JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2013, 64, 5, 579-585
www.jpp.krakow.pl

R. ICIEK[1,3], E. WENDER-OZEGOWSKA[1,4], A. ZAWIEJSKA[1,4], P. MIKOLAJCZAK[2], P.M. MROZIKIEWICZ[2], M. PIETRYGA[1,3], J. BRAZERT[1,3]

PLACENTAL LEPTIN AND ITS RECEPTOR GENES EXPRESSION IN PREGNANCIES COMPLICATED BY TYPE 1 DIABETES

[1]Department of Obstetrics and Women's Diseases, Poznan University of Medical Sciences, Poznan, Poland; [2]Institute of Natural Fibers and Medicinal Plants, Poznan, Poland; [3]Polish Gynecological Society - Section of Ultrasound (Board), Poznan, Poland; [4]Polish Gynecological Society, Section of Diabetes, Obesity and Other Metabolic Disturbances in Pregnancy, Poznan, Poland

Type 1 diabetes mellitus (T1DM) is still associated with increased risk for severe maternal and fetal complications but their pathomechanism remains unclear. We investigated into possible role of placental leptin (LEP) and its receptor gene (LEPR) in T1DM pregnancies. Fourty nine pregnant women with T1DM and singleton pregnancy were enrolled into the study. Control group consisted of 15 healthy pregnant women in uncomplicated, singleton gestation. We observed higher expression of LEP and LEPR in T1DM placentas in comparison to healthy subjects. We also noticed greater expression of LEP and LEPR in T1DM pregnancies with large for gestational age (LGA) and appropriate for gestational age (AGA) fetuses in comparison to small for gestational age (SGA) diabetic fetuses and controls. We found a significant positive correlation between placental LEP and LEPR expression and neonatal birthweight in overweight T1DM subjects. No such a correlation was found in T1DM subjects with normal weight and controls. We conclude that increased placental LEP and LEPR expression may have a role in stimulating fetal overgrowth in T1DM pregnancy.

Key words: *diabetes, pregnancy, leptin, leptin receptor, placenta, obesity, birthweight*

INTRODUCTION

Leptin, an 16 kDa primarily adipose tissue- derived hormone, encoded by ob gene in humans, has been suggested to act as an endocrine signal of energy balance, mainly by inhibiting hypothalamic neuropeptide Y secretion, thus regulating satiety and energy homeostasis in humans (1-3). It has been shown that leptin acts as a peripheral signal for reproduction and fetal growth in pregnancy complicated by T1DM (4). Moreover, leptin level increases in the course of pregnancy, before any increase in maternal body weight occur, probably due to factors different than adiposity, influencing leptin concentration (5). Leptin concentrations rise until the mid-trimester, then rapidly decrease after delivery, suggesting placental contribution to secretion of this hormone (6). Serum leptinemia correlates with maternal body weight (7). Several studies revealed leptin expression in placenta, stomach, endometrium, liver, spleen, lungs, heart and ovaries (8). Gestational leptin secretion increases under hypoxic conditions, *i.e.* diabetes and preeclampsia (9). Both endo- and exogenous insulin modulates leptin expression in human placenta in diabetic pregnancy (10).

Leptin acts *via* its receptors located in the hypothalamus, placenta, stomach, endometrium, liver, spleen, lungs, heart and ovaries (8). These receptors were also found in placenta, making it not only a source of the hormone but also a possible target tissue for leptin (11). This discovery has made leptin a possible new factor involved in fetal development that still remains unclear and need to be clarified by further studies.

The objective of the study was to investigate the placental leptin gene (LEP) and its receptor (LEPR) expression in pregnancies complicated by T1DM. Furthermore, we analyzed the placental expression of studied genes in T1DM placentas from under-, normal- and overweight fetuses and compared with ones from uncomplicated pregnancies.

MATERIAL AND METHODS

Patients

Our study group consisted of 49 T1DM subjects, hospitalized in tertiary level perinatal care unit in the Department of Obstetrics and Women's Diseases between the 2006 and 2012. All participants gave an informed consent and study protocol obtained approval from the Ethics Committee of Poznan University of Medical Sciences No. 1219/06. We also affirm that original studies have been carried out in accordance with the Declaration of Helsinki.

All women were offered a follow-up including at least three admissions to the Department and regular check-ups in our outpatient clinic, according to Polish Gynecological Society - Section of Diabetes, Obesity and Other Metabolic Disturbances in Pregnancy standards of medical care in management of women with diabetes (12). First visit in the Department included

estimation of the gestational age and number of fetuses on vaginal/abdominal ultrasound, according to the Polish Gynecological Society - Ultrasound Section Guidelines on Ultrasound Screening in Uncomplicated Pregnancy (13). Morover, training on intensive insulin therapy, optimal diet and blood glucose control was introduced. In all subjects, we collected data from general, obstetrical and diabetic history, including age at onset, duration of diabetes and the presence of the vascular complications before pregnancy. During each visit in the Department or in the outpatient clinic (at least every two weeks), we collected data on glycemic profile, and blood pressure. HbA_{1C} concentration was estimated every 6 weeks. Once a trimester, all subjects had a retinal examination done and renal function checked. Subjects with BMI of 25 kg/m^2 or more prior the pregnancy were defined as overweight. Fetuses with estimated weight exceeding 95 percentile were defined as large for gestational age (LGA), under 5 percentile as small for gestational age (SGA).

All participants were treated with human insulins and/or rapid acting analogues following a basal-bolus protocol. We adjusted doses according to glucose levels measured by self-control. Target glucose values were set at 3.34–5.0 mmol/l for fasting glycemia and less than 6.67 mmol/l for 2 hours postprandial glucose level. All biochemical parameters were analyzed in Central Laboratory of the University Hospital.

Experimental procedures

We examined 49 placentas from T1DM subjects and 15 from healthy controls. Placental tissue from 49 T1DM women (age 18–36 years old; at 37–40 weeks of gestation) were compared with placentas from 15 healthy controls (age 23–40 years old; 37–40 weeks of gestation). Samples were obtained immediately after delivery from placenta, cleaned from amniotic membranes and maternal deciduae, rinsed in saline, snap frozen in liquid nitrogen and stored in –80°C until assayed.

RNA was isolated from the samples in Institute of Natural Fibers and Medicinal Plants in Poznan, using TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's protocol. The RNA pellets were washed with 70% ethanol, dissolved in DEPC water and stored at –80°C. RNA concentration and purity was determined by measuring the absorbance at 260 and 280 nm in a spectrophotometer (Eppendorf, USA). cDNA was synthesized from 2 µg of total RNA in a total volume of 20 µl using the SuperScriptTM III First-Strand Synthesis System (Invitrogen, USA) and stored at -20°C or used directly for the real-time quantitative PCR (RT-PCR).

Real time-PCR was performed using a LightCycler TM Instrument (Roche, Germany) and Fast Start DNA Master SYBR Green I kit (Roche, Germany) according to the instructions of the manufacturer. We used Oligo 5.0 software to design the following sequences of the primers for LEP, LEPR and GAPDH: LEP; forward 5'-TTG GCC CTA TCT TTT CTA TG-3' reverse 5'-GCA TAC TGG TGA GGA TCT GT-3', LEPR; forward 5'-CAA GAA TTG TTC CTG GGC ACA-3', reverse 5'-TCA GGC TCC AAA AGA AGA GGA-3', GAPDH; forward: 5'- CAA GTG GGG CGA TGC TGG -3', reverse 5'- GCA GAG GGG GCA GAG ATG A -3'. Real time-PCR was carried out in a total volume of 10 µl using Hot Start Tag DNA polymerase. Reaction mixture for quantification of ADM mRNA contained 1 µl

Table 1. Basic characteristics of study group (mean ± S.D).

Parameter	Overall T1DM subjects N=49	Overweight T1DM subjects N=31[A]	Normal weight T1DM subjects N=18[B]	P[1]
Maternal age (years)	27.5 ± 4.5	26 ± 2,7	26 ± 4	NS
T1DM duration (years)	14 ± 4	14 ± 6	16 ± 4	NS
Maternal age at onset of the T1DM (years)	15 ± 8	16 ± 7	14 ± 4	NS
BMI at booking (kg/m^2)	21 ± 9.1	26 ± 5	22 ± 5	<0.05
BMI at delivery (kg/m^2)	28 ± 7.3	31 ± 5	25 ± 5	<0.05
Mean diurnal glycemia at booking (mmol/l)	5.27 ± 2.05	6.32 ± 0.38	3.71 ± 0.35	<0.05
Mean diurnal glycemia at delivery (mmol/l)	5.16 ± 0.94	6.10 ± 0.35	4.82 ± 0.35	<0.05
HbA_{1C} at booking (%)	7.8 ± 2.3	7.7 ± 2.2	6.7 ± 2.3	<0.05
HbA_{1C} at delivery (%)	6.5 ± 0.9	7.5 ± 2.1	6.4 ± 1.2	<0.05
Total cholesterol at booking (mmol/l)	4.49 ± 1.21	6.05 ± 1.16	4.31 ± 0.74	NS
Total triglycerides at booking (mmol/l)	0.80 ± 0.34	1.77 ± 0.38	1.28 ±0.49	<0.05
HDL at booking (mmol/l)	1.89 ±0.54	1.69 ± 0.31	1.43 ± 0.33	NS
LDL at booking (mmol/l)	2.26 ± 0.75	2.31 ± 0.59	1.74 ± 0.23	NS
GFR at booking (mL/min)	120 ± 63	140 ± 45	100 ± 50	<0.05
GFR at delivery (mL/min)	109 ± 46	100 ± 30	90 ± 40	NS
Gestational age at delivery (weeks)	37 ± 2	38 ± 4	40 ± 1	NS
Neonatal birth weight (g)	3205 ± 890	3780 ± 780	3090 ± 200	<0.05
pH umbilical artery	7.22 ± 0.10	7.33 ± 0.9	7.33 ± 1.3	NS
pH umbilical vein	7.26 ± 0.10	7.23 ± 1.2	7.32 ± 2.3	NS
[1] – t-student test for [A] and [B]				

cDNA, 0.4 µl $MgCl_2$ (final concentration 2 mM), 0.25 µl of each primer (final concentration 0.5 µM), 1 µl SYBR Green master mix and 7.1 µl water. Reaction mixture for amplification of GAPDH gene contained 1 µl cDNA, 0.6 µl $MgCl_2$ (final concentration 2.5 mM), 0.25 µl of each primer (final concentration 0.5 µM), 1 µl SYBR Green master mix and 6.9 µl water. The quantitative PCR we monitored by measuring the increase in fluorescence by the binding of SYBR Green I dye to the generated double-stranded cDNA. Complementary DNA was quantified by comparison of the number of cycles required for amplification of unknown samples with those of series of cDNA standard dilutions. The data was evaluated with the Roche Light Cycler Run 5.32 software.

The amount of transcript was evaluated against a standard curve generated by serial dilutions of complementary DNA (cDNA) obtained after reverse transcription of mRNA. The results were standardized against the reference (housekeeping) glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. We used agarose gel electrophoresis and analysis of a melting curve analysis to confirm amplicon size and reaction specificity.

Statistical analysis

We performed statistical analysis using Statistica 10.0 for Windows. All data are presented as mean ± standard error of mean (S.E.M.). Lillefors and Shapiro-Wilk test checked normality of analyzed parameters. As the distribution of variables met criteria for normal distribution, we compared the leptin gene and its receptor expression using analysis of variance (ANOVA) for more than two groups and post hoc tests: least significant difference (LSD) test and Scheffe test to specify the groups in whom the differences are significant. We used the t-student test for comparisons between two groups. To analyze association between leptin/leptin receptor gene expression and fetal birth weight we used the Pearson rank correlation coefficient. Multiple regression analysis was performed to evaluate maternal lipid concentration influence on neonatal birthweight. Statistical significance was accepted at $p<0.05$ for all comparisons.

RESULTS

Characteristics of the study group is presented in the *Table 1* and *2*. The levels of maternal mean daily glycaemia and percentage of HbA_{1C} both at booking and at delivery were significantly higher in overweight subjects as well as the neonatal birthweight. Overweight T1DM subjects were also characterized by the higher serum leptin at delivery than normal weight subjects. Also maternal serum leptin was higher at delivery in women in whom fetal birthweight was estimated as LGA/AGA as compared to SGA and controls.

In *Table 3*, we present placental LEP expression that was significantly higher in the T1DM compared to the controls, whereas no significant differences in placental LEPR expression were found between these groups.

We also assessed the expression of studied genes in relation to newborns birthweight. Placentas of T1DM LGA fetuses presented the highest LEP expression. T1DM AGA and LGA fetuses were characterized by the highest placental LEPR expression as compared to controls and SGA fetuses (*Table 4*). The below data reflect elevated serum leptin level in T1DM subjects, as serum leptin concentration remains in concordance with placental LEP gene expression.

In the next step, we analyzed the possible correlation between placental LEP and LEPR expression and neonatal birthweight in overweight T1DM subjects with pre-pregnancy BMI ≥ 25kg/m^2. We found significant positive correlation between neonatal birthweight and placental LEP and LEPR expression (Pearson $r=0.81$, $p<0.05$ for LEP and $r=0.57$, $p<0.05$ for LEPR respectively in T1DM subjects). The above data is presented in *Fig. 1* and *2*. No such correlations were found in normal weight T1DM subjects.

Since we discovered that overweight and obesity may affect both LEP/LEPR and neonatal birthweight, in the next step we attempted to analyze the possible maternal biochemical factors related to nutritional status, affecting LEP expression in T1DM placenta in overweight subjects. As we identified possible maternal factors that could potentially mediate the effect of BMI on LEP expression and birthweight, we performed multiple

Table 2. Maternal serum leptin at delivery.

Group/Parameter	Overall T1DM subjects N=49	Overweight T1DM subjects N=31	Normal weight T1DM subjects N=18	Control
Maternal serum leptin at delivery[1]	27.70 ± 12.10	33.20 ± 5.63	24.53 ± 3.41	25.11 ± 2.72
Group/Parameter	T1DM + LGA	T1DM + SGA	T1DM + AGA	Control
Maternal serum leptin at delivery (ng/ml)[2]	45.71 ± 9.84	26.33 ± 3.44	30.15 ± 12.14	25.15 ± 2.76

[1] – ANOVA and post-hoc tests

Overall T1DM *vs*. Control	p=NS (LSD, Scheffe)
Overweight T1DM *vs*. Normal T1DM	p=0.041 (LSD, Scheffe)
Overweight T1DM *vs*. Control	p=0.042 (LSD, Scheffe)

[2] – ANOVA, post-hoc tests

T1DM+LGA *vs*. Control	p=0.034 (LSD, Scheffe)
T1DM+LGA *vs*. T1DM+SGA	p=0.041 (LSD, Scheffe)
T1DM+LGA *vs*. T1DM+AGA	p=0.032 (LSD, Scheffe)
T1DM+SGA *vs*. T1DM+AGA	p=NS (LSD, Scheffe)
T1DM+SGA *vs*. Control	p=NS (LSD, Scheffe)
T1DM+AGA *vs*. Control	p=NS (LSD, Scheffe)

regression analyses where lipid concentration were used as explanatory variables.

In the best-fitted model (R - 0.55 for the model), we found an influence of first trimester total triglycerides (positive correlation, B - 0.62, p-value 0.008), first trimester total cholesterol (negative correlation, B - –2.96, p-value 0.004), first trimester LDL and HDL (positive correlation, B - 1.76, p-value 0.009 and B - 1.34, p-value 0.004). Different models, including first and third trimester metabolic parameters (adjusted to HbA1C level at booking and delivery, mean diurnal glycemia at booking and at delivery, GFR at booking), shown no influence on LEP/LEPR expression. The above data are presented in the *Table 5*.

Table 3. LEP and LEPR expression in studied groups.

Group	Placental LEP		p[1]
	Mean(%)	SEM%	
Control (N=15)	100	6.47	<0.05
T1DM (N=49)	150.10	9.30	
Group	Placental LEPR		p[1]
	Mean(%)	SEM%	
Control (N=15)	100	8.90	NS
T1DM (N=49)	119.01	4.89	
[1] – t-student test			

Table 4. Placental LEP and LEPR expression in studied groups in relation to newborns birthweight.

Group	Placental LEP		P[1]		
	Mean%	SEM%			
Control (N=15)	100	6.47	<0.001	<0.001[2]	
T1DM + AGA (N=20)	146.63	16.12			<0.001[5]
T1DM + LGA (N=13)	173.17	20.04			
T1DM + SGA (N=16)	139.33	13.24			
Group	Placental LEPR		P[1]		
	Mean%	SEM%			
Control (N=15)	100	8.90	<0.001	<0.001[3]	NS[4]
T1DM + AGA (N=20)	124.38	8.18			
T1DM + LGA (N=13)	121.81	9.26			
T1DM + SGA (N=16)	111.51	8.51			

[1] – ANOVA, post-hoc tests
[2] - T1DM+AGA, T1DM+LGA, T1DM+SGA *vs*. Control (LSD, Scheffe)
[3] – T1DM+AGA, T1DM+LGA *vs*. Control (LSD, Scheffe)
[4] – Between T1DM, T1DM *vs*. Control (LSD, Scheffe)
[5] - T1DM+LGA *vs*. T1DM+SGA (LSD, Scheffe)

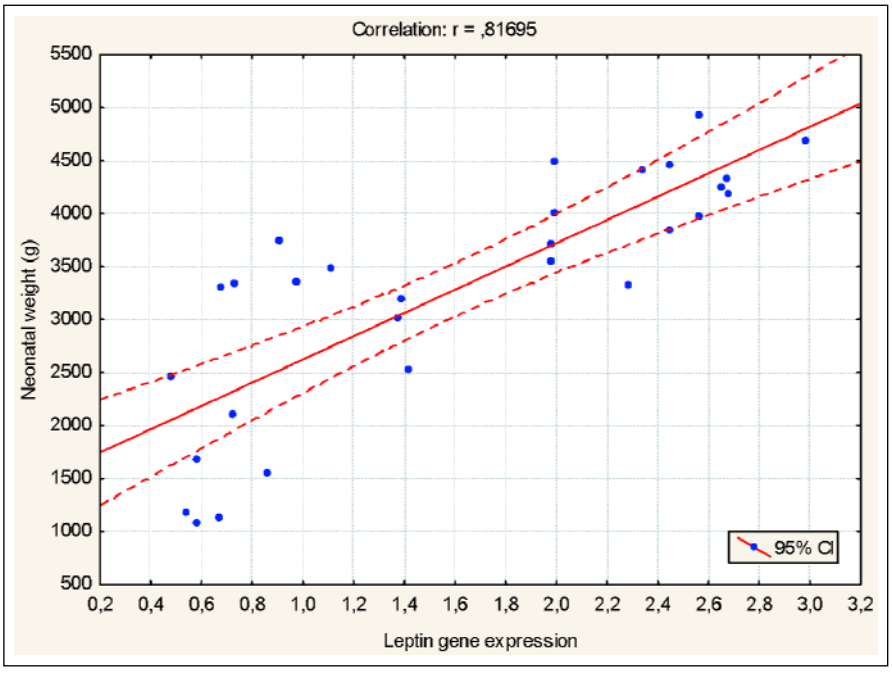

Fig. 1. Placental LEP expression in overweight T1DM subjects in relation to neonatal birthweight (correlation r=0.81, p<0.05).

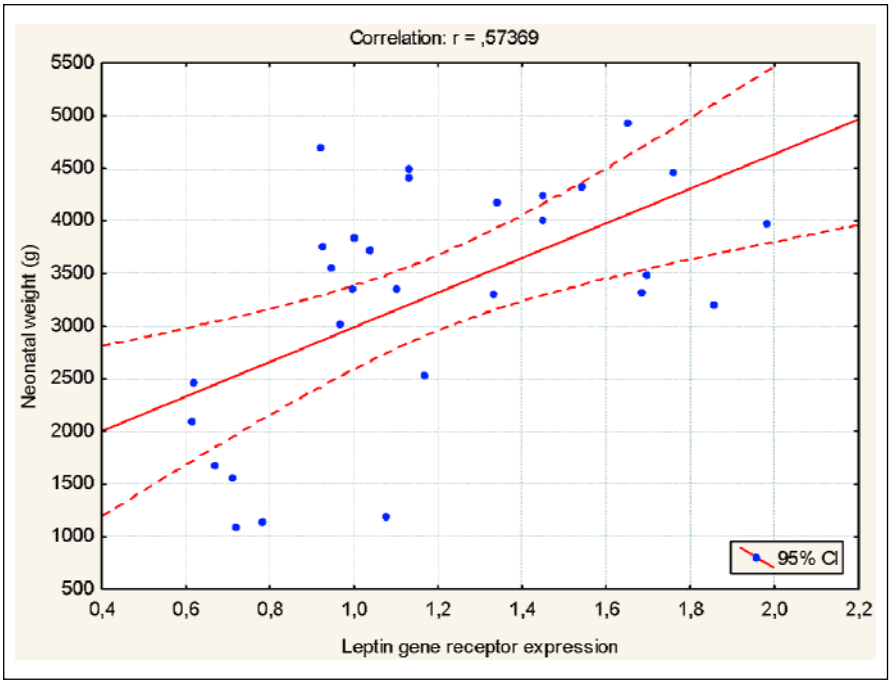

Fig. 2. Placental LEPR expression in overweight T1DM subjects in relation to neonatal weight (correlation r=0.57, p<0.05).

Table 5. Maternal first trimester lipid profile and the LEP expression in T1DM placenta.

Parameter*	R	B	P
Total triglycerides at booking (mg/dl)	0.55577745	0.61631	0.008
Total cholesterol at booking (mg/dl)		–2.95776	0.004
LDL at booking (mg/dl)		1.76571	0.009
HDL at booking (mg/dl)		1.33808	0.004

* - adjusted to maternal BMI.

DISCUSSION

Despite a significant improvement in the management of diabetes, it still constitutes a serious therapeutic problem for women health (14). Diabetes-related complications like microangiopathy or hypertension remains major risk factors for poor perinatal outcome and morbidity (15). Over the last decades we noted tremendous development in diagnostic and therapeutic approaches, but mechanisms underlying the fetal growth and development still needs further studies. In diabetic pregnancy, several factors influencing fetal birthweight have been discovered - glycemic control and lipid profile, maternal nutritional status, weight gain, energy intake and the presence of concomitant disorders influencing placental function like hypertension or preeclampsia (31-33).

In the last decade, much data emerged on leptin in pregnant women population. Many studies generated a common observation that maternal leptinaemia increases in the majority of pregnancy complications, like diabetes, hypertension or preeclampsia (16, 17). There is of note, that all these complications impair placental function. They are also associated with increased maternal insulin resistance.

Confusing data is available on mechanism underlying regulation of placental leptin synthesis and gene expression. In this paper, we attempted to analyze the possible link between several feto-maternal factors affecting placental LEP and LEPR expression - type 1 diabetes, maternal overweight, fetal growth and maternal metabolic disturbances.

Exogenous insulin administered to our patients should also be considered as a possible factor affecting the augmented level of placental LEP expression in diabetic population. This was previously reported by Lepercq, who found 3-5-fold higher placental level of LEP correlating with cord serum leptin (18). In our study, both placental LEP/LEPR expression and serum leptin was higher in T1DM group as compared to controls but also inside T1DM group these differences were significant and dependent of feto-maternal features. This can be considered as an indirect proof for feto-placental leptin synthesis and its contribution to fetal development. Later studies demonstrated higher cord hyperleptinemia in LGA fetuses and lower leptinemia in SGA fetuses. Moreover, significant discrepancies between placental and cord leptin in diabetic pregnancies were also found (19, 20). Latest study of Misra *et al.* demonstrated that maternal overweight and obesity influences fetal growth (21).

We found increased placental LEPR expression in diabetic placentas as compared to healthy subjects, but the results did not reach statistical significance. We observed the disproportion between LEP and LEPR expression in diabetic subjects what we have not seen in healthy women. Uzelac *et al.* (22), in terms of insulin, leptin and androgen receptors in diabetic placenta, previously reported this specific dysregulation - receptor "down-regulation" as the response for increased ligand concentration. In our study, elevated serum leptin in diabetic subjects support this observation.

We also analyzed the possible link between placental LEP/LEPR expression and fetal birthweight. Manderson *et al.*

documented that newborns of both type 1 and gestational diabetic mothers have an increased adiposity, what can influence their birthweight and is correlated with increased cord leptin (23). This led to indirect conclusion that leptin controls fetal growth, although factors which affect adipose tissue in the fetus remain unclear and further investigations into feto-maternal leptinemia are necessary (23-25). In our study, we observed higher LEP expression in placentas of T1DM AGA and LGA fetuses as compared to SGA and controls. Furthermore, increased serum leptin was found in subjects of these groups, even though the SGA diabetic fetuses were characterized by higher LEP expression than controls. This can be the evidence that diabetes per se affects placental LEP expression. Moreover, we also found dysregulation of LEPR, mainly in placentas of T1DM LGA fetuses, what supports Uzelac's hypothesis on "down-regulation" of receptors in response to increased serum leptin (22). It remains unclear, whether lower placental LEPR expression constitutes a compensatory mechanism protecting fetus from macrosomia (26).

LGA infants are characterized by 3-12-fold higher cord leptin concentration than normal and AGA fetuses, therefore leptin was stated as fat-mass content marker in those fetuses. There is also a correlation between cord blood leptin and birthweight in LGA and IUGR fetuses, but no such correlation was found in AGA infants (27, 28).

Interestingly, we observed that overweight diabetic subjects had significantly poorer metabolic control expressed as HbA_{1C} value and mean daily glycemia, both at booking and at delivery. Moreover, overweight diabetic women delivered bigger infants than normal weight subjects. We documented that in overweight diabetic subjects, there was a strong linear correlation between neonatal birthweight and placental LEP expression, what was also documented by elevated serum leptin in this group. In detail, in diabetic placentas of fetuses that exceeded 4000 g, the highest LEP expression was observed. The same linear, yet weaker correlation was found in terms of placental LEPR expression. Studies by Yamashita *et al.* (29), demonstrated that augmentation of placental leptin may have a contribution to fetal growth, independently of maternal glucose control. These results were not confirmed in our study, since obese women with poor metabolic control who delivered bigger infants, were characterized by higher LEP/LEPR expression. It was also widely reported that pregnancy remains leptin-resistance state, despite gestational increase in serum leptin (30).

Since our overweight diabetic women were characterized by the higher lipid concentration in the first trimester (significant only for first trimester triglycerides) and linear correlation was found between LEP/LEPR and birth weight, we proceeded by carrying out a multiple regression model analysis, which revealed that first-trimester triglycerides, LDL, HDL serum concentrations were positively correlated with LEP placental expression. What is interesting, no such relationship was observed in terms of total first trimester cholesterol, which was inversely (B - 2.95) associated with LEP placental expression. What is also interesting, these correlations were independent of maternal first trimester glycemia and BMI. Stepwise multiple regression revealed no changes in B-parameter, while adding these variables to our model. No such relationship was found in terms of LEPR. There is one study by White *et al.* (34), demonstrating that in streptozotocin-induced diabetic rats, augmentation of leptin causes the increase in the level of lipids in the first trimester of pregnancy. The same results were reported by Nelson *et al.*, who found positive association with HDL cholesterol and positive with triglycerides (35). We conclude, that positive correlation between placental LEP expression and first trimester lipid concentration (HDL, LDL and triglycerides) could be the reflection of the response for increased demand for developing fetus. One aspect, which is difficult to explain, is the negative correlation of the maternal first trimester total cholesterol with placental LEP expression. When removing this variable from multiple regression model, it still demonstrates similar, but weaker correlation. On the other hand, the same model in the overall diabetic group, revealed weak positive correlation of all regressors with placental LEP expression (B - 0.63). We conclude, that overweight/obese diabetic group should be increased at least to equal number as in overall group, and re-analyzed.

Leptin is a well-known growth factor for placenta, since the trophic effect of leptin stimulating protein synthesis may be an important factor to support the fetal macrosomia. Leptin stimulates protein synthesis-activating translation in human trophoblastic cells and placenta, working as an autocrine hormone, angiogenesis, growth and immunomodulation mediator. These effects are supported by activation of MAPK and PIK3 pathways promoting the phosphorylation of different proteins *i.e.* RPS6KB1, EIF4E and EIF4EBP1 (36). It was found that MAPK and PI3K activities are required for leptin stimulation of protein synthesis in human trophoblastic cells, since inhibition of both by transfection negative constructs prevented this effect (37).

Recently, it was found that leptin stimulation promotes the over-expression and tyrosine phosphorylation of Sam68 (Src associated in mitosis, 68 kDa) in human trophoblastic JEG-3 cells, suggesting a role for Sam68 in leptin signaling and action in these cells, since down-regulation of Sam68 decreased leptin-stimulated protein synthesis in these cells (38).

Apart from maternal metabolic parameters assessed in the study, there are several pregnancy molecules and hormones, commonly increased in the course of pregnancy, involved in leptin up-regualtion in the placenta. Human HCG and cyclic cAMP enhance leptin by MAPK signaling pathway activation, wheras 17β-estradiol acts *via* MAPK and PI3K signal transduction pathways (39).

To conclude, our data show that feto-maternal factors may influence LEP/LEPR placental expression in T1DM pregnancy, particularly in T1DM + LGA placentas, and these effects are mediated by leptin-stimulated protein synthesis.

Acknowledgements: The study was supported by the scientific grant from Ministry of Science awarded to Dr. Rafal Iciek. The authors are indebted to Mr. Piotr Iciek MA for his language assistance.

Conflict of interests: None declared.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-432.
2. Masuzaki H, Ogawa Y, Sagawa N. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature Med* 1997; 3: 1029-1033.
3. Hauguel-de Mouzon S, Lepercq J, Catalano P. Leptin in the diabetic pregnancy. *Front Diabetes* 2005; 17: 46-57.
4. Iciek R, Wender-Ozegowska E, Seremak-Mrozikiewicz A, *et al.* Leptin gene, leptin gene polymorphisms and body weight in pregnant women with diabetes mellitus type I. *J Physiol Pharmacol* 2008; 59: 19-31.
5. Henson MC, Castracane VD. Leptin in pregnancy: an update. *Biol Reprod* 2006; 74: 218-229.
6. Manderson J, Patterson C, Hadden D, Traub AI, Leslie H, McCance DR. Leptin concentrations in maternal serum and

cord blood in diabetic and nondiabetic pregnancy. *Am J Obstet Gynecol* 2003; 188: 1326-1332.
7. McMillen IC, Edwards LJ, Duffield, Muhlhausler BS. Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity. *Reproduction* 2006; 131: 415-427.
8. Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; 83: 1263-1271.
9. Poston L. Leptin and preeclampsia. *Semin Reprod Med* 2002; 20: 131-138.
10. Kolaczynski JW, Nyce MR, Considine RV, *et al.* Acute and chronic effects of insulin on leptin production in humans: studies in vivo and in vitro. *Diabetes* 1996; 45: 699-701.
11. Henson MC, Swan KF, O'Neil JS. Expression of placental leptin and leptin receptor transcripts in early pregnancy and at term. *Nature Med* 1998; 3: 1029-1033.
12. Wender-Ozegowska E, Bomba-Opon D, Brazert J, *et al.* Rekomendacje Sekcji Cukrzycy, Otylosci i Innych Zaburzen Metabolicznych w Ciazy. Standardy Polskiego Towarzystwa Ginekologicznego postepowania u kobiet z cukrzyca. *Ginek Pol* 2011; 82: 474-479.
13. Pietryga M, Brazert J, Iciek R, *et al.* Rekomendacje Sekcji Ultrasonografii Polskiego Towarzystwa Ginekologicznego w zakresie przesiewowej diagnostyki ultrasonograficznej w ciazy o przebiegu prawidlowym. *Ginek Pol* 2012; 83: 309-315.
14. Langer O, Conway DL. Level of glycemia and perinatal outcome in pregestational diabetes. *J Matern Fetal Med* 2000; 9: 35-41.
15. Ekbom P, Damm P, Feldt-Rasmussen B, Feldt- Rasmussen U, Molvig J, Mathiesen ER. Pregnancy outcome in type 1 diabetic women with microalbuminuria. *Diabetes Care* 2001; 24: 1739-1744.
16. Poston L. Leptin and preeclampsia. *Semin Reprod Med* 2002; 20: 131-138.
17. Stock SM, Bremme KA. Elevation of plasma leptin levels during pregnancy in normal and diabetic women. *Metabolism* 1998; 47: 840-843.
18. Lepercq J, Cauzac M, Lahlou N, *et al.* Overexpression of placental leptin in diabetic pregnancy: a critical role of insulin. *Diabetes* 1998; 47: 847-850.
19. Geary M, Pringle PJ, Persaud M, *et al.* Leptin concentrations in maternal serum and cord blood: relationship to maternal anthropometry and fetal growth. *Br J Obstet Gynaecol* 1999; 106: 1054-1060.
20. Grisaru-Granovsky S, Eitan R, Algur N, Schimmel MS, Diamant YZ, Samueloff A. Maternal and umbilical cord serum leptin concentrations in small-for-gestational-age and in appropriate-for-gestational-age neonates: a maternal, fetal, or placental contribution? *Biol Neonate* 2003; 84: 67-72.
21. Misra VK, Straughen JK, Trudeau S. Maternal serum leptin during pregnancy and infant birth weight: the influence of maternal overweight and obesity. *Obesity* (Silver Spring) 2013; 21: 1064-1069.
22. Uzelac PS, Li X, Lin J, *et al.* Dysregulation of leptin and testosterone production and their receptor expression in the human placenta with gestational diabetes mellitus. *Placenta* 2010; 24: 1223-1229.
23. Harigaya A, Nagashima K, Nako Y, Morikawa A. Relationship between concentration of serum leptin and fetal growth. *J Clin Endocrinol Metab* 1997; 82: 3281-3284.
24. Koistinen HA, Koivisto VA, Andersson S, *et al.* Leptin concentration in cord blood correlates with intrauterine growth. *J Clin Endocrinol Metab* 1997; 82: 3328-3330.
25. Fairbrother UL, Tanko LB, Walley AJ, *et al.* Leptin receptor genotype at Gln223Arg is associated with body composition, BMD, and vertebral fracture in postmenopausal Danish women. *J Bone Miner Res* 2007; 22: 544-550.
26. Lea RG. Placental leptin in normal, diabetic and growth retarded pregnancies. *Mol Human Reprod* 2000; 6: 763-769.
27. Bajoria R, Sooranna SR, Ward BS, Chatterjee R. Prospective function of placental leptin at maternal-fetal interface. *Placenta* 2002; 23: 103-115.
28. Yamashita H, Shao J, Ishizuka T, *et al.* Leptin administration prevents spontaneous gestational diabetes in heterozygous Lepr(db/+) mice: effects on placental leptin and fetal growth. *Endocrinology* 2001; 142: 2888-2897.
29. Mounzih K, Qiu J, Ewart-Toland A, Chehab FF. Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistance state *Endocrinology* 1998; 139: 5259-5262.
30. Nomura RM, Paiva LV, Costa VN, Liao AW, Zugaib M. Influence of maternal nutritional status, weight gain and energy intake on fetal growth in high-risk pregnancies. *Rev Bras Ginecol Obstet* 2012; 34: 107-112.
31. Catalano PM, Thomas AJ, Huston LP, Fung CM. Effect of maternal metabolism on fetal growth and body composition. *Diabetes Care* 1998; 21: 85-90.
32. Wender-Ozegowska E, Zawiejska A, Michalowska-Wender G, Iciek R, Wender M, Brazert J. Metabolic syndrome in type 1 diabetes mellitus. Does it have any impact on the course of pregnancy? *J Physiol Pharmacol* 2011; 62: 567-573. Erratum in: *J Physiol Pharmacol* 2012; 63: 205.
33. White V, Gonzalez E, Capobianco E, *et al.* Modulatory effect of leptin on nitric oxide production and lipid metabolism in term placental tissues from control and streptozotocin-induced diabetic rats. *Reprod Fertil Dev* 2004; 16: 363-372.
34. Nelson SM, Freeman DJ, Sattar N, Johnstone FD, Lindsay RS. IGF-1 and leptin associate with fetal HDL cholesterol at birth: examination in offspring of mothers with type 1 diabetes. *Diabetes* 2007; 56: 2705-2709.
35. Perez-Perez A, Maymo J, Gambino Y, *et al.* Leptin stimulates protein synthesis-activating translation machinery in human trophoblastic cells. *Biol Reprod*. 2009; 81: 826-832.
36. Perez-Perez A, Gambino Y, Maymo J, *et al.* MAPK and PI3K activities are required for leptin stimulation of protein synthesis in human trophoblastic cells. *Biochem Biophys Res Commun* 2010; 396: 956-960.
37. Sanchez-Jimenez F, Perez-Perez A, Gonzalez-Yanes C, Varone CL, Sanchez-Margalet V. Sam68 mediates leptin-stimulated growth by modulating leptin receptor signaling in human trophoblastic JEG-3 cells. *Hum Reprod* 2011; 26: 2306-2315.
38. Maymo JL, Perez AP, Gambino Y, Calvo JC, Sanchez-Margalet V, Varone CL. Leptin gene expression in the placenta-regulation of a key hormone in trophoblast proliferation and survival. *Placenta* 2011; 32(Suppl. 2): 146-153.

Received: July 24, 2013
Accepted: October 10, 2013

Author's address: Dr. Rafal Iciek, Department of Obstetrics and Women's Diseases, Polish Gynecological Society - Section of Ultrasound, Poznan University of Medical Sciences, 33 Polna Street, Poznan, Poland.
E-mail: ralfiho@o2.pl