was stopped with 30% H_2SO_4 and the optical density was read at 3000 ***g***.

Cell culture

Placental BeWo cells were seeded at 26,300 cells/cm^3 on glass coverslips and kept in culture for 24 h with DMEM, high glucose, pyruvate (Gibco)/10% FBS/1% Pent/Strep. Cells were starved in DMEM, high glucose, pyruvate (Gibco)/1% FBS/1% Pent/Strep overnight. Then the cells were incubated with acetic acid buffer (0.2 M acetic acid + 0.5 M NaCl) for 5 min to remove potential serum asprosin from the cell surface. Then cells were fixed with 100% methanol for 10 min and stored in PBS until usage.

Immunofluorescence staining of asprosin in BeWo cells

Fixed cells were stained with Rabbit anti-Human, Mouse Asprosin Polyclonal Antibody (MBS7607159, MyBiosource,

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San Diego, CA, USA). Cells were permeabilized with PBS/0.3%Triton-X (Sigma-Aldrich) for 30 min and blocked for 1 h with SEA BLOCK blocking buffer (37527, Thermo Fisher). Primary asprosin antibody diluted 1:200 in Antibody Diluent (S0809, Agilent Dako) was added to the cells overnight at 4°C. Cells were incubated with secondary Cy™5 AffiniPure Goat Anti-Rabbit IgG (H+L) (AB_2338013, Jackson Immuno Research) for 1 h at room temperature. After that, cells were stained with 1:1000 DAPI for 10 min and embedded in Invitrogen™ Fluoromount-G™ Mounting Medium (Thermo Fisher, 00-4958-02). The negative control was performed the same way without adding a primary antibody. Pictures were taken with the fluorescence and light microscope Olympus BX53 at 100× magnification.

Immunohistochemical staining of asprosin in placental tissue

Paraffin slices were deparaffinated with 3 × 10 min Neo-Clear (109843, Sigma-Aldrich) and a descending ethanol series. Antigen-binding sites of placental tissue were retrieved in citric acid buffer for 35 min cooking in a steam cooker. Slices were permeabilized with PBS/0.3%Triton for 30 min and blocked for 1 h with SEA BLOCK blocking buffer (37527, Thermo Fisher). Primary asprosin antibody (see above), diluted 1:100 in Antibody Diluent (see above), was added to the slices overnight at 4°C. Then the tissue was incubated for 1 h at RT with HRP One-Step Polymer anti-Mouse/Rabbit (ZUC053-100, Zytomed Systems, Berlin, Germany). Subsequently, the Permanent AEC Kit (ZUC054-200s, Zytomed System, Berlin, Germany) was used for final staining. All slices were incubated for 2 min and reaction was stopped by adding aqua dest. Tissue was stained with hematoxylin for 5 min and blued for 10 min with floating tap water. Slides were brought back into organic milieu by an ascending ethanol series followed by 2 × 5 min Neo-Clear and embedded with Neo-Mount (109016, Sigma Aldrich). For the negative control, the primary antibody was changed with antibody diluent. Pictures were taken with the Olympus BX53 light and fluorescence microscope for the determination of asprosin localization and from scanned slides (Nanozoomer S360, Hamamatsu, Hamamatsu City, Japan) for semi-quantitative analysis using ImageJ software. A color threshold was applied to determine AEC stained area (hue 215-255; saturation 25-255; brightness 0-235) and total placental area (hue 0-255; saturation 10-255; brightness 0-235). AEC stained area was normalized to total placental area.

H&E stain in placenta

Placenta slices were deparaffinated as described above. Slides were stained with hematoxylin (Carl Roth, Karlsruhe, Germany) for 5 min and incubated in flowing tap water for 10 min. Thereafter, the tissue was stained with 0.5% eosin G (Carl Roth, Karlsruhe, Germany) for 2 min following an ascending ethanol series and 2 × 5 min Neo-Clear. Samples were embedded with Neo-Mount and pictures were taken at the Olympus BX53 (Olympus).

Statistics

Correlation studies were performed with Spearman correlation analyses in GraphPad Prism software including maternal, fetal and placental asprosin levels and various clinical parameters because of non-Gaussian distribution (weak correlation: Correlation Coefficient $r_S = 0.2–0.39$; moderate correlation: $r_S = 0.4–0.59$; strong correlation: $r_S = 0.6–0.79$; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). Additional bar graphs show relative placental asprosin expression (normalized to the control group) as single data points, mean ± S.D. Mann–Whitney *U*-tests were used for statistical analysis ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

Results

Correlation of maternal and fetal umbilical plasma asprosin levels with clinical parameters

Firstly, we determined the plasma asprosin levels in the maternal blood shortly before and fetal umbilical blood upon delivery (see Fig. 1 for study overview). Asprosin plasma concentrations ranged between 2.93 and 21.64 ng/mL (mean = 8.11 ± 3.80 ng/mL) in maternal and from 1.91 to 20.13 ng/mL (mean = 9.60 ± 3.87 ng/mL) in the fetal umbilical plasma (Table 1). Maternal and fetal plasma asprosin concentrations showed a strong positive correlation (Table 1).

Correlation analyses between asprosin plasma concentrations and numerous clinical characteristics and metabolic parameters, such as age, BMI, GDM diagnosis, blood insulin and glucose, HbA1c, serum triglycerides, cholesterol, LDL, HDL, cholesterol/HDL ratio, cortisol, placental weight, week of childbirth, sex of the child, birth weight, birth weight percentile and birth length, did not reveal significant results (Table 1). Still, a significant reduction of plasma asprosin levels was observed in maternal and fetal umbilical plasma if mothers reported

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Table 1 Correlation analysis of human maternal and fetal umbilical plasma asprosin with maternal clinical parameters and neonatal birth information from ELISA analysis. Spearman correlation analysis: weak correlation (W): $r_S = 0.2–0.39$; moderate correlation (M): $r_S = 0.4–0.59$; strong correlation (S): $r_S = 0.60–0.79$.

Clinical parameter	Mean ± s.d. Min./max.	Maternal asprosin r_S	Maternal asprosin *P*-value	Maternal asprosin *n*	Fetal umbilical asprosin r_S	Fetal umbilical asprosin *P*-value	Fetal umbilical asprosin *n*
Maternal asprosin	8.11 ± 3.80 2.93/21.64	X	X	X	**0.776$_S$**	**<0.0001**[b]	77
Fetal umbilical asprosin	9.60 ± 3.87 1.91/20.13	**0.776$_S$**	**<0.0001**[b]	77	X	X	X
Age (years)	33 ± 5 21/43	0.145	0.208	77	0.152	0.186	77
BMI (kg/m^2)	26.56 ± 5.77 16.21/41.32	0.119	0.303	NW = 36 OW = 25 OB = 16	−0.028	0.812	NW = 36 OW = 25 OB = 16
GDM	X	0.150	0.194	Non-GDM = 56 GDM = 21	0.038	0.743	Non-GDM = 56 GDM = 21
Exercise before pregnancy	X	**0.232$_W$**	**0.0421**[a]	Yes = 38 No = 39	0.106	0.359	Yes = 38 No = 39
Smoking before pregnancy	X	**−0.293$_W$**	**0.0102**[a]	Yes = 35 No = 41	**−0.288$_W$**	**0.0116**[a]	Yes = 35 No = 41
Insulin (mU/L)	13.35 ± 9.02 0.50/61.40	0.085	0.482	71	0.089	0.459	71
Glucose (mg/dL)	72 ± 13 35/103	0.062	0.606	72	0.090	0.452	72
HbA1c (%)	5.6 ± 1.1 4.6/13.9	−0.067	0.592	67	−0.168	0.175	67
Triglycerides (mg/dL)	252 ± 83 78/524	0.039	0.743	72	0.043	0.719	72
Cholesterol (mg/dL)	257 ± 59 86/500	−0.028	0.818	72	0.116	0.331	72
LDL (mg/dL)	154 ± 51 34/361	0.022	0.871	57	0.099	0.464	57
HDL (mg/dL)	69 ± 17 31/119	0.149	0.270	57	0.208	0.121	57
Cholesterol/HDL	3.9 ± 0.9 1.8/6.7	0.007	0.958	57	−0.030	0.823	57
Cortisol (µg/L)	285 ± 98 1/583	−0.071	0.569	67	0.020	0.872	67
Placental weight (g)	713.2 ± 154.83 418.2/1221.2	0.015	0.899	77	−0.002	0.984	77
Gestational age at birth (week)	39.2 ± 0.7 37.1/41.1	−0.153	0.183	77	−0.171	0.137	77
Sex of child	X	0.048	0.679	♀ = 39 ♂ = 38	0.108	0.352	♀ = 39 ♂ = 38
Birth weight (g)	3501 ± 517 2100/4620	0.096	0,405	77	0.045	0.697	77
Birth weight percentile (%)	54 ± 28 1/99	0.105	0.368	75	0.084	0.472	75
Birth length (cm)	52 ± 3 45/62	−0.010	0.931	77	−0.152	0.186	77

[a]$P < 0.05$; [b]$P < 0.0001$; Significant results were indicated in bold.
GDM, gestational diabetes mellitus; NW, normal weight (BMI <25); OB, obese (BMI >30); OW, overweight (BMI, 25–30).

smoking before pregnancy compared to non-smoking mothers (Table 1). Additionally, there was a weak but significant positive correlation between the level of exercise before pregnancy and maternal plasma asprosin concentration (Table 1).

However, when stratifying for BMI (NW: BMI <25; OW: BMI = 25–30; OB: BMI >30) and GDM diagnosis (yes or no), an increase in maternal and fetal plasma asprosin of normal-weight and obese mothers was observed in the GDM group (Fig. 2A and B). Solely the overweight group did not show any significance (Fig. 2A and B). There was no difference in maternal and fetal asprosin upon different GDM-treatments (Fig. 2C and D).

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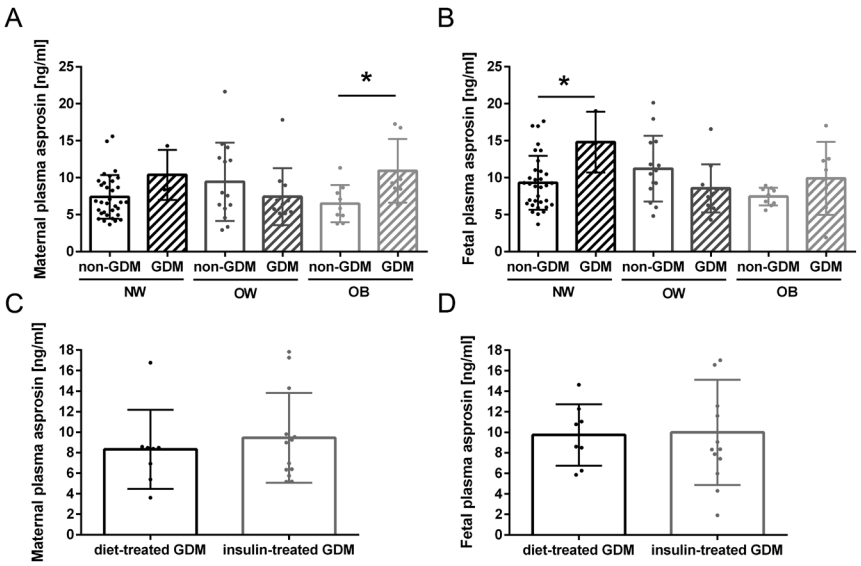

Figure 2
Maternal and fetal plasma asprosin levels upon overweight/obesity and GDM. (A and B) Maternal (A) and fetal (B) plasma asprosin levels stratified by BMI and GDM. (C and D) Maternal (C) and fetal (D) plasma asprosin levels stratified by GDM treatment (diet vs insulin). Statistics were performed with GraphPad Prism using two-way ANOVA and Mann–Whitney *U*-test (*P < 0.05). GDM, gestational diabetes mellitus.

Asprosin expression pattern in human placental cells and tissue

To explore whether placental cells might be a source of asprosin, cultured chorionic BeWo cells were stained with the anti-asprosin antibody (Fig. 3A). In the absence of an external asprosin source, two distinct staining patterns were detectable in cultivated chorionic cells: dotted cytosolic asprosin and asprosin with a rather fibrillar appearance in the extracellular space (Fig. 3A; white arrows). To assess the pattern of immunoreactivity in human placental tissue, IHC stainings for asprosin were performed (Fig. 3B; IHC). Orientation within the tissue was achieved by the distinction of the maternal and fetal site of the placenta, identified by characteristic cell structures. Asprosin immunoreactivity was mainly detected in endothelial cells of fetal blood vessels, both in the villous parenchyma as well as in the chorion (Fig. 4B Villous parenchyma, Chorion; black arrows). Also, the epithelium of the amnion displayed immunoreactivity (Fig. 4B Amnion; black arrow). Additionally, immune cells all over the placenta displayed marked asprosin immunoreactivity (Fig. 4B Chorion, Amnion, Decidua; white arrows). In the maternal part of the placenta, individual decidual cells (Fig. 4B Decidua; gray arrow) also showed a distinct pattern of immunoreactivity.

Possible correlation between placental asprosin immunoreactivity and maternal BMI and GDM

We next performed asprosin immunohistochemistry in a subset of placentas of normal weight, overweight and obese women with and without GDM in our study (Fig. 4A). To assess whether placental asprosin in the villi relates to its plasma levels, Spearman correlation analysis was performed. There was a positive correlation of maternal but not fetal umbilical plasma asprosin concentrations with placental asprosin immunoreactivity (Table 2). Additionally, there was a positive correlation between placental asprosin immunoreactivity and BMI (Table 2), especially significant in women with GDM (Fig. 4B). GDM alone did not affect placental asprosin content (Table 2), but interestingly, maternal circulating serum insulin correlated with placental asprosin immunoreactivity (Table 2). When comparing treatment strategies (dietary vs insulin treatment) in pregnant women with GDM, a significant increase in placental asprosin immunoreactivity occurred with insulin treatment (Fig. 4C).

Discussion

In our study, we set out to investigate the potential relation of maternal and fetal umbilical plasma asprosin concentrations to BMI, GDM and other clinical parameters in our CPC-Study. Even though we could not find a significant correlation between any single clinical parameter and plasma asprosin concentrations, the diagnosis of GDM in BMI subgroups seemed to affect asprosin concentrations in both, maternal and fetal umbilical plasma: obese pregnant women (BMI >30) and the fetal umbilical blood of normal-weight women (BMI <25) display a significant increase in plasma asprosin levels with maternal GDM; the fetal and maternal counterparts show same tendencies. While the influence of BMI in pregnancy was never addressed before, it was shown that asprosin blood levels are increased with

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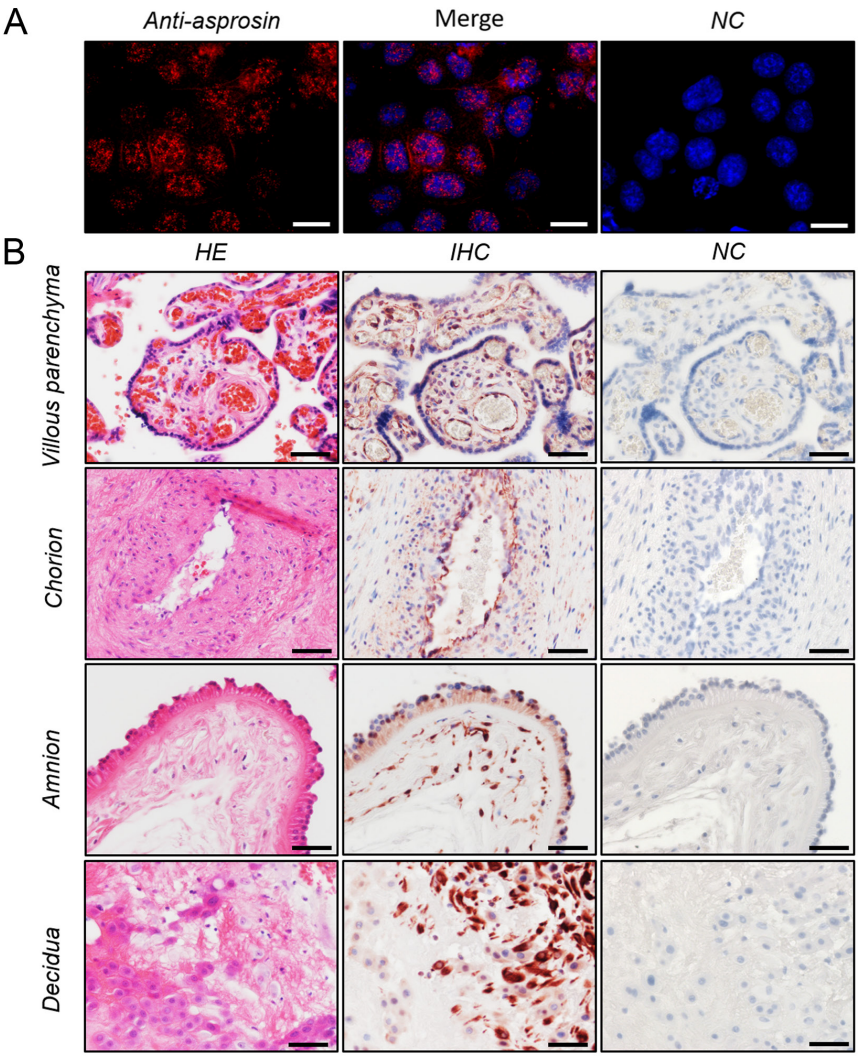

Figure 3
Immunofluorescence and -histochemistry staining of asprosin in placental cells and tissue. (A) Immunofluorescent staining of endogenous asprosin in placental BeWo cells with asprosin antibody and negative control (stained solely with Cy5 goat-anti-rabbit secondary antibody). White arrows point to fibrillar asprosin stainings. Scale bar = 20µm. (B) IHC AEC staining of asprosin in different placental areas with respective H&E-G staining and NC. Black arrows display endothelial cells, white arrows show immune cells and the gray arrow points to a decidual cell. Scale bar = 50 µm. H&E, hematoxylin and eosin; IHC, immunohistochemical; NC, negative control.

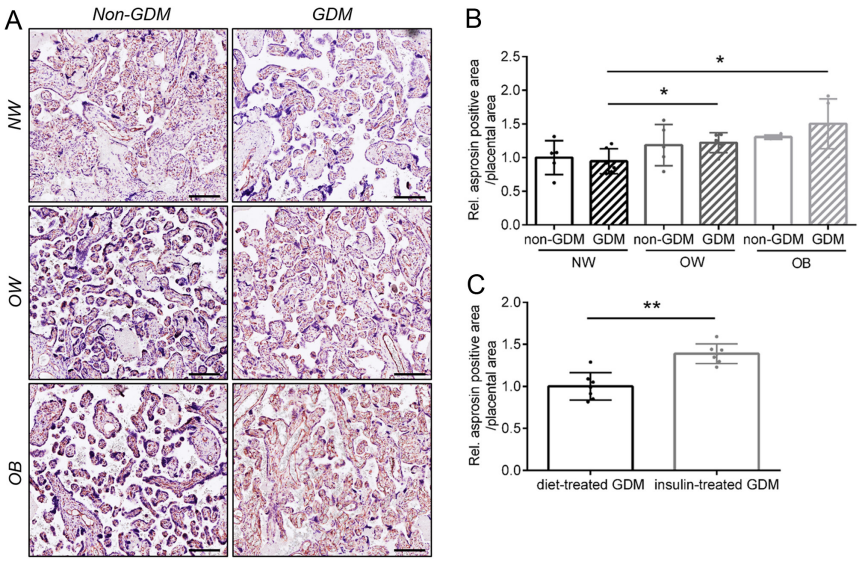

Figure 4
Analysis of asprosin levels in the placental villous parenchyma. (A) Exemplary immunohistochemical AEC+Hematoxylin staining of placental tissue for asprosin from normal weight (NW; BMI <25), overweight (OW; BMI >25 and <30) and obese (OB; BMI >30) pregnant women segregated by non-GDM and GDM patients. Scale bar = 100 µm. (B) Relative placental asprosin immunoreactivity normalized to non-GDM/normal weight level and segregated by BMI and GDM diagnosis. (C) Relative placental asprosin immunoreactivity in GDM patients. Patients were either treated by diet or with insulin and values are normalized to the diet-treated group. Statistics were performed with GraphPad Prism using Mann–Whitney *U*-test (*P < 0.05; **P < 0.01). GDM, gestational diabetes mellitus; NW, normal weight; OB, obese; OW, overweight.

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Table 2 Correlation analysis of human placental asprosin with maternal clinical parameters and neonatal birth information from IHC analysis. Spearman correlation analysis: weak correlation (W): $r_S = 0.2–0.39$; moderate correlation (M): $r_S = 0.4– 0.59$; strong correlation (S): $r_S = 0.60–0.79$.

Clinical parameter	Mean ± s.d.	Placental asprosin		
	Min./Max.	r_S	*P*-value	*n*
Maternal asprosin	9.23 ± 3.55 4.48/17.82	**0.459**$_M$	**0.0209**[a]	25
Fetal umbilical asprosin	10.93 ± 3.92 5.39/19.00	0.249	0.230	25
Age (years)	34 ± 4 26/43	0.187	0.322	30
BMI (kg/m^2)	27.74 ± 6.66 16.21/41.32	**0.684**$_S$	**<0.0001**[b]	NW = 11 OW = 10 OB = 9
GDM	X	0.000	>0.999	Non-GDM = 14 GDM = 16
Exercise before pregnancy	X	–0.143	0.450	Yes = 14 No = 16
Smoking before pregnancy	X	–0.005	0.978	Yes = 5 No = 25
Insulin (mU/L)	14.6 ± 11.4 4.7/61.4	**0.482**$_M$	**0.0126**[a]	26
Glucose (mg/dL)	73 ± 11 44/93	0.204	0.307	27
HbA1c (%)	5.8 ± 1.6 4.9/13.9	0.350	0.0580	30
Triglycerides (mg/dL)	258 ± 91 78/452	–0.362	0.0637	27
Cholesterol (mg/dL)	249 ± 48 180/357	–0.237	0.234	27
LDL (mg/dL)	145 ± 43 77/243	–0.126	0.606	19
HDL (mg/dL)	67 ± 16 36/98	0.235	0.332	19
Cholesterol/HDL	3.9 ± 1.0 2.4/6.7	–0.206	0.399	19
Cortisol (µg/L)	301 ± 89 183/583	–0.247	0.225	26
Placental weight [g]	722.1 ± 139.0 468.2/1024.2	0.025	0.895	30
Gestational age at birth (week)	39.2 ± 0.6 37.4/40.1	0.288	0.122	30
Sex of child	X	0.004	0.983	♀ = 11 ♂ = 19
Birth weight (g)	3658 ± 429 2990/4660	0.301	0.107	30
Birth weight percentile (%)	63 ± 24 12/99	0.258	0.169	30
Birth length (cm)	53 ± 3 49/62	0.114	0.550	30

[a]$P < 0.05$; [b]$P < 0.0001$; Significant results were indicated in bold.
GDM, gestational diabetes mellitus; NW, normal weight (BMI <25); OB, obese (BMI >30); OW, overweight (BMI, 25–30).

GDM in both, rodents (17) and women (13, 14). Asprosin is known to trigger the glucose release from the liver (1). Thus, increased asprosin levels during pregnancy might contribute to increased blood glucose concentrations in GDM.

Although several metabolic parameters and patient characteristics of our cohort did not correlate with asprosin plasma concentrations, two external factors seem to have an influence on asprosin levels: exercise and smoking before pregnancy. With regard to exercise and asprosin levels, so far rather contradictory data were published. On one hand, it was shown by several groups that acute aerobic exercise decreases asprosin levels in humans (18, 19, 20) and rats (21). On the other hand, acute anaerobic exercise

was shown to correlate with increased asprosin levels in women (22). Here, we see a positive correlation between the number of exercise days per week (0, 1, 2, 3 or more) during pregnancy and maternal asprosin plasma concentrations. Thus, there might be a so far undescribed dose-dependent long-term effect of chronic exercise on plasma asprosin concentrations. However, since it was not the focus of our study, we have no further information regarding the exact type and level of impact of exercise before pregnancy in our cohort. Therefore, further conclusions would be premature.

Interestingly, we found a novel correlation between asprosin plasma levels and smoking. In mothers who smoked before pregnancy, we found decreased asprosin concentrations in maternal and fetal umbilical plasma. Asprosin was shown to be an orexigenic hormone produced by white adipose tissue (1, 2) and smoking often has been demonstrated to be connected to anorexigenic effects (23, 24). Thus, decreased asprosin levels in women who started smoking before pregnancy match reports on several other adipokines that are affected by nicotine and might convey the anorexigenic effects of smoking (25). However, in our study set-up, there is a supposed 9-month phase of nicotine abstinence before the determination of plasma asprosin. Therefore, a direct effect of nicotine on circulating asprosin in our cohort seems to be rather unlikely but should be addressed in future studies.

Confirming previous reports, we see a strong positive correlation between maternal and fetal umbilical plasma asprosin levels in our cohort (13, 14). This suggests that asprosin might pass the placenta and that changes in maternal asprosin plasma levels might exert direct metabolic effects in the fetus. Additionally, asprosin might affect the placental function and therefore cause secondary consequences for the fetus. So far, very little is known about placental asprosin expression, content, or transport. By assessing asprosin immunoreactivity in placentas from our cohort and relating it to asprosin concentrations in maternal and fetal umbilical plasma, we aimed at contributing to a better understanding of asprosin's role during pregnancy. We found a positive correlation between placental asprosin immunoreactivity and maternal plasma asprosin levels, which may indicate that maternal plasma asprosin accumulates in placental tissue. In addition, the placenta might serve as a source of circulating asprosin in the mother and might thereby contribute to plasma asprosin concentrations – a concept that is supported by our finding of asprosin production in cultured chorionic cells.

We detected placental asprosin immunoreactivity in various parts of the placenta. It was stained in endothelial cells of fetal vessels in the villous parenchyma and the chorion. Former studies have shown that placental endothelial cells produce many different chemokines (26) and function as conditional innate immune cells (27). Furthermore, we detected asprosin in decidual cells in the maternal side of the placenta, which are also known to play a role in immune regulation (28). Since asprosin seems to play a role in inflammation in other tissues (29, 30), it may be possible that the production/storage of asprosin in endothelial and decidual cells contributes to inflammatory processes in the placenta, similar to leptin which is a known autocrine and paracrine regulator of placental tissue (31). Of note, also endothelial cells in the amnion and macrophages in all parts of the placenta displayed marked asprosin immunoreactivity. As macrophages are known sources for pro-inflammatory adipokines (32) and have been described to accumulate in placentas of pregnancies complicated by GDM (33), production or release of asprosin by placental macrophages might contribute to several placental pathologies. However, both, endothelial cells and macrophages are known to commonly display unspecific immunoreactivity (34), so the detected signals in these cells should be interpreted with caution. Overall, the pattern of placental asprosin immunoreactivity indicates that asprosin seems to be mainly produced in placental cells that contribute to inflammation and immune regulation, two mechanisms contributing largely to known pregnancy complications, such as the increased risk of preterm birth, fetal growth restriction, placental pathologies and hypertensive disorders leading to profound consequences in the fetus (35, 36).

It was suggested before that placental asprosin immunoreactivity might be associated with GDM (14). In our cohort, we cannot confirm this finding. However, we can report a novel association between placental asprosin immunoreactivity and maternal BMI. And interestingly, the increase of placental asprosin immunoreactivity with rising BMI is stronger in women with GDM than in women without GDM. GDM is known to modulate insulin and leptin signaling during pregnancy (37), which might suggest that asprosin, as a strong metabolic regulator, is also affected by GDM. Additionally, we found that increased individual insulin levels correlated with increased placental asprosin immunoreactivity suggesting an interdependence between asprosin and insulin in general and during pregnancy. As asprosin displays an overlap with glucagon's molecular pathways and effects, a mechanistic link between asprosin and insulin seems plausible.

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In conclusion, our study contributes several novel aspects to the ongoing investigation of asprosin's role in metabolism. The correlation of placental asprosin expression with the extent of maternal obesity as well as the characterization of potential placental transport mechanisms for asprosin deserve special attention in the future. In particular, an in-depth assessment of a potential molecular interplay between insulin and asprosin might advance the development of preventive and therapeutic measures against the health consequences of obesity and diabetes – not only during pregnancy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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