Placental expression of FADS1, FADS2, FADS3 desaturases in selected pregnancy pathologies

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Abstract

Background: The period of intrauterine development is a key period in human development. Its progress largely depends on the function of the placenta, which is responsible for the transportation and biosynthesis of fatty acids. Desaturation enzymes play a key role in placental fatty acid metabolism. The 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 Desaturase 1 (FADS1), Fatty Acid Desaturase 2 (FADS2) and Fatty Acid Desaturase 3 (FADS3) in women who gave birth to: appropriate for gestational age (AGA), large for gestational age (LGA), small for gestational age (SGA), intrauterine growth restriction (IUGR) and preterm birth (PTB) infants.

Method: The study took place at the Tychy Specialist Hospital in Poland. 34 pregnant women aged 21-37 years old took part. The placental samples were taken from a site about 2-3 cm away from the umbilical cord attachment. The collected tissue sections were stored, according to the manufacturer's protocol, in RNAlater (Sigma-Aldrich, St Louis, MO, USA), until required for molecular analysis. The expression profile of FADS1, FADS2 and FADS3 was determined by RT-qPCR.

Results: In terms of the FADS1 and FADS2 genes, there was no difference in the expression between the groups. However, differences in the expression of the FADS3 gene were found. Analysis of the transcriptional activity of the FADS1, FADS2 and FADS3 genes in most of the examined groups showed significant differences.

Conclusions: These findings suggest that the transcriptional activity of 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.

Background

Intrauterine foetal development is a critical period in human development. Subsequent quality of life, health, susceptibility to diseases, intelligence and many other factors are dependent on this foetal stage [1]. The placenta plays a key role during pregnancy. It controls the metabolic processes on the hormonal pathway between the interface of the mother and child organisms - including oxygenation and the supply of building and energy substrates to the foetus [1,2]. Placental abnormalities lead to premature births - with varying degrees of malnutrition (e.g. Intrauterine Growth Restriction (IUGR)), small for gestational age (SGA) babies, or Large for Gestational Age (LGA) births. 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, brain, development of cell membranes, structure and function of the retina as well as fulfilling many other metabolic and structural functions [3,4]. 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 [5,6], the release of FA from deposits in the mother's tissue [7], the

endogenous biosynthesis of FA by the mother and later - foetal FA synthesis [8]. The placenta is primarily responsible for the maternal-foetal metabolism of FA; this includes both transportation of the FA from mother to foetus, as well as placental biosynthesis and FA 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 [5,9,10]. Some placental disorders can impair FA metabolism and this may lead to intrauterine foetal development disorders and a predisposition to the development of 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 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 family members at 11q12-q13.1 [11]. 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) respectively are formed [12]. The third representative of desaturases is the Delta (13) Desaturase (D13D) enzyme (EC 1.14.19.-) encoded by the FADS3 gene. D13D, in turn, 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 [13]. 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 as well as DHA - key lipids for the development of the foetal nervous system [14], loss of control over the metabolism of inflammatory lipids such as prostaglandin E2 - critical for acute inflammatory response and maintenance of epithelial homeostasis [15] and metabolic disorders, the effects of which may take some time to appear, such as diabetes, lipid disorders, cardiovascular diseases, etc. [16]. The processes controlled by these desaturases are extremely important from the point of view of the intrauterine development of the foetus and, especially, 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.

Methods

Study population

The research was carried out 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 from all the participants in writing. 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 can be found in Table 1.

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/m^2)	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					

To obtain a homogeneous group of women, the following inclusion criteria were applied [2,17]:

- Polish nationality (excluding naturalised Polish citizens); single pregnancy; pregnancy I-III (consider parity);
- 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 [2,17]:

- Chronic diseases occurring in the women 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^{th} – 90^{th} 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. Group AGA was a control group.
- LGA Group (LGA – large for gestational age, n=10): healthy mothers who gave birth to large for gestational age neonates (bw>90^{th} 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 1/8cs; Apgar score 8/9.
- 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 < 10^{th} percentile). Age (y) 29,1 ± 5,1; BMI (kg/m^2) 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 all ultrasound studies of the pregnant women foetal growth restriction was found (bw <10^{th} percentile). Age (y) 25,6 ± 5,7; BMI (kg/m^2) 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 6/7/8.
- 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^{th}–90^{th} Age (y) 27,8 ± 4,1; BMI (kg/m^2) 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 12^{th} and 14^{th} weeks of gestation, the second between the 20^{th} and 22^{nd} weeks and the third one between the 32^{nd} and 33^{rd} weeks.

Using ultrasound scans, the foetal weight and length were determined primarily according to the gestational age. The 27th gestational week was the crucial week. The dimensions obtained determined the appropriate way to proceed with the foetus. Although the results of Doppler flow are not considered significant below the 30 week term, this test was performed on the foetuses at the 27th week. The foetal dimensions, taken every 6-7 days were 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 carried out on the foetus. The radiological criteria for hypotrophy or intrauterine foetal growth inhibition (IUGR) was an ultrasound assessment of foetal weight and length and the conversion of this data into a growth chart.

The ultrasound was used to measure the bi-parietal diameter (BPD), head circumference (HC), abdomen circumference (AC) and femur length (FL). These are the standard parameters of so-called basic foetal biometry.

Collection of the placentas

The placental samples were taken from a site about 2-3 cm away from the umbilical cord attachment. For the research, we wanted to standardise the site of collection to the place where, having established the highest metabolic activity and a strong RNA expression, there was the largest blood supply to the placenta. In our opinion, taking samples from different places would mean that the results would not give a full picture of the expression of the genes tested. With our selection criteria for the place of collection, we now have knowledge of how the quantitative process works in the this part of the placenta. Samples were taken immediately after the birth, then transported in ice to the laboratory. The transport time did not exceed one hour. The samples were then weighed and placed in the reagent (immersed in 1 ml RNAlater for 48 hours at 4℃ and then snap frozen).

RT-qPCR

Collected tissue sections were stored, according to the manufacturer's protocol, in RNAlater (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 profile of *FADS1*, *FADS2* and *FADS3* was determined in the presence of β-actin as an endogenous control by the RT-qPCR method using the SensiFAST SYBR No-ROX One-Step kit (Meridian Life Science Inc., Memphis, TN, USA) and sequence detector 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), 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 (Kruskall-Walis 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.

Comparing the β-actin mRNA copy number in the control and study groups, no statistically significant differences were found, 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 significantly differ statistically ($p > 0.05$). 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 in order to determine exactly where there were significant differences between the groups.

There were significant statistical differences ($p < 0.05$) between:

AGA - IUGR; In the AGA group, average values of FADS3 M = 9149.67 were recorded, while in the IUGR group M = 4713.50. The AGA group achieved statistically significant ($p < 0.05$) higher results than the IUGR group (Fig. 1).

AGA - PTB; In the AGA group, average values of FADS3 M = 9149.67 were recorded, while in the PTB group M = 967.33. The AGA group achieved statistically significant ($p < 0.05$) higher results than the PTB group (Fig. 1).

LGA - PTB; In the LGA group, average values of FADS3 were M = 8487.60, while in the PTB group M = 967.33. The LGA group achieved statistically significant ($p < 0.05$) higher results than the PTB group (Fig. 1).

SGA - PTB; In the SGA group, average values of FADS3 M = 8580.83 were recorded, while in the PTB group M = 967.33. The SGA group achieved statistically significant ($p < 0.05$) higher results than the PTB group (Fig. 1).

IUGR - PTB; In the IUGR group, average values of FADS3 were M = 4713.50, while in the PTB group M = 967.33. The IUGR group achieved statistically significant ($p < 0.05$) higher results than the PTB group (Fig. 1).

In the next part of the analysis, the transcriptional activity of the FADS1, FADS2 and FADS3 genes was compared within each of the research groups (AGA, LGA, SGA, IUGR, PTB).

AGA Group

Friedman's test showed significant differences ($p < 0.05$) statistically between the expression levels of these genes within the AGA group. Bonferroni’s post hoc test was conducted in order to determine exactly where the differences between these gene expressions were significant,. The test showed that statistically significant ($p < 0.05$) differences between them were, for example:

FADS1 - FADS3; For FADS1 the average was M = 2640.78 while for FADS3 the average was M = 9149.67; The AGA group had a significantly higher level of FADS3 than of FADS1 (Fig. 2).

FADS2 - FADS3; For FADS2 the average was M = 494.89 while in FADS3 the average was M = 9149.67; The AGA group had a significantly higher level of FADS3 than of FADS2 (Fig. 2).

LGA Group

Friedman's test showed statistically significant differences ($p < 0.05$) between the expression level of these genes within the LGA group. The Bonferroni test showed that there were statistically significant ($p < 0.05$) differences between gene expression - for example:

FADS1 - FADS2; For FADS1 the average was M = 2663.20 while for FADS2 the average was M = 8090.90; The LGA group had a significantly higher level of FADS2 than of FADS1 (Fig. 3).

FADS1 - FADS3; For FADS1 the average was M = 2663.20, while for FADS3 the average was M = 8487.60; The LGA group had a significantly higher level of FADS3 than of FADS1 (Fig. 3).

SGA Group

Friedman's test showed statistically significant differences ($p < 0.05$) between the expression levels of these genes within the SGA group. Tukey's post hoc test was performed in order to determine exactly where there were significant differences between gene expression. Tukey's test showed that there were statistically significant ($p < 0.05$) differences between gene expression - for example:

FADS1 - FADS3; For FADS1 the average was M = 2587.17, while for FADS3 the average was M = 8580.83; The SGA group had a significantly higher level of FADS3 than of FADS1 (Fig. 4).

IUGR Group

Friedman's test showed statistically significant differences ($p < 0.05$) between the expression levels of these genes within the IUGR group. Tukey's post hoc test showed that statistically significant ($p < 0.05$) differences between gene expression were:

FADS1 - FADS3; For FADS1 the average was M = 671.75 while for FADS3 the average was M = 4713.50; The IGUR group had a significantly higher level of FADS3 than of FADS1 (Fig. 5).

FADS2 - FADS3; For FADS2 the average was M = 2,123.00 while for FADS3 the average was M = 4,713.50; The IGUR group had a significantly higher level of FADS3 than ofFADS2 (Fig. 5).

PTB Group

The test that was performed showed no statistically significant differences ($p > 0.05$) between gene 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 (3500g) [18].

These processes occur most vigorously in the second and third trimesters (the anabolic period) and will happen even if the mother is malnourished [19-21]. 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. The third trimester is a catabolic period. 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 [21,23]. 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 of all, there is no catabolic period, secondly, 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 results of testing the expression of three genes which encode strategic desaturases controlling the formation of n-3 and n-6. In the AGA, LGA, SGA, PTB and IUGR groups that were studied, no significant differences in transcriptional activity of the FADS1 and FADS2 genes were observed. This may mean that LC-PUFA biosynthesis and pro-inflammatory and anti-inflammatory cytokines are functioning relatively normal. However, this is not absolutely certain because we have not studied the polymorphism of the FADS1 and FADS2 genes as a factor that could have an effect on foetal development. Nevertheless, studies have shown differences in the transcriptional activity of the FADS3 gene in the tests on placenta. Women who gave birth to healthy children on time (AGA) and women who gave birth to children with only minor problems - SGA and LGA had higher FADS3 transcriptional activity than in the PTB and IUGR (higher level of problems) groups. The result is difficult to interpret because FADS3 and the desaturase encoded by it have not been well researched. It is known that the transcriptional activity of the gene in tissues is significantly different between males and females (mice, rats). FADS3 encoded desaturase can introduce, like any other desaturase, a double bond into the FA chain but other potential functions should be considered. In the world of living matter, desaturases perform hydroxylation [24,25], acetylenation and epoxidation [26] reactions. Such substances as etherlipid [27], sphingolipid [25] and cholesterol [28] can also be substrates for desaturases. Therefore, its potential physiological role can be broad, especially when the fact that D13D exists in at least three isoforms [24] is taken into account. One concept which could explain the lower transcription activity of FADS3 in the PTB and IUGR groups is the specific structure of the gene promoter. Regions binding factors NF-κB [29, 30], MYCN [31] and p63 protein [24] were identified in it, suggesting that FADS3 is the presumed target gene for these factors. It is known that NF-kB, MYCN and p63 are involved in cell pathways associated with proliferation or apoptosis. Studies exist which demonstrate the impact of IUGR on these pathways. For example, IUGR disrupts NF-κB-regulated proangiogenic targets in foetal pulmonary artery endothelial cells, which leads to the abnormal metabolism of extracellular matrix components and, as a result, interferes with pulmonary angiogenesis [32]. In the placenta of pregnancies complicated with IUGR, in which the processes of apoptoptosis are stronger than in a healthy placenta [33], a significantly higher NF-κB expression can be observed [34]. It is not known why the rise in NF-κB

does not cause an increase in the expression of FADS 3 in the IUGR placenta; in fact, exactly the opposite happens. Higher NF-κB activity is accompanied by lower FADS 3 transcriptional activity. It is likely that NF-κB inhibitors increase during IUGR, or the chromatin is remodelled in such a way that it becomes inaccessible to the FADS 3 promoter. 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 and foetal circulation. Changes in the F / M ratio were observed in IUGR in SGA and PTB children [2,16]. This work also thoroughly analyses the transcriptional activity of FADS genes in the AGA, LGA, SGA, PTB, and IUGR groups. Except for the PTB group which had the lowest number of samples, no differences in FADS gene expression were observed. In the other groups there were differences in expression between all genes, only in the LGA group there were no differences between FADS2 and FADS3. Comparing the average FADS between the AGAs and LGAs, it can be assumed that the lack of differences in the LGA group was due to increased FADS2 activity and slightly reduced FADS3 activity. LGAs are a group of children who, in addition to increased body weight (> 90 percentile), have increased body fat. Both of these, of course, are involved in fat metabolism, so with the increased fat mass of the child, changes in D6D and D13D activity are highly likely, although surprising, for example, in the case of the FADS3 gene product. It has been previously shown that the increased expression of FADS3 in adipose tissue is characteristic of hyperlipidemia [35]. Our research shows that this is the opposite for the placenta. So, perhaps the reduced placental expression of FADS3 in LGA children is a type of compensatory mechanism that regulates foetal fat metabolism. From a clinical point of view, however, it is more interesting to see a significant increase in the transcriptional activity of the FADS2 gene encoding desaturase 6, catalysing the reactions of the main biosynthesis pathway n-3 and n-6. 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, such as sunflower-seed oil, safflower oil or corn oil, which contain large amounts of LA, then less DHA is produced from ALA as a result of n-6 desaturase inhibition leading to decreased EPA biosynthesis. The n-6 pool then increases, which could be a risk factor for the development of LGA [1,36]. Furthermore, it may have an influence on the metabolism of medium-chain fatty acids (MCFA), especially miristic acid (C14: 0) and lauric acid (C12: 0), which have a significant impact on the conversion of EPA to DHA [37]. This, in turn, in addition to placental biosynthesis LC-PUFA, can disturb the specified hierarchy DHA> AA> LA> ALA defining the order of transport of the acids across the placental barrier [38,39].

Conclusions

The placenta fulfils a hormonal, nutritional and metabolic role. Its task is to control the development of the foetus, although the hormonal-metabolic mechanisms occurring in the placenta also affect, to a large extent, 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 pre-existing acids with shorter carbon chains and which either lack or have fewer double bonds. Desaturases are involved in these processes. This is an important group of enzymes because they maintain the balance of levels of n-3 and n-6 FAs,

have 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 groups we examined. It was only in the FADS3 gene that differences were discovered. Its lowest activity was observed in the placenta of women who gave birth to premature babies. In this group, no differences were observed in the transcriptional activity of the tested FADS1, FADS2 and FADS3. However, in the SGA group, differences were revealed only between the FADS3 and FADS1 genes. The AGA, LGA and IUGR groups had a similar expression profile. The FADS3 gene dominated and the FADS1 gene had the lowest activity, although the LGA group did not show differences between the FADS3 and FADS2 genes. The IUGR group had the lowest transcriptional activity of all genes, while maintaining statistical differences between them. The largest number of differences in gene activity were observed in the placenta of women who gave birth to children with a mild degree of disorder - i.e. PTB and SGA children.

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 genes. 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.

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

Declarations

Ethics approval and consent to participate

The research was carried out with the approval of the Komisja Bioetyczna (The Ethics Committee) in Bielsko-Biala under approval no: 2016/02/11/4. Informed consent was obtained from all participants in writing.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

RB performed laboratory tests and statistical analysis, created the figures and was the major contributor in writing the manuscript. UM and NZ performed laboratory tests and reviewed the manuscript. IU-W collected samples, analysed and interpreted the patient data and reviewed the manuscript. MD reviewed and edited the manuscript. WP-W, AP, MH, JB and CP collected data. All authors read and approved the final manuscript.

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Figures

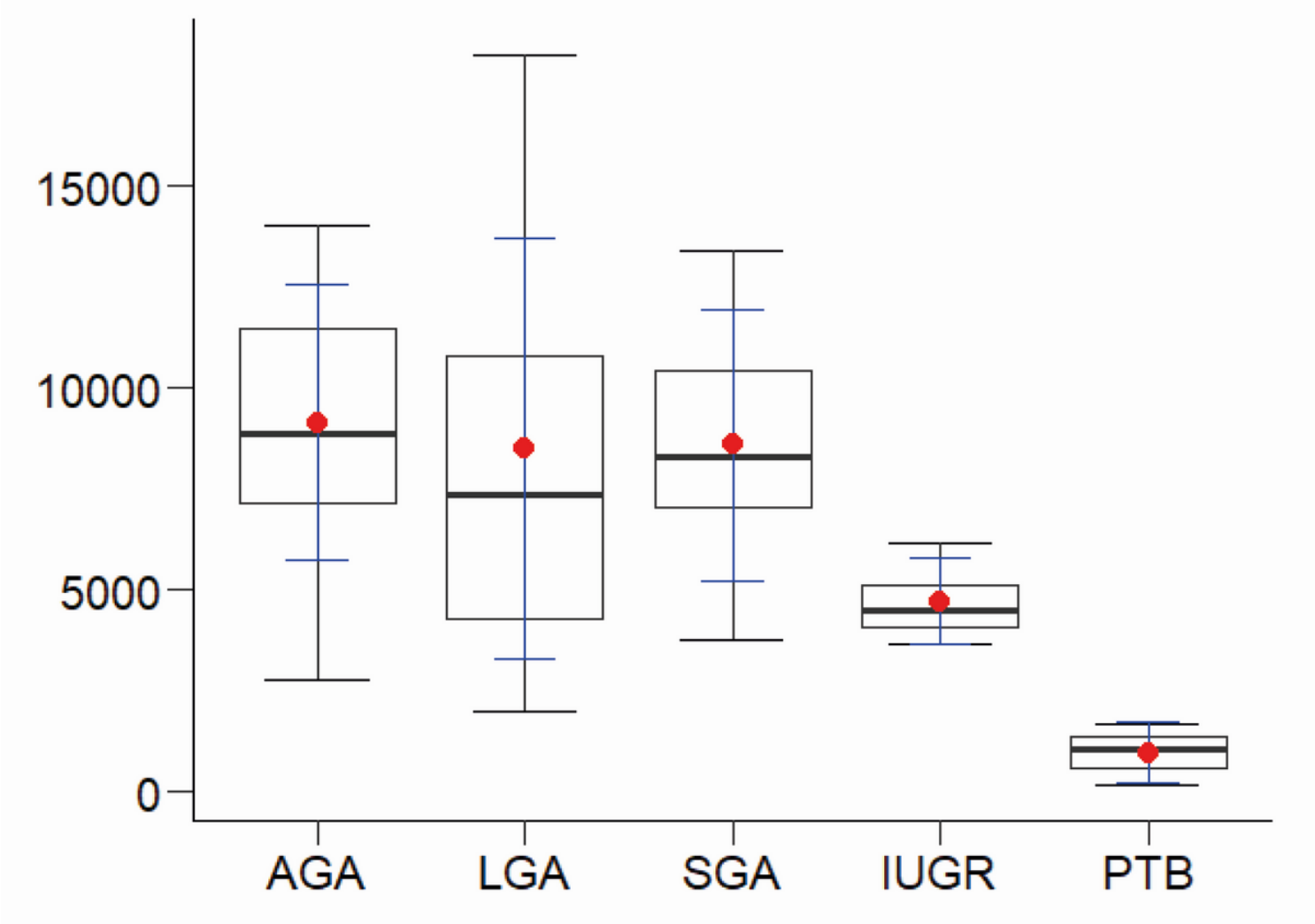

Figure 1

Box plot for FADS3. The y-axis represents the number of mRNA copies/1µgRNA.

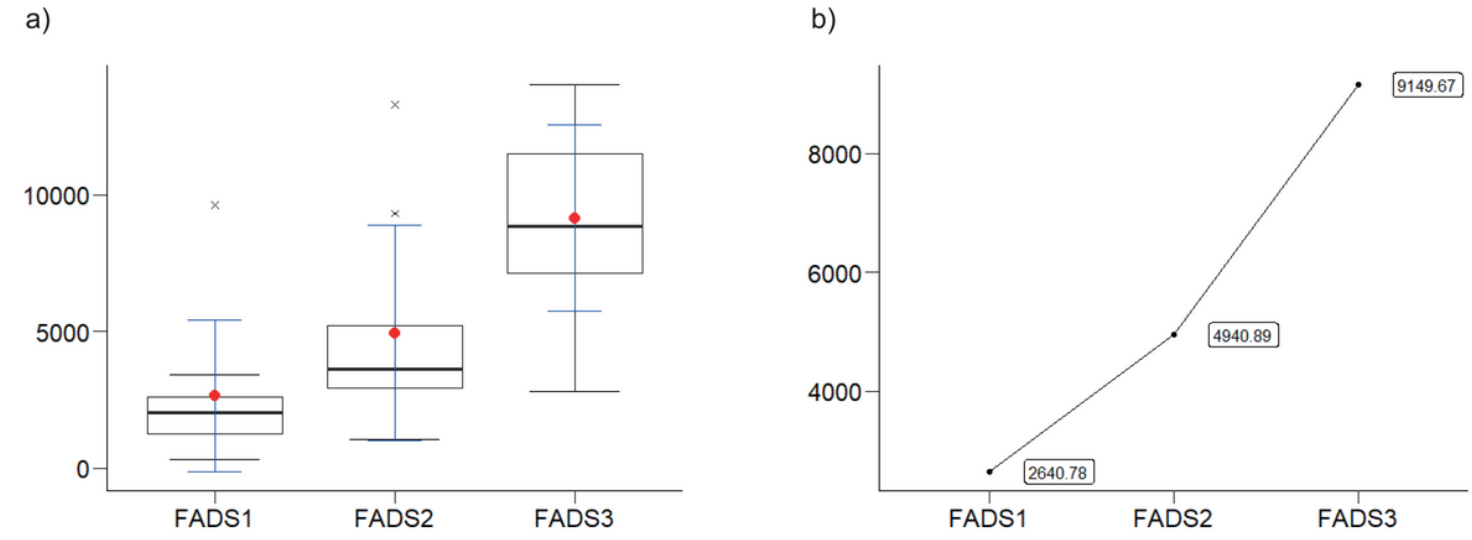

Figure 2

Levels of expression of FADS1, FADS2 and FADS3 genes in AGA group presented as: a). a box plot where the y-axis represents the number of mRNA copies/1µgRNA and b). a line graph showing the average value for the expression of FADS1, FADS2 i FADS3 genes., where the y-axis represents the number of mRNA copies/1µgRNA

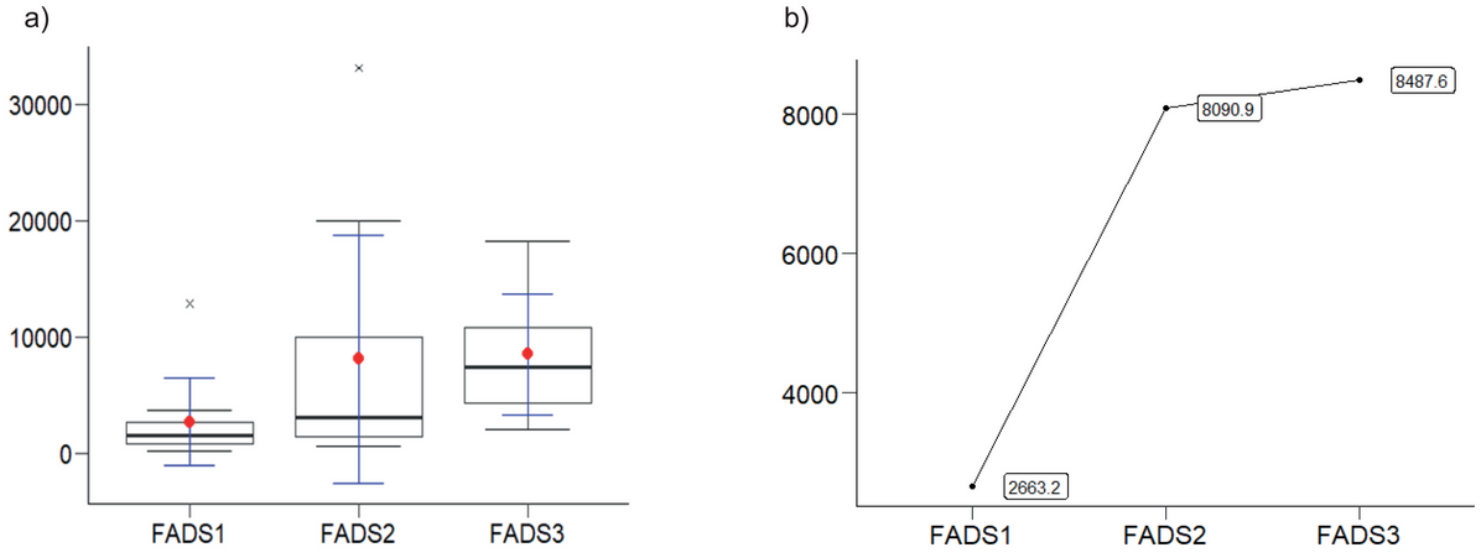

Figure 3

Levels of expression of FADS1, FADS2 and FADS3 genes in LGA group presented as: a). a box plot where the y-axis represents the number of mRNA copies/1µgRNA and b). a line graph showing the average value for the expression of FADS1, FADS2 i FADS3 genes, where the y-axis represents the number of mRNA copies/1µgRNA

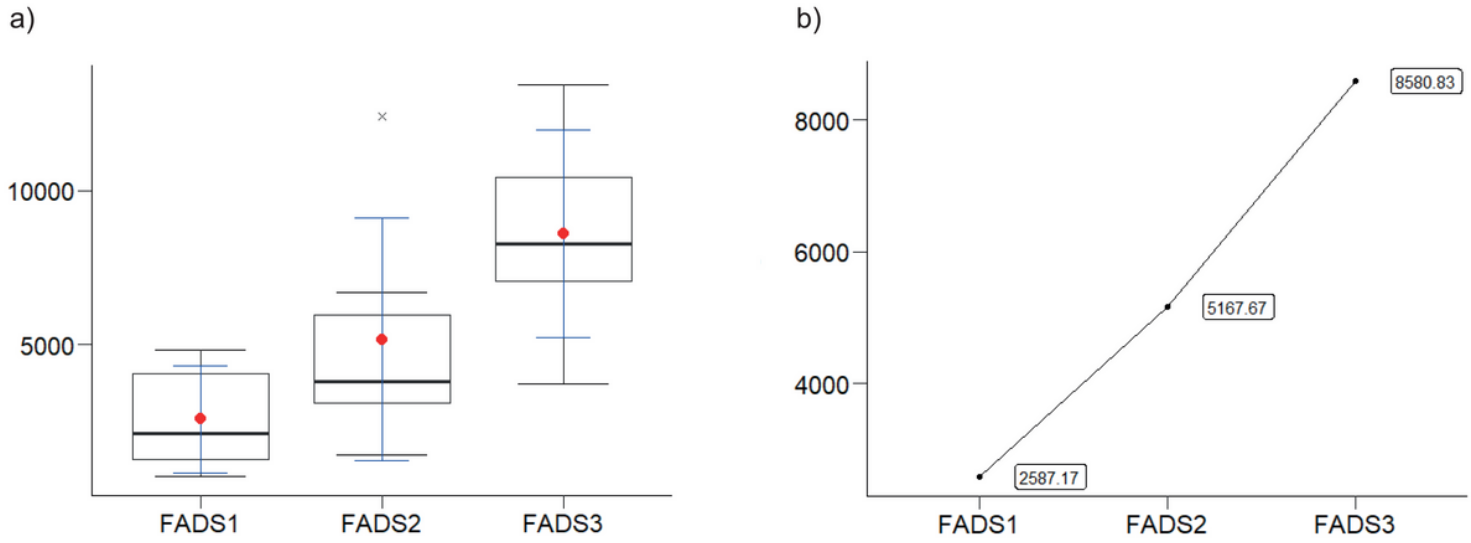

Figure 4

Levels of expression of FADS1, FADS2 and FADS3 genes in SGA group presented as: a). a box plot where the y-axis represents the number of mRNA copies/1µgRNA and b). a line graph showing the average value for the expression of FADS1, FADS2 i FADS3 genes, where the y-axis represents the number of mRNA copies/1µgRNA

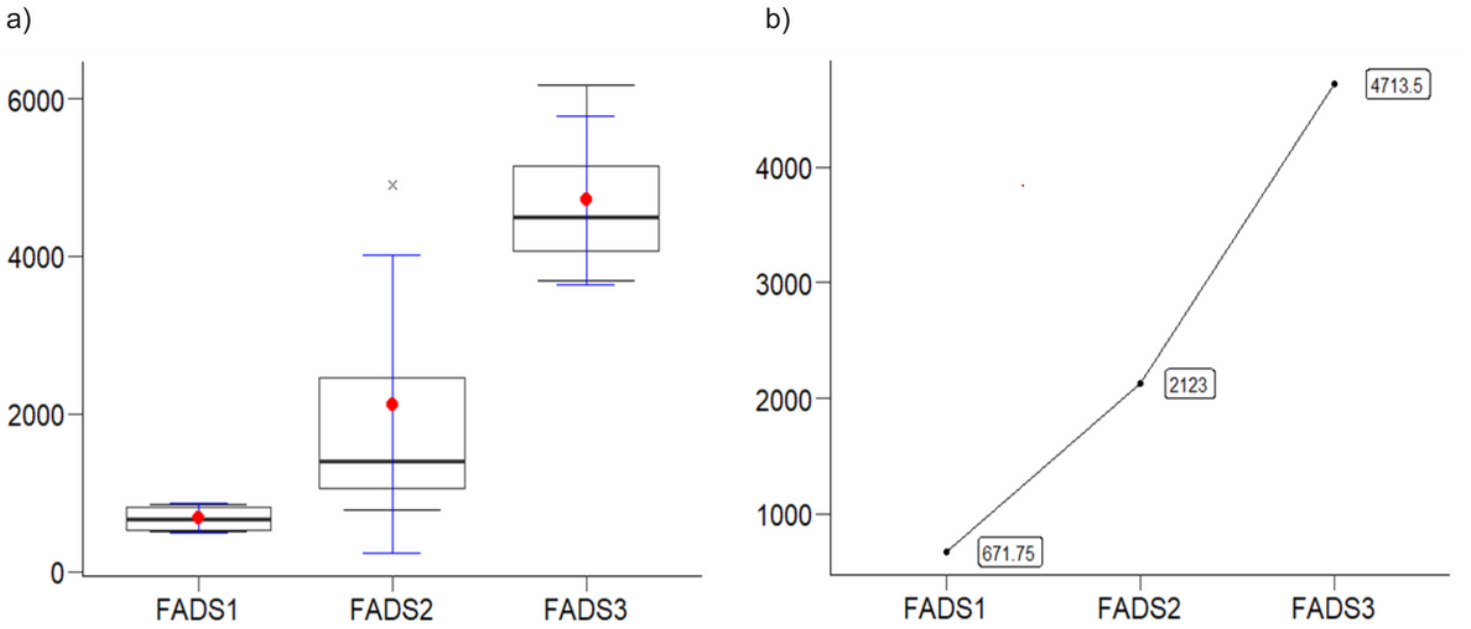

Figure 5

Levels of expression of FADS1, FADS2 and FADS3 genes in IUGR group presented as: a). a box plot where the y-axis represents the number of mRNA copies/1µgRNA and b). a line graph showing the average value for the expression of FADS1, FADS2 i FADS3 genes, where the y-axis represents the number of mRNA copies/1µgRNA