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EFFECT OF SHORT-LASTING UNDERNUTRITION OF GILTS DURING PERI-CONCEPTIONAL PERIOD ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN BLOOD PLASMA DURING PERI-IMPLANTATION PERIOD*

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Abstract

In gilts, the period of early pregnancy occurring from the time of fertilization to the beginning of implantation is sensitive to any environmental disruptions, including an unbalanced diet of a future mother. Previously, we found that due to the undernutrition in gilts during this period, the endocrine intrauterine microenvironment and DNA methylation in the uterus have been changed. These distortions may diminish the success of pregnancy. In this study we focused on the influence of a restricted diet used in gilts during the first days of pregnancy on their biochemical and haematological parameters in peripheral blood. The applied restrictive diet *vs.* normal diet covered only 70% of the nutritional demands of early pregnant gilts. Normal (n = 4 gilts) or restrictive (n = 5 gilts) diets were used from the day of the first signs of the estrus until day 9 of pregnancy and biochemical and haematological parameters in blood plasma were determined during peri-implantation period, e.g. on days 15 to16 of pregnancy. In restrictive *vs.* normal fed gilts significantly lower plasma phosphorus, calcium and total cholesterol as well as the tendency to increasing concentrations of triglicerydes and asparate aminotranserase were found. Haematological parameters did not differ between the studied gilts. Thus, it seems that the availability of nutritional factors became suboptimal in restrictively fed early pregnant gilts. Even short-lasting undernutrition of females during the peri-conceptional period may cause a disruption of biochemical homeostasis during the peri-implantation period and probably affect the success of pregnancy.

Keywords: undernutrition, biochemical parameters, haematological parameters, early pregnancy, gilts.

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INTRODUCTION

In mammals, the nutritional status of females, future mothers, has a huge impact on the success of reproduction as well as on the health of future offspring (Tsuma et al. 1996, Belkacemi et al. 2010, Fleming et al. 2012). Any shortfalls of the diet composition or malnutrition of early pregnant females may disrupt pregnancy and adversely alter embryo-fetal development (Prunier, Quesnel 2000, Fleming et al. 2011, 2012). Importantly, gestational restrictive diet can also have long-lasting effects and may cause diseases of adult offspring, including humans (Roseboom et al. 2001, Yajnik, Deshmukh 2008). Thus, in the last years, the mechanisms of the influence of gestational diet on reproduction and health in humans have been intensively investigated using different animal models (Oliver et al. 2005, Lillycrop 2011, Watkins et al. 2011). The pig appears to be a valuable model for studies of different aspects of human physiology, including reproductive processes, therefore it was used in this study.

Early pregnancy in pigs includes several important reproductive processes, e.g. transport of gametes and their final maturation, fertilization and formation of zygotes, development of resulting early embryos and their transport into uterine horns (Bazer, Thatcher 1997). In pigs, the fertilization of oocytes takes place in an ampulla-isthmic connection of the oviduct, where resulting embryos stay for 2-3 days after fertilization (Hunter 2012). During the subsequent days of gestation, early embryos are transferred into the uterus and the implantation process begins on the fifteenth day of pregnancy. Thus, the proper microenvironments and the availability of nutrients both in oviducts and in the uterus are necessary for the maintenance of early pregnancy. Many components of the oviduct and uterine microenvironments have to be supplied with the mother's diet. Moreover, changes of the endocrine milieu of early pregnant gilts adapt female reproductive organs to pregnancy and maternal recognition of pregnancy in pigs occurs on days 12 to 13 after fertilization (Spencer et al. 2004, Geisert et al. 2012). Maternal recognition of pregnancy turns on the mechanism protecting the functional *corpora lutea* to continue production of progesterone. This steroid hormone has an essential role in the maintenance of pregnancy (Franczak, Kotwica 2010).

In previous research, the high sensitivity of an early pregnancy period to any interference of environmental factors, including maternal diet, has been demonstrated (Ashworth et al. 2009, Belkacemi et al. 2010). Biochemical and haematological profiles in peripheral blood of sows within the physiological range are essential for the maintenance of homeostasis and health required for efficient reproduction (Odink et al. 1990, Elbers et al. 1991). The measurement of these parameters in pigs may be useful also as an indicator of the effect of dietary components as well as an unbalanced diet on the disruption of homeostasis (Štukelj et al. 2010, Chmielowiec-Korzeniowska et al. 2012, Korniewicz et al. 2012).

We hypothesized that restricted diet provided during a short but crucial peri-conceptional period affects biochemical and/or haematological parameters in peripheral blood of females, future mothers, at the beginning of implantation. Thus, in the present study a restrictive diet, as a negative environmental factor, was used in gilts until day 9 of early pregnancy to determine its effect on biochemical and haematological parameters during the peri-implantation period. A novel aspect of our study is the assumption that even short-lasting undernutrition used in females during unique peri-conceptional time may affect biochemical profiles in their peripheral blood.

MATERIAL AND METHODS

The experiment was approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn. The experimental gilts were housed at a private pig farm in Filice. Before the onset of the experiment, nine gilts (PGW × PL; body weight 90-100 kg) were fed with normal balanced diet according to the Nutrient Requirements of Pigs (1993). From the first signs of the second estrus, gilts were kept in individual cages and for two consecutive days were naturally mated. The day of the second mating was designated as the first day of pregnancy and the gilts were then randomly assigned to a group fed balanced diet (n = 4) and to a group fed restricted diet (n = 5). The balanced and restrictive feeding lasted from the first signs of the second estrus until day 9 of pregnancy. Then, from day 10 until the slaughter on days 15 to 16 of gestation, all gilts received normal, balanced diet designed for early pregnant gilts (*Nutrient Requirements of Pigs* 1993). The composition of the diet for both experimental groups was the same, however the restricted diet covered only 70% of the demand for early pregnant gilts. During the slaughter on days 15 to 16 of pregnancy all the gilts were pregnant, as confirmed by the presence of embryos in uterine flushings. Moreover, no clinical problems were observed in the gilts fed normally or restrictively. During the slaughter, blood samples were taken from all the gilts and used for analysis of biochemical and haematological parameters.

Twenty five biochemical and ten haematological parameters in peripheral blood of the gilts were determined automatically, using the following apparatuses: Cobas c501, Cobas e411 and Cobas 601 (all Roche, Switzerland) for biochemical analysis and Sysmex XT1800i (Sysmex, Japan) for haematological analysis. Biochemical determinations included: plasma ions concentrations – phosphorus, calcium, sodium, chlorides and potassium, renal parameters – urea, uric acid, creatinine and total protein, hepatic parameters – aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and gamma glutamyl transpeptidase (GGTP), cardiac parameters – creatine kinase and troponin, intestino-pancreatic parameters – amylase, lipase and glucose, lipid parameters – total cholesterol, triglicerydes, high density lipoproteins (HDL) and low density lipoproteins (LDL), thyroid axis hormones – thyreotropin (TSH) and free thyroxine (FT_4). The following haematological indices were analysed: white blood count (WBC), red blood cells count (RBC), level of haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red blood cells distribution width (RDW-CV), platelet cell count (PLT) and immunoglobulin E (IgE) concentration.

Individual results were log-transformed. Statistical differences between parameters obtained in the group fed with normal diet and the group fed restricted diet were analysed by the Student's *t*-test (Statistica, StatSoft Inc, Tulsa, OK, USA). Differences with p values ≤ 0.05 were considered as statistically significant. All data were expressed as mean ± S.E.M.

RESULTS AND DISCUSSION

According to our knowledge, these are the first data concerning the effect of short-lasting undernutrition of gilts during the peri-conceptional period on biochemical and haematological parameters in blood plasma during the peri-implantation period. Therefore, the direct comparison of the current results and results published previously is difficult.

The haematological parameters (Table 1) in the females studied remained within the reference range determined for pigs (WINNICKA 2011, VER-

Table 1

Haematological parameters in peripheral blood of gilts on days 15-16 of pregnancy (mean ± SEM). The gilts were fed normal or restrictive diet from day of the first signs of the estrus until day 9 of pregnancy. Blood samples were collected on days 15-16 of pregnancy

Parameters	Gilts fed normal diet (n = 4)	Gilts fed restricted diet (n = 5)	Significance of differences
WBC (10^9 L^{-1})	19.7 ± 1.8	20.5 ± 1.1	n.s.
RBC (10^{12} L^{-1})	8.98 ± 0.07	8.54± 0.35	n.s.
HGB (mmol L^{-1})	9.54 ± 0.16	9 ± 0.34	n.s.
HCT (1/1)	0.48 ± 0.01	0.45 ± 0.02	n.s.
MCV (fl)	53.56 ± 1.05	53.25± 0.83	n.s.
MCHC (mmol L^{-1})	19.8 ± 0.11	19.8 ± 0.32	n.s.
MCH (fmol)	1.06 ± 0.01	1.05 ± 0.03	n.s.
RDW-CV (%)	24.03 ± 0.14	21.63 ± 0.49	n.s.
PLT (10^9 L^{-1})	249.66 ± 60.29	112.5± 40.67	n.s.
IgE (IU mL^{-1})	< 0.1	< 0.1	n.s.

MCV – mean corpuscular volume (mean cell volume), MCHC – mean corpuscular hemoglobin concentration (mean cell hemoglobin concentration), MCH – mean corpuscular hemoglobin (mean cell hemoglobin), RDW-CV – red blood cell distribution width, n.s. – non-significant differences heyen et al. 2007). Although we did not find any effect of a diet on haematological changes in pigs, our most important observations concern changes in biochemical parameters including blood plasma concentrations of phosphorus, calcium and total cholesterol (Table 2). It should be emphasized that multiple factors may affect these parameters. Apart from the feeding (Chmielowiec-Korzeniowska et al. 2012, Korniewicz et al. 2012), the age of animals, gender, breeding, reproductive period, housing, health and season may affect biochemical profile of animals (Tumbelson, Kalish 1971, Odink et al. 1990, Elbers et al. 1991).

Interestingly, our experiment demonstrated that short-lasting undernutrition caused significantly lower plasma concentrations of phosphorus (p = 0.02), calcium (p = 0.01; Table 2) and total cholesterol (p = 0.05; Figure 1*a*)

Table 2

Biochemical parameters in peripheral blood of gilts on days 15 to16 of pregnancy. The gilts were fed normal or restrictive diet from day of the first signs of the estrus until day 9 of pregnancy. Blood samples were collected on days 15 to 16 of pregnancy. Selected parameters are expressed as means ± SEM

Parameters	Gilts fed normal diet (n = 4)	Gilts fed restricted diet (n = 5)	Significance of differences
Ions concentration (mmol L^{-1})			
Phosphorus (mmol L^{-1})	3.15 ± 0.3	2.41 ± 0.12	p=0.02
Calcium (mmol L^{-1})	3.26 ± 0.05	2.85 ± 0.12	p=0.01
Sodium (mmol L^{-1})	149.75 ± 2.21	145.2 ± 2.85	n.s.
Chlorides (mmol L^{-1})	97.75 ± 0.85	100 ± 1.64	n.s.
Potassium (mmol L^{-1})	10.81 ± 0.48	9.5 ± 1.05	n.s.
Renal parameters			
Urea (mmol L^{-1})	4.52 ± 0.6	4.77 ± 0.2	n.s.
Uric acid (mmol L^{-1})	0.003 ± 0.0018	0.003 ± 0.0012	n.s.
Creatinine (μmol L^{-1})	132.6 ± 6.2	141.4 ± 11.5	n.s.
Total protein (g L^{-1})	73.5 ± 2.9	70.2 ± 1.5	n.s.
Hepatic parameters			
AST (U L^{-1})	38.5 ± 3.2	54.6 ± 6.4	p=0.07
ALT (U L^{-1})	64.25 ± 3.8	74.2 ± 15.2	n.s.
ALP (U L^{-1})	153 ± 9.3	136.6 ± 20.7	n.s.
Total bilirubin (μmol L^{-1})	1.7 ± 0.0	1.7 ± 0.0	n.s.
GGTP (U L^{-1})	57.25 ± 2.8	51.8 ± 7.6	n.s.
Cardiac parameters			
Creatine kinase (U L^{-1})	3445 ± 1135.14	2154.4 ± 447.52	n.s.
Troponin (mg L^{-1})	7.25 ± 1.5	8.8 ± 2.9	n.s.
Intestino-pancreatic parameters			
Amylase (U L^{-1})	2278.75 ± 258.68	2150.6 ± 337.58	n.s.
Lipase (U L^{-1})	6.5 ± 0.5	6.6 ± 0.68	n.s.
Glucose (mmol L^{-1})	7.73 ± 0.5	7.65 ± 0.5	n.s.

AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGTP – gamma glutamyl transpeptidase, n.s. – differences non-significant

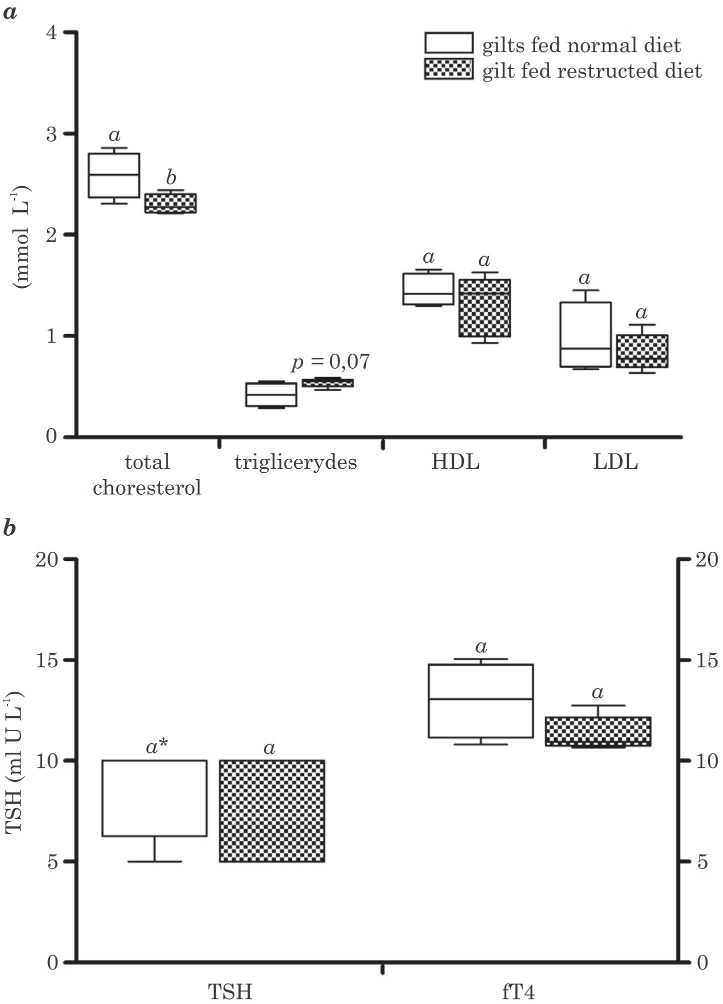

Fig. 1. The mean (± SEM) plasma concentrations: *a* – total cholesterol, triglicerydes, high density lipoproteins (HDL), low density lipoproteins (LDL), *b* – tyreotropine (TSH) and free thyrosine (fT4) – in gilts fed normal (n = 4) or restricted diet (n = 5) from day of the first signs of the estrus until day 9 of pregnancy. The parameters were determined on days 15 to 16 of gestation,* ^{a,b} – different lower case letters designate significant ($p \leq 0.05$) difference between gilts fed normal and gilts fed restricted diet

in restrictively fed gilts compared to data in gilts fed normal diet. Both phosphorus and calcium are important as regulators of the cellular signalling pathways, thus their low availability can disrupt the functioning of these pathways. During early pregnancy, the unique dialogue between developing embryos and maternal oviductal and uterine cells begins (Bazer, Thatcher 1997). This dialogue remains under control of hormones, cytokines and growth factors acting through specific receptors and intracellular signalling pathways as well as being potentially affected by phosphorus and calcium (Franczak et al. 2006, 2013*a*,*b*, Geisert et al. 2012). It is important to note that calcium is also a direct regulator of the fertilization process. The intracellular increases of the calcium concentration occur in oocytes and spermatozoids shortly after the first contact between the gametes (Ito, Kashiwazaki 2012). Thus, limited availability of phosphorus and calcium in early pregnant gilts may affect inconveniently processes taking place during this period. Interestingly, similarly to our results, a low phosphorus concentration in blood plasma due to the restrictive diet was observed in primi- and multiparous sows from high producing pig herds (Verheyen et al. 2007).

We demonstrated a significantly lower cholesterol plasma concentration ($p \leq 0.05$) and the tendency to a higher concentration ($p = 0.07$) of triglicerydes in restrictively compared to gilts fed by normal diet (Figure 1*a*). This observation suggests that a restricted diet, even short-lasting, generates changes in lipid metabolism due to undernutrition. However, the differences in HDL and LDL as well as of TSH and fT4 plasma concentrations between the groups of gilts studied were not observed (Figure 1*b*). Although lipid metabolism is regulated mainly by hormones of the thyroid axis, it seems that in the present study increased concentrations of cholesterol and triglicerydes in restricted gilts directly resulted from the availability of dietary components (Migdał et al. 2003, Sechman et al. 2007). The influence of a diet on cholesterol metabolism was also demonstrated by Altman et al. (2013). The authors used long-lasting protein restriction in pregnant pigs and found that the diet induced a decrease in the expression of hepatic *HMGCR* gene in 95-day old offspring (Altman et al. 2013). The *HMGCR* gene encodes the rate-limiting enzyme responsible for cholesterol synthesis. During early pregnancy in pigs, cholesterol is required as the substrate for progesterone and estradiol-17*β* synthesis. Estradiol-17*β* is important for the reorganization of the uterine activity during the maternal recognition of pregnancy, while progesterone is necessary for the maintenance of pregnancy (Spencer et al. 2004). Thus, decreased cholesterol availability, demonstrated in this study in restrictively fed gilts, may interfere with the course of early pregnancy events.

In this study, the renal, hepatic, cardiac and intestino-pancreatic parameters did not differ significantly between normally and restrictively fed gilts (Table 2). The exception is tendency to a higher ($p = 0.07$) AST plasma level in restrictively compared to normally fed gilts. This tendency in the AST level in gilts fed restricted diet implicates the mobilization of body reserves in these animals.

In this study, the changes of lipid metabolism in gravid gilts were demonstrated as a result of short-lasting restricted diet. In other study, long-term feeding of an unbalanced diet during pregnancy caused disruption of protein and lipid metabolism in organisms of gravid pigs (Korniewicz et al. 2012). The decrease of total proteins, globulins and urea serum concentrations was demonstrated in these sows. However, contrary to our results, the serum levels of total and LDL cholesterol increased but AST decreased in comparison to normal diet fed sows. Thus, it seems that the duration of an unbalanced diet affects the intensity of metabolic changes in the organism of pregnant pigs. It is important to note that our results indicate that application of a short-lasting unbalanced diet during the peri-conceptional period may disturb some biochemical parameters in females during the peri-implantation period.

Haematological parameters of studied gilts are presented in Table 1. Data analysis showed no significant differences ($p > 0.05$) in any of these parameters or in the IgE plasma level between the two groups of gilts. Thus, the restrictive diet used in early pregnant gilts did not disrupt their haematological homeostasis. Interestingly, in the other studies conducted on pigs, some haematological parameters changed due to the growth rate (Tumbleson, Kalish 1971), health (Odink et al. 1990, Elbers et al. 1991) and fattening season (Chmielowiec-Korzeniowska et al. 2012).

CONCLUSIONS

The results of the present study indicate that restricted diet used even during the short-lasting but unique period of early pregnancy may decrease plasma concentrations of phoshorus, calcium and total cholesterol in gravid females. Decreased maternal availability of these components in blood plasma may result in the creation of suboptimal conditions for early pregnancy. In consequence, the processes of fertilization, maternal recognition and maintenance of pregnancy as well as the development of early embryos may be disturbed. Thus, unbalanced diet applied in early pregnant gilts can be anegative environmental factor, which triggers changes in biochemical parameters and may affect the quality of reproduction. Although the current study was performed on a pig model, the results implicate a possibility of adverse effects of unbalanced diet (e.g. slimming), used by women during the periconceptional period, on reproductive processes during early pregnancy.

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