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The impact of gestational diabetes on changes in the concentration of selected hormones regulating food intake in umbilical cord blood, and the development of obesity in children in later life

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Abstract

Introduction and objective. Gestational diabetes mellitus (GDM) is a metabolic disease affecting an increasing number of women. The disease not only affects the health of mothers, but may also contribute to metabolic problems in children, both at the stage of foetal life and in the future, including increasing the risk of obesity or type 2 diabetes. Therefore, it is important to conduct research to get closer to fully defining the problem and developing therapies or methods of preventing GDM.

Materials and method. The study used umbilical cord blood from 540 women (260- healthy and 280 – GDM) and commercially available ELISA and RIA tests to investigate the interaction between leptin, ghrelin (active and total) and insulin in cord blood, and its correlation with anthropometric parameters of newborns as well as pregnancy week on the day of delivery, weight, BMI and growth of mothers.

Results. It was found that in healthy mothers there were statistically significant correlations between maternal pre-pregnancy weight in relation to leptin (positive) and glucose (negative), a positive correlation between active ghrelin concentration and gestational week at delivery, and a positive level of leptin to the chest circumference of newborns, and a negative of glucose levels to the chest circumference of newborns at birth in the healthy group. In the GDM group, a positive correlation was found of total ghrelin to the abdominal circumference of the newborns at birth, and a positive of leptin concentration and thigh circumference of the newborns

Conclusions. It was concluded that altered hormonal profiles during the prenatal period may have long-term consequences for the health of children. The study also indicated the need for further research in this area to better understand the causes and consequences of GDM r.

Key words

insulin, umbilical cord blood, leptin, ghrelin, gestational diabetes mellitus (GDM)

INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition in which there is glucose intolerance, leading to elevated blood glucose levels in pregnant women. The influence of gestational diabetes on the health of the baby can be multifaceted [1, 2]. One possible factor having a potential negative impact are changes in the concentration of hormones (including the hormones regulating appetite) that accompany this pathology. Interestingly, some hormones involve both the mother's peripheral blood and changes in umbilical cord blood [3–5]. However, the relationship between changes in the concentration in mother and child is still under investigation.

The main hormones responsible for regulating the appetite process include leptin and ghrelin [6, 7]. Leptin, produced by adipose tissue, influences the feeling of satiety, while ghrelin,

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secreted mainly by the stomach, increases the feeling of hunger. The discovery in 1994 of adipocyte-derived leptin led to a new look at adipose tissue in the context of its role not only as a passive energy store, but one of the most significant endocrine tissues which may regulate many processes in the organism, including the inhibiton of food intake [8, 9]. Ghrelin, discovered 5 years later, which is not a hormone derived from adipose tissue, and the main source of which is the gastrointestinal pathway (mainly the stomach); unlike leptin, ghrelin is considered one of the strongest peripheral stimulators of food intake [10]. Both these hormones, as potent regulators of food intake, seem to be particularly important for the regulation of metabolism at foetal and infant age. Moreover, for many years the literature has indicated that there are potential relationships between these hormones and their role in regulating food intake and energy management [11, 12]. Previous studies suggest that children born to mothers with gestational diabetes may be at greater risk of developing obesity in later life. One possible mechanism responsible for this effect is thought to be an altered hormonal profile in umbilical cord blood affecting the metabolism and regulation of appetite in children (including contributing to increased appetite and satiety disorders), which may lead to excessive food intake and obesity. Because of this fact, it is crucial to understand the impact of gestational diabetes on changes in the concentration of hormones responsible for regulating food intake and the potential relationships between them.

Abnormal regulation of the synthesis or secretion of these hormones can contribute to metabolic disorders, such as obesity and its subsequent associated conditions.

OBJECTIVE

The aim of the study was to evaluate the influence of selected hormones responsible for the regulation of appetite in the cord blood of newborns of mothers with gestational diabetes on the possible development of obesity in later life, and to establish the potential relationship between the concentration of selected hormones (ghrelin and leptin) with anthropometric parameters, the weight gain of children from birth to 36 months of age, as well as maternal parameters (pre-pregnancy weight, gestational weight gain and Hbd).

MATERIALS AND METHOD

Ethics. The study was conducted on Caucasian women who were patients at the Gynaecologic and Obstetrical University Hospital in Poznań, west-central Poland, due to planned delivery. The research was conducted according to the principles of the Declaration of Helsinki. The protocol was approved by the Clinical Research Ethics Committee of Poznań University of Medical Sciences (Approval No. 997/18). The material for the study consisted of 2 groups of women, one group with normal weight (BMI 18.5–24.9 kg/m2), and the other of women who were obese according to WHO standards (BMI value of ≥30 kg/m2). All pregnancies were carried to term and delivered naturally. Vaginal-assisted delivery and caesarean section were excluded from the study.

Characteristics of the study groups. A total of 540 women (mothers) and 540 of their children participated in the study. On the basis of the physical examination performed in the emergency room and the results of the additional examinations, the pregnant women were divided into 2 groups – healthy, and with diabetes (GDM); 260 were found to have no disease, and 280 were found to have gestational diabetes during pregnancy. Gestational diabetes was diagnosed in patients based on the WHO criteria from 2013 [13], which were implemented into the Polish Treatment Standards [14, 15]. The main criterion for admission to the study was the diagnosis, treatment and control of gestational diabetes. Other inclusion and exclusion criteria were established for each group (Tab. 1).

The deliveries took place at the Gynaecological-Obstetrical Clinical Hospital of the Karol Marcinkowski Medical University in Poznań. Cord blood samples were collected during the deliveries. An interview with the women was conducted with the participation of midwives during the women's stay in the delivery room. On the basis of a questionnaire, information on the mother's health was obtained, while after the birth, data on basic anthropometric parameters of the newborn were obtained.

Collection of blood samples. Umbilical cord blood collected at birth was properly preserved for storage. The blood was centrifuged for 15 minutes at 3,500 rpm at 4 °C to separate the serum. The serum was then transferred to new eppendorf tubes and aliquoted. Samples were stored at -80 °C until assays were performed.

Table 1. Inclusion and exclusion criteria in the research group

HEALTHY	
Inclusion criteria	**exclusion criteria**
healthy pregnant women	co-morbidities during pregnancy
normal course of pregnancy (information obtained from medical records)	poor health of the newborn
delivery between 38th – 42nd week of pregnancy	delivery earlier or later than between 38th – 42nd weeks of pregnancy
voluntary agreement to participate in the study as evidenced by written consent	lack of consent to participate in research
GDM	
Inclusion criteria	**exclusion criteria**
established gestational diabetes	no known gestational diabetes
normal course of pregnancy apart from the finding of gestational diabetes (information obtained from medical records)	co-morbidities other than gestational diabetes
delivery between 38th – 42nd week of pregnancy	delivery earlier or later than between 38th – 42nd weeks of pregnancy
voluntary agreement to participate in the study as evidenced by written consent	poor health of newborn and lack of consent to participate in research

Anthropometric measurements of children. Iimmediately after birth, the newborns were measured and weighed in the delivery room by expert medical staff, according to current standards, The following measurements were taken: weight and length, circumference of the head, chest, abdomen, thigh and arm.

RIA and ELISA assays. Immunoenzymatic (ELISA) and radioimmunoenzymatic (RIA) assays were used to determine changes in the concentration of selected hormones, ELISA for Insulin and leptin and RIA for ghrelin. Determinations were performed according to the test manufacturer's instructions.

A DRG Insulin ELISA Kit (Cat. No. EIA-2935, DRG Instrumrnts GmbH, Germany) was used for the assays, with reference values of 2–25 μIU/ml, for an assay sensitivity of 1.76 μIU/ml. A DRG Leptin (Sandwich) ELISA Kit (Cat. No. EIA-2395, DRG Instruments GmbH, Germany) was used to determine leptin concentrations, with reference values for females of 7.36±3.73 ng/ml and males of 3.84±1.79 ng/ml and an assay sensitivity of 1 ng/ml. Using RIA assays, changes in the concentration of 2 forms of ghrelin were determined – both the active form and total ghrelin.

A radioimmunoassay method using the Ghrelin (Active) Radioimmunoassay (RIA) Kit (Cat. No. GHRA-88HK, LINCO Research, St. Charles MO, USA) was used to determine active ghrelin concentrations in patients' blood serum; for assay sensitivity = 7.8 pg/ml. Total plasma ghrelin concentration was determined by radioimmunoassay using the Ghrelin (Total) Radioimmunoassay (RIA) Kit (Cat. No. GHRT-89HK, Millipore, –? – USA); for assay sensitivity = 93 pg/ml.

The colour intensity of the sample from Elisa assays was measured using a Synergy 2 microplate reader (Biotek, –? – USA) on a wavelength 450 nm. The intensity of gamma radiation from samples during the RIA test was measured via the Wizard2 5-Detector Gamma Counter (Perkin Elmer, –? – USA)

Glucose serum level. The concentration of glucose in serum was measured using a commercially available colorimetric assay, Glucose Oxidase Reagent Set (Cat. No. G7521–100, Pointe Scientific, –? – USA). The colour intensity of the sample was measured on a wavelength 500 nm as described above.

Development of children up to 36 months of age. The development of children up to the age of 36 months was documented through the responses given by mothers via a telephone survey. Due to the absence of many measurements through the lack of their entry in the child's health booklet during follow-up visits to the paediatrician, critical moments were chosen for the study of weight gain: 6th, 12th, 24th and 36th months of a child's life. The results presented in this study are part of a research project carried out by the Preventive Research Institute of the Kalisz Academy and the Department of Maternal and Child Health of the Karol Marcinkowski Medical University in Poznań.

Statistical analysis. Statistical analysis were performed using Statistica 10 StatSoft software; the Shapiro-Wilk test was used to check the normality of the distribution; the Student's t-test or Mann-Whitney U test was used for the calculations of statistical differences between groups ($p<0.05$ and $p<0.01$). To assess the degree of rank correlation, Spearman's rank coefficient was used with a statistical significance level of $p < 0.05$.

RESULTS

The group of women with gestational diabetes before pregnancy had a slightly higher mean body weight (65.71±16.57), compared to the mean body weight of women in the Healthy Group (62.62±9.11). In contrast, they had a marginally lower mean body weight on the day of delivery (77.54±16.78), compared to the Healthy Group (78.29±9.21). The mean weight gain of women in the Healthy Group was significantly higher (15.68±6.14) than in the GDM group (11.82±3.41). The mean BMI values of women before pregnancy in the 2 groups did not differ. The anthropometric parameters of the children in both groups differed at each stage. From weight to circumference measurements, children in the Healthy Group were significantly larger compared to children of mothers with GDM (Tab. 7 and 8).

Hormone concentrations vs. selected maternal parameters. Statistically significant correlations were found between maternal pre-pregnancy weight in relation to leptin and glucose concentrations in a group of healthy mothers (Tab. 9).

The correlation of leptin (p=0.027) to maternal pre-pregnancy weight was positive, i.e. the higher the maternal pre-pregnancy weight, the higher the leptin concentration in the cord blood of the child. The correlation of glucose (p=0.017) to maternal weight before pregnancy was negative, meaning that the higher the maternal weight before pregnancy, the lower the cord blood glucose concentration of the newborn. For the group of women with gestational diabetes, no statistically significant correlations were observed.

In both the group of healthy women and the group of women with gestational diabetes, there was no statistically significant correlation of women's weight gain during pregnancy to hormones in the umbilical cord blood of the newborns (Tab. 10).

A positive statistically significant correlation was observed for active ghrelin concentration (p=0.025) and gestational week at delivery in the group of healthy women. No statistically significant correlation was observed in the group of women with gestational diabetes (Tab. 6).

Comparison of anthropometric parameters of infants with hormones in cord blood. No statistically significant correlations were observed between neonatal birth weight and cord blood hormone concentrations in either the group of women with gestational diabetes or the healthy group (Tab. 12).

Neither group showed statistically significant correlations between head circumference (Tab. 13) and arm circumference (Tab. 16) and hormone concentrations in the umbilical cord blood of newborns.

There was a positive correlation of leptin levels (p=0.027) at a statistically significant level to the chest circumference of newborns in the healthy group. The same group also showed a statistically significant (p=0.017) negative correlation of glucose levels to the chest circumference of newborns at birth. In the group of women with gestational diabetes, no statistically significant correlation was shown (Tab. 9).

Table 2. Descriptive statistics of the healthy group [n=260]

Healthy group – mothers	**Mean±SD**	**Median**	**Min.**	**Max.**
Pre-pregnancy weight [kg]	62.62±9.11	62.00	46.00	81.00
Weight on day of delivery [kg]	78.29±9.21	78.00	60.00	92.00
Current body weight [kg]	65.55±10.57	64.00	48.00	81.00
Growth [m]	1.66±0.04	1.65	1.62	1.74
Pregnancy weight gain [kg]	15.68±6.14	16.00	3.00	27.00
BMI before pregnancy [kg/m^2]	22.72±3.08	23.05	17.53	28.36
Pregnancy week on the day of delivery	39.54±1.8	40	38	41
Healthy group – children	**Mean±SD**	**Median**	**Min.**	**Max.**
Birth weight [g]	4027.50±604.56	4160.00	2340.00	4540.00
Head circumference [cm]	35.75±1.36	36.00	33.00	38.00
Chest circumference [cm]	36.08±1.78	36.00	33.00	40.00
Abdominal circumference [cm]	35.25±2.60	34.50	32.00	41.00
Thigh circumference [cm]	17.04±1.21	17.00	14.00	19.00
Arm circumference [cm]	13.04±1.10	13.00	12.00	15.00
Body weight at 6 months of age [g]	8820.00±607.00	8900.00	8000.00	9800.00
Body weight at 12 months of age [g]	12060.00±1132.55	12300.00	10300.00	13200.00
Body weight at 24 months of age [g]	14570.00±1358.96	14250.00	13000.00	17300.00
Body weight at 36 m.p.h. [g]	16745.00±2269.91	16150.00	14000.00	21000.00
Hormone concentrations in cord blood	**Mean±SD**	**Median**	**Min.**	**Max.**
Ghrelin Active [pg/ml]	38.78±13.78	33.51	18.84	63.97
Ghrelin total [pg/ml]	936.82±558.48	798.17	285.07	2485.35
Insulin [uIU/ml]	9.07±5.02	10.48	1.86	16.6
Leptin [ng/ml]	18.63±9.10	21.00	1.87	35.5
Glucose [mg/dl]	64.55±20.26	59.70	36.1	103

Table 3. Descriptive statistics GDM group [n=280]

Healthy group – mothers	**Mean±SD**	**Median**	**Min.**	**Max.**
Pre-pregnancy weight [kg]	65.71±16.57	61.25	46.00	112.00
Weight on day of delivery [kg]	77.54±16.78	72.25	54.00	120.00
Current body weight [kg]	63.64±10.64	62.00	48.00	87.00
Growth [m]	1.65±0.04	1.64	1.58	1.75
Pregnancy weight gain [kg]	11.82±3.41	11.25	8.00	20.00
BMI before pregnancy [kg/m^2]	22.75±3.33	21.94	17.47	28.63
Pregnancy week on the day of delivery	39.43±0.94	40.00	38.00	41.00
Healthy group –children	**Mean±SD**	**Median**	**Min.**	**Max.**
Birth weight [g]	3495.00±431.75	3395.00	3020.00	4450.00
Head circumference [cm]	34.36±1.50	34.50	32.00	37.00
Chest circumference [cm]	34.64±1.95	34.50	32.00	39.00
Abdominal circumference [cm]	31.50±8.39	33.00	3.00	37.00
Thigh circumference [cm]	16.00±1.18	16.00	15.00	19.00
Arm circumference [cm]	12.04±1.34	12.00	10.00	15.00
Body weight at 6 months of age [g]	7461.82±1001.82	7300.00	6000.00	9600.00
Body weight at 12 months of age [g]	17481.82±24736.69	10200.00	8500.00	92000.00
Body weight at 24 months of age [g]	12527.27±882.15	12500.00	11500.00	14800.00
Body weight at 36 m.p.h. [g]	14536.36±874.38	14800.00	13000.00	16000.00
Hormone concentrations in cord blood	**Mean±SD**	**Median**	**Min.**	**Max.**
Ghrelin Active [pg/ml]	42.77±35.48	34.84	17.34	162.34
Ghrelin total [pg/ml]	1002.86±612.03	800.84	301.74	2671.80
Insulin [uIU/ml]	11.26±6.87	10.26	3.41	23.29
Leptin [ng/ml]	18.20±9.20	17.40	4.50	41.22
Glucose [mg/dl]	68.84±27.11	66.10	20.10	127.00

Table 4. Correlations between maternal pre-pregnancy weight and cord blood hormone concentrations

	Healthy Group		GDM Group	
Mother's weight before pregnancy [kg]	r Pearson	p	R Spearman	p
Active ghrelin [pg/ml]	-0.24	0.132	0.46	0.835
Total ghrelin [pg/ml]	0.41	0.261	0.04	0.321
Insulin [uIU/ml]	0.34	0.158	0.46	0.215
Leptin [ng/ml]	0.15	**0.027**	0.57	0.069
Glucose [mg/dl]	-0.68	**0.017**	-0.13	0.369

Table 5. Correlations between women's weight gain during pregnancy and cord blood hormone concentrations

	Healthy Group		GDM Group	
Pregnancy weight gain [kg]	r Pearson	p	r Pearson	p
Active ghrelin [pg/ml]	-0.44	0.789	0.72	0.438
Total ghrelin [pg/ml]	-0.61	0.95	0.32	0.78
Insulin [uIU/ml]	0.33	0.852	0.37	0.772
Leptin [ng/ml]	-0.26	0.503	0.00	0.286
Glucose [mg/dl]	-0.40	0.632	-0.14	0.493

Table 6. Correlations between gestational week at delivery and cord blood hormone concentrations

	Healthy Group		GDM Group	
Hbd	r Pearson	p	r Pearson	p
Active ghrelin [pg/ml]	0.30	**0.025**	0.50	0.733
Total ghrelin [pg/ml]	0.43	0.116	0.24	0.746
Insulin [uIU/ml]	-0.34	0.417	-0.08	0.126
Leptin [ng/ml]	-0.16	0.631	0.15	0.383
Glucose [mg/dl]	0.53	0.327	-0.02	0.796

Table 7. Correlations between neonatal birth weight and cord blood hormone concentrations

	Healthy Group		GDM Group
Birth weight [g]	R Spearman	p	R Spearman
Active ghrelin [pg/ml]	-0.37	0.716	0.36
Total ghrelin [pg/ml]	0.38	0.88	0.10
Insulin [uIU/ml]	0.48	0.158	0.09
Leptin [ng/ml]	0.40	0.478	0.64
Glucose [mg/dl]	-0.52	0.11	0.13

Table 8. Correlations between head circumference at birth and cord blood hormone concentrations

	Healthy Group		GDM Group	
Head circumference [cm]	r Pearson	p	r Pearson	p
Active ghrelin [pg/ml]	-0.55	0.688	0.43	0.246
Total ghrelin [pg/ml]	0.04	0.153	0.18	0.976
Insulin [uIU/ml]	0.36	0.778	0.10	0.658
Leptin [ng/ml]	0.13	0.888	0.32	0.188
Glucose [mg/dl]	-0.23	0.246	0.28	0.822

Table 9. Correlations between thoracic circumference at birth and cord blood hormone concentrations

	Healthy Group		GDM Group	
Chest circumference [cm]	r Pearson	p	r Pearson	p
Active ghrelin [pg/ml]	-0.35	0.132	0.20	0.631
Total ghrelin [pg/ml]	0.22	0.261	0.12	0.238
Insulin [uIU/ml]	0.44	0.158	0.03	0.298
Leptin [ng/ml]	0.18	**0.027**	0.29	0.491
Glucose [mg/dl]	-0.16	**0.017**	-0.04	0.122

Table 10. Correlations between abdominal circumference at birth and cord blood hormone concentrations

	Healthy Group		GDM Group	
Abdominal circumference [cm]	r Pearson	p	R Spearman	p
Active ghrelin [pg/ml]	-0.26	0.944	0.011	0.522
Total ghrelin [pg/ml]	0.03	0.171	0.084	**0.013**
Insulin [uIU/ml]	0.20	0.163	-0.115	0.183
Leptin [ng/ml]	0.26	0.315	0.591	0.235
Glucose [mg/dl]	-0.02	0.625	0.038	0.619

Table 11. Correlations between thigh circumference at birth and cord blood hormone concentrations

	Healthy Group		GDM Group	
Thigh circumference [cm]	R Spearman	p	R Spearman	p
Active ghrelin [pg/ml]	-0.34	0.967	0.534	0.507
Total ghrelin [pg/ml]	0.42	0.953	0.186	0.843
Insulin [uIU/ml]	0.39	0.668	-0.178	0.292
Leptin [ng/ml]	0.52	0.325	0.094	**0.034**
Glucose [mg/dl]	0.20	0.143	0.321	0.853

Table 12. Correlations between arm circumference at birth and cord blood hormone concentrations

	Healthy Group		GDM Group	
Arm circumference [cm]	R Spearman	p	r Pearson	p
Active ghrelin [pg/ml]	-0.01	0.451	-0.15	0.367
Total ghrelin [pg/ml]	0.44	0.941	-0.14	0.065
Insulin [uIU/ml]	0.23	0.653	0.07	0.653
Leptin [ng/ml]	0.44	0.736	0.28	0.092
Glucose [mg/dl]	0.38	0.512	0.17	0.908

Table 13. Correlations between body weight at 6.month of age and cord blood hormone concentrations

	Healthy Group		GDM Group	
Body weight at 6 months [g]	r Pearson	p	r Pearson	p
Active ghrelin [pg/ml]	0.02	0.948	-0.15	0.056
Total ghrelin [pg/ml]	0.37	0.998	-0.24	0.73
Insulin [uIU/ml]	0.28	0.676	-0.54	0.95
Leptin [ng/ml]	0.34	0.81	0.03	0.974
Glucose [mg/dl]	-0.42	0.463	-0.03	0.538

Table 14. Correlations between body weight at 12 months of age and cord blood hormone concentrations

	Healthy Group	GDM Group
Body weight at 12 months [g].	r Pearson	R Spearman
Active ghrelin [pg/ml]	0.00	0.24
Total ghrelin [pg/ml]	-0.27	-0.17
Insulin [uIU/ml]	-0.11	-0.26
Leptin [ng/ml]	0.23	0.03
Glucose [mg/dl]	-0.60	-0.23

Table 15. Correlations between body weight at 24 months of age and cord blood hormone concentrations

	Healthy Group	GDM Group	
Body weight at 24 months [g]	r Pearson	R Spearman	p
Active ghrelin [pg/ml]	-0.15	0.04	0.65
Total ghrelin [pg/ml]	-0.42	0.30	0.107
Insulin [uIU/ml]	-0.08	0.18	0.063
Leptin [ng/ml]	0.51	-0.07	0.394
Glucose [mg/dl]	-0.48	0.42	0.282

Table 16. Correlations between body weight at 36 months and cord blood hormone concentrations

	Healthy Group	GDM Group	
Body weight at 36 months [g]	r Pearson	r Pearson	p
Active ghrelin [pg/ml]	-0.06	-0.49	0.344
Total ghrelin [pg/ml]	-0.41	0.04	0.316
Insulin [uIU/ml]	-0.16	0.02	0.184
Leptin [ng/ml]	0.55	-0.22	0.09
Glucose [mg/dl]	-0.24	0.30	0.395

The gestational diabetes group showed a positive correlation of total ghrelin concentration with the abdominal circumference of the newborns at birth, which was statistically significant (p=0.013). No such correlation was shown in the statistically significant healthy group (Tab. 10).

In the group of mothers with gestational diabetes, a statistically significant positive correlation of leptin concentration with the thigh circumference of the newborns was shown. No such statistically significant correlation was shown in the healthy group (Tab. 11).

Children's weight gain at 36 months of life vs. hormones in cord blood. There was no correlation at the level of statistical significance between the increase in body weight at the different stages of child development (6, 12, 24, 36 months of age) and the concentrations of hormones in the umbilical cord blood of the newborns studied (Tab. 13–16).

DISCUSSION

Diabetes during pregnancy is a growing problem worldwide. The incidence of type 2 diabetes is increasing at an alarming rate and is occurring in increasingly younger individuals, causing more women of childbearing age to develop type 2 diabetes. In addition, the incidence of gestational diabetes mellitus (GDM) is also found to have increased. The observed increase probably reflects the well-documented obesity epidemic [16]; therefore, it seems to be extremely important to search for the pathogenesis of this disease, as well as potential therapeutic targets both in adults and in infants and children. The presented study has demonstrated that statistically significant correlations were found between maternal pre-pregnancy weight in relation to leptin (positive correlation) and glucose (negative correlation) concentrations in a group of healthy mothers. A positive statistically significant correlation was also observed for active ghrelin concentration and gestational week at delivery in the group of healthy women. Moreover, a positive correlation of leptin levels at a statistically significant level to the chest circumference of newborns in the healthy group, and negative correlation of glucose levels to the chest circumference of newborns at birth. In the GDM group a positive correlation was shown between total ghrelin concentration and abdominal circumference of the newborns at birth, and a statistically significant positive correlation of leptin concentration and thigh circumference of the newborns.

The beginning of research on the impact of gestational diabetes on the foetus and subsequent development of children date back to the mid-20th century. The hypothesis of excessive foetal nutrition or teratogenicity indirectly related to the provision of excess energy, proposed in 1950s by Pederson, postulated that intrauterine exposure of the foetus of a woman with gestational diabetes and hyperglycaemia causes permanent changes in the foetus, leading to malformations, higher birth weight, and an increased risk of developing type 2 diabetes and obesity in later life. In 1980, this hypothesis was extended to include the possibility that free fatty acids (FFAs), ketone bodies and amino acids may influence increased foetal size [17, 18]. With subsequent discoveries of mechanisms regulating metabolism at various stages, further elements were added, (and still being added) allowing for a deeper understanding of the changes accompanying diabetes (including gestational diabetes)[19].

Since the 1990s, an increasing role has been assigned to hormonal imprinting, which involves hormones/peptides and proteins responsible for regulating metabolism, including food intake [15, 20]. However, so far, most studies have focused on the correlation between the concentration of the studied variables between the mother and the child (cord blood). The presented study focuses on searching for a correlation between changes in the hormonal profile in the umbilical cord blood and other variables, such as athropometric data of the mother and child, as well as body weight of the child at 6, 12, 24 and 36 months of age.

Interestingly, the current study did not show statistically significant differences between the concentration of tested hormones in the umbilical cord blood in the group of children from healthy mothers, and from mothers with GDM (data not shown). This is consistent with previous literature data [21], but despite this, interesting interactions were observed that varied depending on the study group. The first interesting observation made during the presented study was the data that did not show greater growth in offspring from women with GDM, which is very often described in the literature. However, it should be noted that these data do not contradict those found in the literature. The role of diabetes exposure *in utero* on infant and child development, later obesity and type 2 diabetes has been examined in the Pima Indian Study which

noted that children from mothers with gestational diabetes were large for their gestational age (LGA) at birth, and at all ages were heavier compared to children from non-diabetic mothers [22, 23]. Similar results were obtained by Silverman et al. (1991), wh0 reported excessive growth in the offspring of women with diabetes during pregnancy. Moreover these same children at 8 years of age, on average, were 30% heavier than the expected weight for their growth, compared to children from non-diabetic mothers[24].

On the other hand, not all studies have shown such a clear link between GDM exposure and childhood obesity. Gillman et al. (2003) examined obesity and overweight among 9 – 14-year-old offspring of mothers with gestational diabetes with data collected using a questionnaire. In the study, foetal exposure to GDM was also associated with a 40% increase in adolescent overweight; however, these findings were attenuated when subsequent comparisons were made with birth weight and maternal BMI [25]. Moreover, it is very difficult to compare studies because many factors may influence both the prevention and earlier development of metabolic complications in children born to mothers with GDM. One such variable is the duration of breastfeeding, because literature data indicate that it may partially reduce obesity risk in children exposed to GDM *in utero,* but a longer duration seems necessary to achieve its protective effect against obesity [26]. Due to the multitude of environmental variables, it is difficult to clearly determine the impact of GDM in mothers on the future size of offspring, and the key influence seems to be the environment and population in which the children grow up [27].

In the current study, statistically significant correlations were found between maternal weight before pregnancy in relation to leptin and glucose concentrations in the group of mothers without gestational diabetes. The correlation of leptin ($p=0.027$) to maternal pre-pregnancy weight was positive, i.e. the higher the maternal pre-pregnancy weight, the higher the leptin concentration in the cord blood in the child. This agrees with literature reports that leptin concentrations are directly proportional with body fat [28–30]. The relationship between ghrelin and insulin is controversial, which also translates into glucose levels in cord blood. The results depend on the conditions under which the studies were conducted. As an example, *in vivo* studies in humans have reported an increase in glycaemia and a decrease in insulin; while other studies report opposite results. However, some results indicate that ghrelin concentration is 40% higher in neonates small for gestational age (SGA), compared to neonates with appropriate for their gestational age, AGA and LGA. [31–33], which also suggest role of ghrelin in child development [34]. Moreover, placental ghrelin and leptin expression is not associated with do not seem to be altered is neither associated with nor seems to be altered in severe maternal obesity, but only with BMI at delivery in obese women [35].

The variability of literature data regarding the role and relationships between leptin, ghrelin and insulin does not allow us to clearly determine their role in the later development of the child, both as a whole and in the context of the current study. However, it is hoped that they will add information that will allow the solving of problems related to metabolic diseases in mothers and children in the future.

Although the study contains a relatively large amount of data obtained using large research groups, there are nevertheless many weaknesses. The main weaknesses include, among others: lack of data on changes in the concentration of tested hormones during development, insufficient data on children's development (only sample data), lack of determination of selected substances in mothers' blood. However, it is hoped that these weaknesses will not obscure the new knowledge gained during the study

CONCLUSION

The results do not conclusively establish whether the hormones responsible for regulating appetite can be biomarkers of neonatal obesity in women with gestational diabetes. The formulation of such a conclusion should be approached very cautiously due to the very small study population. It is undeniable that hormones regulate appetite and influence the metabolic programming of neonatal adipose tissue during critical periods of life. Based on the results of the study, despite the lack of correlation of hormones with birth weight and further development up to 3 years of age, a correlation was shown between thigh circumference and leptin concentration in the GDM group and, interestingly, between abdominal circumference and total ghrelin concentration.

The influence of appetite-regulating hormones in the cord blood of newborn mothers with gestational diabetes on the possible development of obesity in children later in life is an area of intensive research. An altered hormonal profile during the prenatal period may have long-term consequences for the health of children. Understanding the mechanisms of action of these hormones and their impact on the development of childhood obesity, may lead to prevention and intervention strategies to reduce the risk of obesity in this group of children. Further research is needed to better understand these relationships and develop effective preventive interventions.

Contribution statement. MW designed the study, performed experiments, obtained the data and consent of the Ethics Committee and wrote the manuscript. HK, PAK, contributed to the study design, edited, supported and critically revised the manuscript, and contributed to the discussion. ZCM, AC, ARD, MGD, researched data. EPO edited, supported and critically revised the manuscript and contributed to the discussion. All authors have given final approval for the current version to be published.

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