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Regular paper

Placental expression of Fatty Acid Desaturases 1, 2 and 3 in selected pregnancy pathologies

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Intrauterine development is a key period in human life. The foetal progress largely depends on the function of the placenta, whose responsibility is transportation and biosynthesis of fatty acids. Desaturation enzymes play a key role in placental fatty acid metabolism. Expression of genes coding for desaturases may be associated with pregnancy abnormalities. The objective of this study was to determine the transcriptional activity of the placental genes Fatty Acid Desaturases 1, 2 and 3 (FADS 1, 2 and 3) in women who gave birth to the infants appropriate for gestational age, large for gestational age, small for gestational age, with intrauterine growth restriction and born preterm. 34 pregnant women aged 21–37 years old participated in the study. The placental samples were taken from a site located 2–3 cm away from the umbilical cord attachment. The collected tissue sections were stored in RNAlater according to the manufacturer's protocol, until required for molecular analysis. The expression profiles of *FADS1*, *FADS2* and *FADS3* were determined with RT-qPCR. There was no difference in *FADS1* and *FADS2* expression between the groups. However, the differences in the expression of the *FADS3* were found. Analysis of the *FADS1*, *FADS2* and *FADS3* transcription showed significant differences between most of the examined groups. Our findings suggest that the transcriptional activity of *FADS* genes changes with the severity of intrauterine disorders and is associated with foetal lipid disorders linked to a greater accumulation of fat in the foetal tissues.

Keywords: placenta, foetal development, desaturases, fatty acids, gene expression

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Abbreviations: AA, arachidonate; AC, abdomen circumference; AGA, appropriate for gestational age; ALA, alpha-linolenic acid; BPD, bi-parietal diameter; D13D, Delta (13) Desaturase; D5D, Delta-5 Desaturase; DGLA, dihomo-gamma-linoleoate; EFAs, essential fatty acids; EPA, eicosapentaenoate; ETA, eicosatetraenoate; FA, Fatty acids; FADS1, Fatty Acid Desaturase 1; FADS2, Fatty Acid Desaturase 2; FADS3, Fatty Acid Desaturase 3; FL, femur length; GLA, gamma-linoleate; HC, head circumference; IUGR, intrauterine growth restriction; LA, linoleic acid; L-CPUFA, long-chain polyunsaturated fatty acids; LGA, large for gestational age; MCFA, medium-chain fatty acids; PTB, preterm birth; SGA, small for gestational age

INTRODUCTION

Intrauterine foetal development is a critical period in human development. Subsequent health, susceptibility to diseases, intelligence, and many other factors are dependent on this foetal stage (Bobiński & Mikulska, 2015). The placenta plays a key role during pregnancy. It controls metabolic processes on the hormonal pathway between the organisms of the mother and child – including oxygenation and supplying the foetus with building and energy substrates (Bobiński *et al*., 2013; Bobiński & Mikulska, 2015). Placental abnormalities lead to premature births with varying degrees of foetus malnutrition (e.g., intrauterine growth restriction, IUGR), as well as the birth of small for gestational age (SGA) or large for gestational age (LGA) babies. Fatty acids (FA) are one of the most important ingredients that determine proper intrauterine development. They are responsible for the structure of the child's nervous system, development of cell membranes, structure and function of the retina as well as fulfilling many other metabolic and structural functions (Helland *et al*., 2003; Gale *et al*., 2008). The diversity of FA functions results from the high heterogeneity of their structure, determined by the number of carbon atoms and the number and location of unsaturated bonds in the carbon chain. The source of FAs for the developing foetus is the mother's diet (Herrera, 2002; Cetin *et al*., 2009a), the release of FA from deposits in maternal tissue (Prentice & Golberg, 2000), the endogenous biosynthesis of FA by the mother and later – foetal FA synthesis (Clandinin *et al*., 1981). The placenta is primarily responsible for the maternal-foetal metabolism of FA; this includes transportation of the FA from mother to foetus, as well as placental FA biosynthesis and modification. Placental transfer is determined by numerous factors, such as the mother's health, condition of the foetus, transport efficiency of the placenta and diet during pregnancy (Cetin *et al*., 2002; Haggarty 2002; Cetin *et al*., 2009a). Some placental disorders can impair FA metabolism, and this may lead to intrauterine foetal developmental disorders and a predisposition to numerous diseases after birth. Changes in the activity of the enzymes responsible for the desaturation of essential fatty acids (EFAs) seem to be particularly important in the maternal-foetal homeostasis. The first of these is delta-5 desaturase (D5D) [EC 1.14.19.44], encoded by the fatty acid desaturase 1 (FADS1) gene.

This gene is clustered with its family members at 11q12-q13.1 (Lattka *et al*., 2010). This desaturase plays one of the key roles in the biosynthesis of long-chain polyunsaturated fatty acids (L-CPUFA) of both the n-3 and n-6 families. D5D introduces a cis double bond at carbon 5 into dihomo-gamma-linoleoate (DGLA) (20: 3n-6) and eicosatetraenoate (ETA) (20: 4n-3) to generate arachidonate (AA) (20: 4n-6) and eicosapentaenoate (EPA) (20: 5n-3), respectively. The second important enzyme in the biosynthesis pathway of L-CPUFA is delta-6-desaturase [EC 1.14.19.3]. It introduces a double cis bond at carbon 6 in linoleic acid (LA) (18: 2n-6) and alpha-linolenic acid (ALA) (18: 3n-3). As a result of this reaction, gamma-linoleate (GLA) (18: 3n-6) and stearidonate (18: 4n-3) are formed, respectively (GeneCards The Human Gene Database, Weizmann Institute of Science, Israel., 2020a). The third representative of desaturases is the delta (13) desaturase (D13D) enzyme (EC 1.14.19.-) encoded by the FADS3 gene. D13D introduces a cis double bond in (11E)-octadecenoate (trans-vaccenoate) at carbon 13 to generate (11E, 13Z)-octadecadienoate, likely participating in the biohydrogenation pathway of LA (GeneCards The Human Gene Database, Weizmann Institute of Science, Israel., 2020b). Under normal conditions, the activity of these enzymes remains in a delicate dynamic balance, maintaining the biosynthesis of L-CPUFA n-3 and n-6 at the appropriate level. Disturbance of enzymatic activity, which may be caused by altered transcriptional activity of FADS genes, may contribute to the loss of control over the biosynthesis of the membrane phospholipids and DHA – the key lipids for the development of the foetal nervous system (Otto *et al*, 1997), as well as the loss of control over the metabolism of inflammatory lipids, such as prostaglandin E2 ,which is critical for acute inflammatory response, maintenance of epithelial homeostasis (Challis *et al*., 2002) and metabolic disorders, such as diabetes, lipid disorders, cardiovascular diseases, etc. (Cetin *et al*., 2002). The processes controlled by these desaturases are extremely important for the intrauterine development of the foetus, especially for the structure of its nervous system. Therefore, the aim of the study was to analyse the expression (at transcription level) of the FADS1, fatty acid desaturase 2 (FADS2), and fatty acid desaturase 3 (FADS3) genes.

MATERIALS AND METHODS

Study population

The research was conducted with the approval of the Ethics Committee in Bielsko-Biala under approval no: 2016/02/11/4. All relevant guidelines and regulations were adhered to, and informed consent was obtained in writing from all the participants. The study population consisted of 34 women who gave birth at the Provincial Specialist Hospital No. 1 in Tychy, Poland. The pregnant women were recruited for the study during their first visit to the hospital. The women were between 21 and 37 years of age. A description of the study population is provided in Table 1.

To obtain a homogeneous group of women, the following inclusion criteria were applied (Bobiński *et al*., 2013; Waksmańska *et al*., 2017):

– Polish nationality (excluding naturalised Polish citizens); single pregnancy; pregnancy I-III (parity considered);

– Stable socioeconomic status; married, secondary level or higher education; living in a highly industrialised urban region, both the women and their husbands having a steady job;

– Consenting to participate in the study.

The following exclusion criteria were applied (Bobiński *et al*., 2013; Waksmańska *et al*., 2017):

– Chronic diseases occurring before pregnancy, such as pre-gestational diabetes; pathologies during the course of pregnancy such as infections during pregnancy (any kind of infection in the perinatal period, such as fever, respiratory infections, urinary infections, etc.); miscarriages and/or premature birth resulting in the death of the child or developmental anomalies in the foetus;

– AIDS and sexually transmitted diseases;

– Adherence to a vegetarian diet, Mediterranean diet, or any other special diet;

– Lack of consent by the mother to take part in the research programme or withdrawal of consent during the study.

Women who participated in the research programme were classified into five groups according to the following criteria:

– AGA Group (AGA – appropriate for gestational age, n=9): healthy mothers, routine and uneventful pregnancy, full-term delivery neonates (bw 10–90th percentile). Age (y) 27.5±4.3; BMI (kg/m^2) 22.6±4.2; delivery (week) 39.6±1.1; neonatal weight (g) 3542.1±387.7; placental weight (g) 503.4±108; mode of delivery 8n/1cs; Apgar score 9–10. AGA served as the control group.

– LGA Group (LGA – large for gestational age, n=10): healthy mothers who gave birth to large for gestational age neonates (bw>90th percentile). Age (y) 29.2±5.2; BMI (kg/m^2) 35.6±4.5; delivery (week) 41.8±1.3; neonatal weight (g) 4056.8±411.2; placental weight (g) 693.3±124.9; mode of delivery 1n/8cs; Apgar score 8–9.

Table 1. Characteristics of the study population.

	AGA	LGA	SGA	IUGR	PTB
Age (Y)	27.5±4.3	29.2±5.2	29.1±5.1	25.6±5.7	27.8±4.1
BMI (kg/m2)	22.6±4.2	35.6±4.5	23.8±4.7	37.3±7.2	23.3±4.4
Delivery (week)	39.6±1.1	41.8±1.3	38.2±1.1	34.5±1.9	36.8±1.0
Neonatal weight (g)	3542.1±387.7	4056.8±411.2	2297.5±149.9	1975.6±258.9	2398±432.8
Placental weight (g)	503.4±108	693.3±124.9	356.4±108	423.4±88.8	323.8±102
Mode of delivery	8n/1cs	1n/8cs	6n	6cs	3n
Apgar Score	9/10	8/9	9/10	6/7/8	9/10

n=natural delivery, cs=caesarean section. Apgar Score measured after 1, 3 and 5 minutes.

– SGA Group (SGA – small for gestational age, n=6): healthy mothers who gave birth to full-term, but small for gestational age, neonates (neonatal weight <10th percentile). Age (y) 29.1±5.1; BMI (kg/m²) 23.8±4.7; delivery (week) 38.2±1.1; neonatal weight (g) 2297.5±149.9; placental weight (g) 356.4±108; mode of delivery 6n; Apgar score 9–10.

– IUGR Group (IUGR – intrauterine growth restriction, n=6): mothers who gave birth to babies showing characteristics of intrauterine growth restriction. In this group, all ultrasound studies showed foetal growth restriction (bw <10th percentile). Age (y) 25.6±5.7; BMI (kg/m²) 37.3±7.2; delivery (week) 34.5±1.9: neonatal weight (g) 1975.6±258.9; placental weight (g) 423.4±88.8; mode of delivery 6cs; Apgar score 6–8.

– PTB Group (PTB – preterm birth age, n=3): mothers who gave birth prematurely at 32–36 weeks' gestation to neonates whose weight fell in the 10–90th percentile. Age (y) 27.8±4.1; BMI (kg/m²) 23.3±4.4; delivery (week) 36.8±1.0; neonatal weight (g) 2398±432.8; placental weight (g) 323.8±102; mode of delivery 3n; Apgar score 9–10.

Women eligible for the study underwent three ultrasound examinations. The first ultrasound test was performed between the 12th and 14th weeks of gestation, the second between the 20th and 22nd weeks of gestation and the third one between the 32nd and 33rd weeks of gestation.

Using ultrasound scans, the foetal weight and length were determined primarily according to the gestational age. The dimensions obtained at the 27th gestational week were crucial and determined the appropriate way to proceed with the delivery. Although usually the results of Doppler flow are not considered significant for foetuses younger than 30-weeks, this test was performed too on the foetuses at the 27th week. The foetal dimensions were taken every 6–7 days and marked on a growth chart. If the foetus was between the 10th and 3rd percentile and its gestational age was above 27 weeks further ultrasound scans were conducted to assess hypotrophy or intrauterine foetal growth inhibition (IUGR) according to the radiological criteria. The ultrasound was used to measure the standard parameters of so-called basic foetal biometry, including the bi-parietal diameter (BPD), head circumference (HC), abdomen circumference (AC), and femur length (FL). The ultrasound estimation of foetal weight and length were converted into a growth chart.

Collection of the placentas

The placental samples were collected from a site approximately 2–3 cm away from the umbilical cord attachment. For the research, we wanted to standardise the site of collection to the place where blood supply to the placenta was the highest, ensuring the highest metabolic activity and a strong RNA expression. In our opinion, taking samples from a different site would not give a full picture of the expression of the genes tested. The samples were collected immediately after birth and transported on ice to the laboratory. The transport time did not exceed one hour. The samples were then weighed, immersed in 1 ml RNAlater for 48 hours at 4°C and then snap frozen.

RT-qPCR

Collected tissue sections were stored in RNAlater according to the manufacturer's protocol (Sigma-Aldrich, St Louis, MO, USA) until required for molecular analysis. Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of the obtained extracts were assessed using a MaestroNano MN-913 nano-spectrophotometer (MaestroGen, Inc., USA). The expression profiles of *FADS1*, *FADS2* and *FADS3* were determined in the presence of β-actin as an endogenous control by RT-qPCR using SensiFAST SYBR No-ROX One-Step kit (Meridian Life Science Inc., Memphis, TN, USA) and Opticon DNA Engine Sequence Detector (MJ Research Inc., Watertown, MA, USA) according to the manufacturer's instructions. The reaction was carried out using primer pairs specific for the sequence of each gene tested: *FADS1* (forward: 5' ATGATTACCTTCTACGTCCG 3', reverse: 5' TCAATGTGCATGGGAATATG 3', amplicon length – 149 bp), *FADS2* (forward: 5' GATGAATCACATCGTCATGG 3', reverse: 5' GTGCTCAATCTGGAAGTTAAG 3', amplicon length – 139 bp), *FADS3* (forward: 5' CAACATCTTCCACAAAGACC 3', reverse: 5' CTGGTTGTAGGGTAGGTATC 3', amplicon length – 109 bp), *ACTB* (forward: 5' TCACCCACACTGTGCCCATCTACGA 3', reverse: 5' CAGCGGAACCGCTCATTGCCAATGG 3', amplicon length – 295bp) purchased in Sigma-Aldrich (St Louis, MO, USA). The thermal profile of the RT-qPCR reaction included the following steps: reverse transcription (45°C for 10 minutes), activation of the polymerase (95°C for 2 minutes), 41 cycles consisting of denaturation (95°C for 5 seconds), annealing (60°C for 10 seconds), and elongation (72°C for 5 seconds). A standard curve was plotted for every run, based on which the Opticon DNA Engine Sequence Detector (MJ Research Inc., Watertown, MA, USA) calculated the mRNA copy numbers of studied genes in each sample. The curves were drawn based on the quantitative standard – β-actin (TaqMan DNA Template Reagent kit, Applied Biosystems, Foster City, CA, USA) at five different concentrations (400, 800, 2000, 4000, 8000 copies of *ACTB* cDNA). Each run included positive and negative controls and was completed by melting curve analysis of each sample to confirm the specificity of the reaction. The endogenous control assessment together with the melting curve analysis were the basis for including the results of the studied genes in the comparative analysis. The results are presented as the number of mRNA copies per 1 μg of total RNA.

The calculations were made in the statistical environment R ver.3.6.0, PSPP program and MS Office 2019. Parametric tests (analysis of variance ANOVA) or their non-parametric equivalents (Kruskal-Wallis test) were used to analyse the quantitative variables broken down into groups. The *T*-test and Wilcoxon pairs test were also used. The selection of tests was based on the distribution of variables, which was verified by the Shapiro-Wilk test.

There were no significant differences in β-actin mRNA copy number between the control and study groups, which indicates that β-actin can be used as an endogenous control in this experiment.

RESULTS

FADS1 and *FADS2*

After checking the assumptions of normality, a non-parametric Kruskal-Wallis test was performed to compare the median in individual groups. In terms of the *FADS1* and *FADS2* genes, the groups did not differ

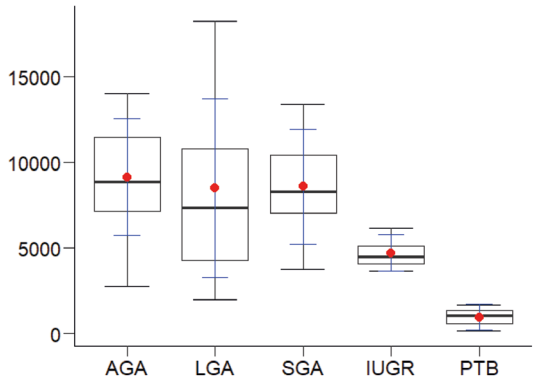

Figure 1. Box plot for *FADS3* expression.
The y-axis represents the number of mRNA copies/1 μg RNA.

significantly ($p>0.05$). The AGA, LGA, SGA, IUGR and PTB groups were therefore similar in terms of the distribution of *FADS1* and *FADS2*.

FADS3

After checking the assumptions of normality of the distribution, it turned out to be possible to use the parametric analysis of variance ANOVA, to compare the average of the dependent variable in the individual groups. In the *FADS3* range, the groups significantly differed statistically ($p<0.05$). Tukey's post hoc test was performed to determine the groups whose means differed significantly.

Significant statistical differences ($p<0.05$) were observed between the following groups: AGA and IUGR, AGA and PTB, LGA and PTB, SGA and PTB, and IUGR and PTB (shown in Fig. 1). In the AGA group, the average values of FADS3 M were: 9149.67 in the AGA group; 4713.50 for IUGR; 967.33 for PTB, 8487.60 for LGA; and 8580.83 for SGA.

In the next part of the analysis, the transcription levels of the *FADS1*, *FADS2* and *FADS3* genes were analysed within each of the research groups (AGA, LGA, SGA, IUGR, PTB) using Friedman's test and Bonferroni's post hoc test.

AGA Group

Friedman's test showed statistically significant differences ($p<0.05$) between *FADS1*, *FADS2* and *FADS3* expression levels within the AGA group. The mean mRNA levels were M=2640.8 mRNA copies/1 μg RNA for *FADS1*, M=494.9 mRNA copies/1 μg RNA for *FADS2*, and M=9149.7 mRNA copies/1 μg RNA for *FADS3* (shown in Fig. 2).

Bonferroni's post hoc test was conducted to determine which of the comparisons between groups were statistically significant ($p<0.05$). Such differences were detected between FADS1 and FADS3 and between FADS2 and FADS3 groups.

LGA Group

Friedman's test showed statistically significant differences ($p<0.05$) between *FADS1*, *FADS2* and *FADS3* expression levels within the LGA group.

The mean mRNA level for *FADS1* was M=2663.2 copies/1 μg, for *FADS2* 8090.9 copies/1 μg RNA, and for *FADS3* M=8487.6 copies/1 μg RNA (shown in Fig. 3).

The Bonferroni test identified that statistically significant ($p<0.05$) comparisons were between FADS1 and FADS2, and between FADS1 and FADS3 groups.

SGA Group

Friedman's test showed statistically significant differences ($p<0.05$) between *FADS1*, *FADS2* and *FADS3* expression levels within the SGA group. Tukey's post hoc test was performed to determine which of the comparisons between groups were statistically significant ($p<0.05$). Such differences were detected between FADS1 and FADS3.

The mean mRNA level for FADS1 was M=2587.2 copies/1 μg, and M=8580.8 copies/1 μg RNA for FADS3 (shown in Fig. 4).

IUGR Group

Friedman's test showed statistically significant differences ($p<0.05$) between *FADS1*, *FADS2* and *FADS3* expression levels within the IUGR group.

The mean mRNA levels were M=671.8 copies/1 μg for *FADS1*, 2123 copies/1 μg RNA for *FADS2*, and

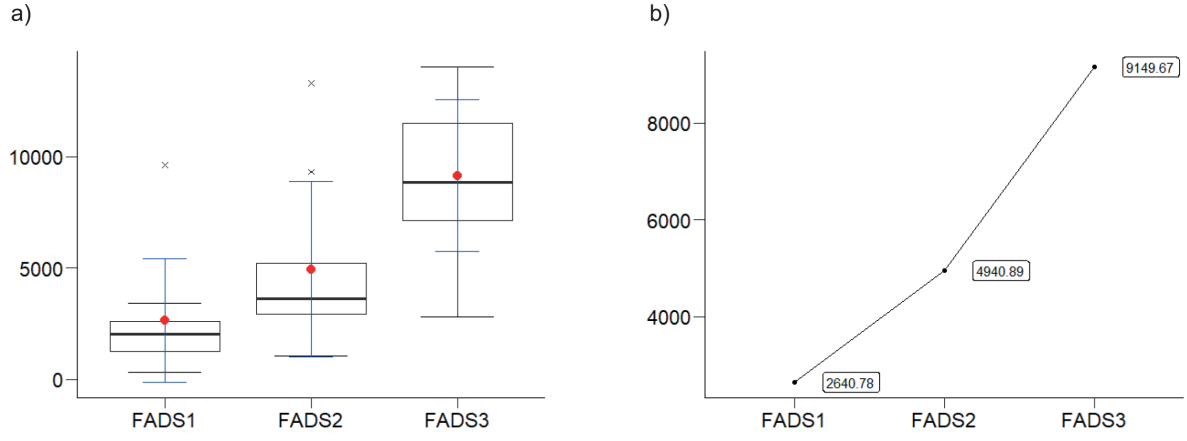

Figure 2. Expression of *FADS1*, *FADS2* and *FADS3* in the AGA group presented as:
(**a**) a box plot where the y-axis represents the number of mRNA copies/1 μg RNA and (**b**) a line graph of the average expression values, where the y-axis represents the number of mRNA copies/1 μg RNA

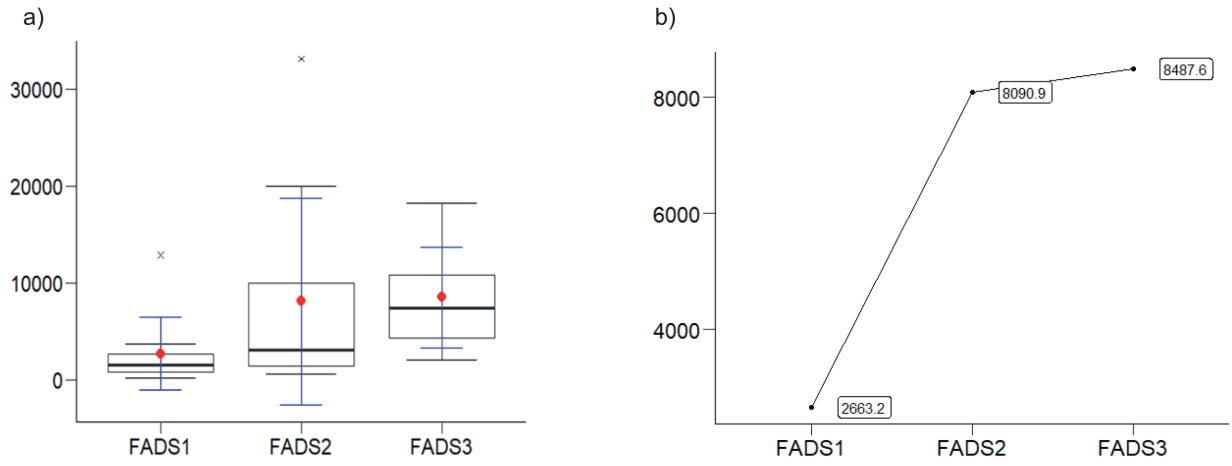

Figure 3. Expression of FADS1, FADS2 and FADS3 in the LGA group presented as:
(**a**) a box plot where the y-axis represents the number of mRNA copies/1 µg RNA and (**b**) a line graph of the average expression values, where the y-axis represents the number of mRNA copies/1 µg RNA

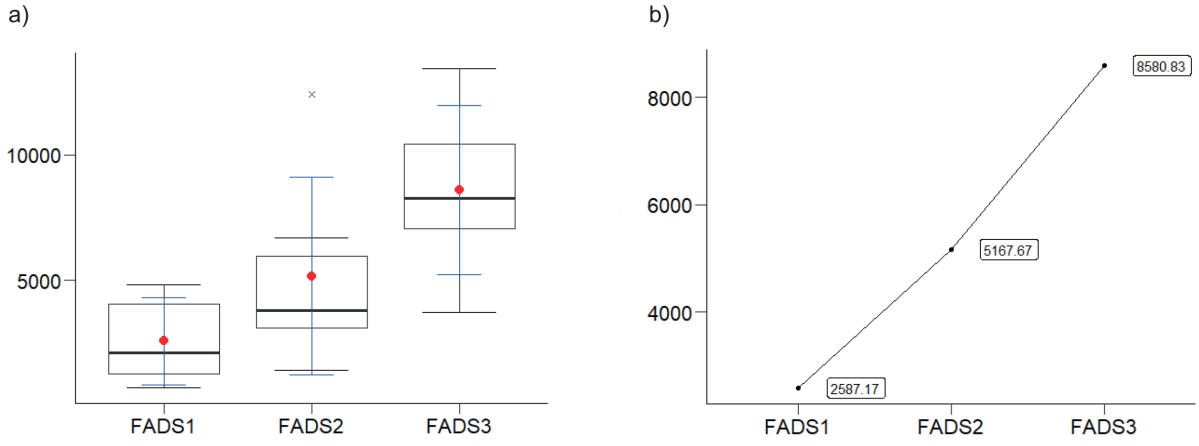

Figure 4. Expression of *FADS1*, *FADS2* and *FADS3* in the SGA group presented as:
(**a**) a box plot where the y-axis represents the number of mRNA copies/1 µg RNA and (**b**) a line graph of the average expression values, where the y-axis represents thmRNA copies/1 µgRNA

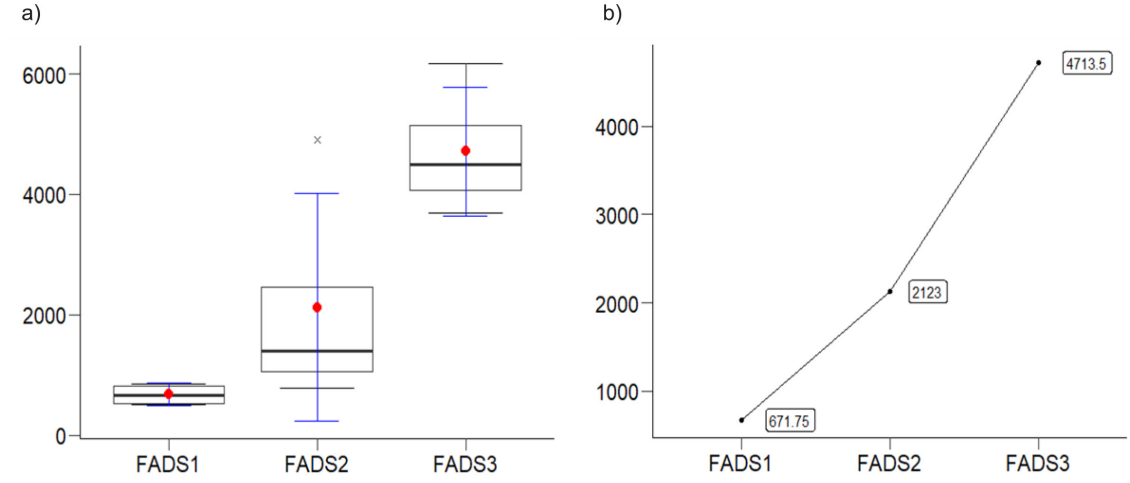

Figure 5. Expression of FADS1, FADS2 and FADS3 in the IUGR group presented as:
(**a**) a box plot where the y-axis represents the number of mRNA copies/1 µgRNA and (**b**) a line graph of the average expression values, where the y-axis represents the number of mRNA copies/1 µg RNA

for M=4713.5 copies/1 μg RNA for *FADS3* (shown in Fig. 5).

The Tukey's post hoc test identified that statistically significant ($p<0.05$) comparisons were between FADS1 and FADS3, and between FADS2 and FADS3.

PTB Group

The Friedman's test showed no statistically significant differences ($p<0.05$) between *FADS1*, *FADS2* and *FADS3* expression in the PTB group. Tukey's post hoc test confirmed there were no differences.

DISCUSSION

Fat is one of the key ingredients necessary for proper foetal development. During pregnancy, a mother's body deposits fat in an amount which corresponds approximately to the baby's weight (3500 g) (Hytten & Chamberlain, 1974). These processes occur most vigorously in the first and second trimester and will happen even if the mother is malnourished (Prentice & Golberg, 2000; Herrera, 2002; Herrera *et al*., 2006). In the second trimester (the anabolic period), the concentration of phospholipids, non-esterified FAs and triglycerides increases in the mother's circulation. This mechanism is associated with an insulin-dependent decrease in lipoprotein lipase activity in adipose tissue and subsequent insulin resistance. As a result of these processes, part of the accumulated fat is transferred to the foetus via the placenta. In contrast, the third trimester is a catabolic period for the mother. Increased lipolysis in the mother's adipose tissue is associated with decreased sensitivity of insulin receptors, which are hormonally controlled by progesterone, cortisol, prolactin and leptin (Cousins, 1991; Herrera *et al*., 2006; Catov *et al*., 2007). As a result, in comparison to the anabolic period, even greater amounts of fat, including FAs, reach the placenta. The dynamics of changes in fat content in the foetus is different from that found in the mother. First, there is no catabolic period in the foetus, and second, the anabolic period begins much later than the mother's – between 20 and 22 weeks of pregnancy. Complicated maternal-placental-foetal fat metabolism, especially of FAs and their derivatives, continues to be controlled by numerous factors, including enzymes whose expression is regulated, *inter alia*, at the level of transcription. This paper presents the analysis of expression of three genes encoding the strategic desaturases, which control the formation of n-3 and n-6 FA. In the AGA, LGA, SGA, PTB and IUGR groups that were studied, no significant differences in mRNA levels of the *FADS1* and *FADS2* were observed. This could imply that LC-PUFA biosynthesis and the function of pro- and anti-inflammatory cytokines are unaffected in neither group. However, since we have not studied the polymorphism of the *FADS1* and *FADS2* genes as a factor that could influence foetal development, this conclusion is not irrefutable. Our studies have shown differences in the placental mRNA expression level of the *FADS3*. Women who gave birth to healthy children in due time (AGA) and women who gave birth to children with only minor problems (SGA and LGA), had higher *FADS3* mRNA expression levels in the placenta than women in the PTB and IUGR (higher level of problems) groups. This result is difficult to interpret because *FADS3* and the desaturase encoded by it have not yet been sufficiently researched. It is known that *FADS3* transcriptional activity in tissues is significantly different between male and female mice and rats. *FADS3* encoded desaturase can introduce a double bond into the FA chain like any other desaturase but other potential functions of the enzyme should be considered as well. Desaturases are known to perform hydroxylation (Mizutani *et al*., 2004; Blanchard *et al*., 2011), acetylenation and epoxidation (Lee *et al*., 1998). Substances such as etherlipid (Paltauf & Holasek, 1973), sphingolipid (Mizutani *et al*., 2004) and cholesterol (Nusblat *et al*., 2005) can also be substrates for desaturases. Therefore, FADH3 potential physiological role can be broad, especially considering that D13D exists in at least three isoforms (Blanchard *et al*., 2011). One concept that could explain the lower transcription activity of *FADS3* in the PTB and IUGR groups is the specific structure of the gene promoter. It contains target sites for NF-κB (Szczesna-Skorupa *et al*., 2004; Yan *et al*., 2007), MYCN (Alaminos *et al*., 2003) and p63 protein (Blanchard *et al*., 2011), suggesting that *FADS3* expression can be regulated by these factors. It is known that NF-κB, MYCN and p63 are the components of intracellular pathways associated with proliferation and apoptosis. Several studies have demonstrated the relatedness of IUGR and these pathways. For example, in IUGR, the NF-κB-regulated proangiogenic targets in foetal pulmonary artery endothelial cells are disrupted, which leads to the abnormal metabolism of extracellular matrix components and, as a result, interferes with pulmonary angiogenesis (Dodson *et al*., 2018). In pregnancies complicated with IUGR, the processes of apoptosis in placenta are stronger than in a healthy placenta (Erel *et al*., 2001) and a significantly higher NF-κB expression can be observed (Aban *et al*., 2004). It is not known why the increase in NF-κB expression does not cause an increase in the expression of *FADS3* in the IUGR placenta; in fact, exactly the opposite is observed. Higher NF-κB expression is accompanied by lower *FADS3* transcriptional activity. It is possible that NF-κB inhibitors increase during IUGR, or the chromatin is remodelled in such a way that the *FADS3* promoter becomes inaccessible for NF-κB. Changes in FADS 3 activity in the course of IUGR may also be related to the functioning of the membrane transport system, which is responsible for maintaining the correct FA ratio in the maternal (M) and foetal (F) circulation. Changes in the F/M ratio were observed in IUGR, SGA and PTB children (Cetin *et al*., 2002; Bobiński *et al*., 2013).

This work also analyses *FADS* genes expression within the AGA, LGA, SGA, PTB, and IUGR groups. Except for the PTB group, which had the lowest number of samples, and in which no differences in *FADS* gene expression were observed, there were differences in expression between *FADS1, FADS2* and *FADS3* in the remaining groups. In the LGA group there were no differences between *FADS2* and *FADS3* transcript levels Comparing the average *FADS* mRNA expression between the AGAs and LGAs, it can be assumed that the lack of differences in the LGA group was due to an increased *FADS2* and slightly reduced *FADS3* transcript levels. LGAs are a group of children who, in addition to the increased body weight (>90 percentile), have also increased body fat. Both are involved in fat metabolism, so with the increased fat mass of the child, changes in D6D and D13D activity are highly likely, although the case of the *FADS3* gene product is surprising. It has been previously shown that the increased expression of *FADS3* in adipose tissue is characteristic of hyperlipidemia (Plaisier *et al*., 2009). Our research shows that this is the opposite for the placenta. Perhaps, the reduced placental expression of *FADS3* in LGA pregnancies is a type of compensatory mechanism that regulates foetal fat

metabolism. From a clinical point of view, however, it would be more interesting to see a significant increase in the transcriptional activity of the *FADS2* gene encoding desaturase 6, catalysing the reactions of the n-3 and n-6 main biosynthesis pathway. One of the factors (although not studied in this research) that could affect *FADS2* expression is the pregnant woman's diet. With an ample supply of plant oils, which contain high proportion of LA, such as sunflower seed oil, safflower oil or corn oil, less DHA is produced from ALA as a result of n-6 desaturase inhibition leading to a decreased EPA biosynthesis. The n-6 FA pool then increases, which could be a risk factor for the development of LGA (Llanos *et al.*, 2005; Bobiński & Mikulska, 2015). Furthermore, it may have an influence on the metabolism of medium-chain fatty acids (MCFA), especially myristic acid (C14: 0) and lauric acid (C12: 0), which have a significant impact on the conversion of EPA to DHA (Legrand *et al.*, 2020). This, in turn, in addition to placental biosynthesis of LC-PUFA, can disturb the specified hierarchy DHA>AA> LA>ALA defining the order of transport of the acids across the placental barrier (Cetin *et al.*, 2009a; Cetin and Alvino, 2009b).

CONCLUSION

The placenta fulfils hormonal, nutritional and metabolic roles. Its task is to control the development of the foetus, but the hormonal-metabolic placental processes affect, to a large extent, also the body of a pregnant woman. Fatty acids play a key role in these mechanisms. Some of them are transported through the placental barrier, others undergo biosynthesis in the placenta. Often, placental biosynthesis involves the elongation and desaturation processes of the pre-existing FAs with shorter carbon chains, which either lack or have fewer double bonds. Desaturases are involved in these processes. This important group of enzymes maintain the balance of n-3 and n-6 FAs levels, has a significant role in the development of the nervous system and cell membranes, and affect general maternal-placental-foetal homeostasis. Our studies have shown that the transcriptional activity of the *FADS1* and *FADS2* genes remain at similar levels in the pregnancy groups we examined. It was only in the *FADS3* gene expression that the differences were discovered. *FADS3* lowest mRNA levels were observed in the placenta of women who gave birth to premature babies. In this group, also no differences were observed in the *FADS1*, *FADS2* and *FADS3* mRNA levels. In the SGA group, expression differences were observed between the *FADS3* and *FADS1* genes only. The AGA, LGA and IUGR groups had a similar expression profile, in which the *FADS3* gene had the highest and the *FADS1* gene had the lowest expression except for the difference between *FADS2* and *FADS3* expression, which was observed in AGA and IUGR, but not LGA. . The IUGR group had the lowest expression of all tested genes, while maintaining differences between their expression. The largest number of differences in *FADS* mRNA levels were observed in the placenta of women who gave birth to children with a mild degree of disorder, i.e., PTB and SGA.

Considering the number of FAs undergoing maternal-placental-foetal transformation and the complexity of their metabolism, it is difficult to unequivocally interpret changes in the transcriptional activity of genes encoding desaturases in selected pregnancy pathologies. Many factors regulate these processes and one of the most important is - not studied in this work - the expression of genetic variants of the *FADS1*, *FADS2* and *FADS3*. Their analysis would provide further data for the assessment of maternal-placental-foetal FA changes, crucial for the proper development of the foetus, the emergence of metabolic or genetic risk factors and an improvement in the profile of prevention and treatment of foetal developmental abnormalities.

Declarations

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