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Alpha-ketoglutarate partially protects newborns from metabolic changes evoked by chronic maternal exposure to glucocorticoids

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Abstract: Foetal and neonatal time is very important for the growth and development of the mechanisms involved in the programming of metabolic processes in adult life. In foetal life, critical developmental time-windows for different key factors determining the programming of metabolic processes persisting for a longer time in later life were discovered. Dexamethasone (Dex), a synthetic glucocorticoid is administered in pregnancy to reduce mortality in preterm infants. However, other studies did not examine the effects of maternal simultaneous dexamethasone treatment with alpha-ketoglutarate (AKG) on glucose, total cholesterol, triacylglycerol and other metabolic markers in the blood serum of newborns. This study shows that exposure to maternal dexamethasone excess during the last 45 days of pregnancy resulted in reduced body weight by 26% in newborns. Moreover, when dexamethasone was administered with AKG, body weight was reduced by only 13.5% when compared with the control group. Total cholesterol concentrations in sows and their newborns in the Dex groups were higher by 81% and 79% compared with the control values in sows and in newborns, respectively. Triacylglycerol serum concentrations were higher by 54% in sows from the Dex group and 58% in newborn piglets born by these mothers. Glucose concentration was higher by 142% in newborns after maternal dexamethasone treatment, compared with the control group. Serum glucose concentration remained unchanged in sows after simultaneous dexamethasone administration with AKG, but in newborns a 2-fold increase was observed. These foetal metabolic changes after maternal treatment with dexamethasone might be linked not only with long lasting metabolic disturbances, but also with more frequent coronary heart and cardiovascular diseases in later life. Our results indicate that maternal AKG administration to sows during the last 45 days of pregnancy protects newborns from metabolic disturbances induced by dexamethasone acting at this time and influencing developmental programming of metabolic processes which may persist or appear in later life.

Keywords: Apha-ketoglutarate, dexamethasone, piglets, prenatal life, cholesterol

INTRODUCTION

Foetal and neonatal time is very important for the growth and development of the mechanisms involved in the programming of metabolic diseases in adult life [1, 2, 3]. In foetal life, significant developmental windows were found with specific sensitivity for some factors as a key predisposing for abnormalities in the metabolic processes persisting in later life. Glucocorticoids are used for the treatment of numerous diseases, as well as for the promotion of organ maturation and prevention of preterm delivery [4]. In both humans and animals, prenatal exposure to glucocorticoids plays a major role in the pathogenesis of intrauterine growth restriction (IUGR) [3, 5, 6]. Glucocorticoids treatment may change the original programming of organs, especially those in developmental phases, which can result in long-term changes in metabolism [7]. These changes are part of a growing list of IUGR-related diseases, including type 2 diabetes mellitus, cardiovascular diseases, hypertension, polycystic ovary syndrome and obesity linked with the regulation of energy metabolism and food intake [3, 7, 8, 9, 10, 11, 12]. These consequences are strictly connected with the maternal metabolic state as a crucial determinant for alterations in the metabolic and endocrine functions of foetuses and newborns [5, 6, 8, 9, 10].

Alpha-ketoglutarate (AKG) is a precursor of glutamine which is converted via glutamate to alpha-ketoglutarate in the citric acid cycle [13, 14]. Glutamine serves as oxidative fuel for rapidly dividing cells such as lymphocytes and fibroblasts [14]. This most abundant free amino acid is released in the intestinal lumen by protein digestion [15]. Glutamine plays an important role in the inter-organ flow of nitrogen in ammoniagenesis and carbon between carbohydrates and proteins, participates in many reactions in the body, and is essential for glucose homeostasis [14].

Considering the negative effects of glucocorticoids treatment, especially during foetal development when individual sensitivity for metabolic and hormonal factors is very high, it is important to investigate effectors decreasing such disadvantages. Among these factors, AKG may be included,

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and the effects of its prenatal action on dexamethasone treated sows and their foetuses should be checked. The lack of knowledge about the relationship between maternal treatment in selected periods of pregnancy with glucocorticoids and alpha-ketoglutarate on biochemical markers in blood serum in the mother and newborns stimulated us to undertake this study. To evaluate the effects of Dex on based processes of metabolism at the end of the pregnancy in sows, biochemical analysis in blood serum in newborns and parturient sows were determined after Dex administration – alone or simultaneously with AKG – to sows during the last 45 days before delivery.

MATERIALS AND METHODS

This study was approved by the Local Ethics Committee on Animal Experiments at the Agricultural University in Lublin, Poland.

Procedure with pregnant sows. The study was carried out on 18 sows of the Large Polish White breed, housed individually in pens under standard rearing conditions (controlled and constant temperature and humidity and a controlled 12:12 h light-dark cycle) with free access to fresh water and fed twice daily with standard commercial diets for pregnant sows. All sows used in the experiment were clinically healthy. The experimental procedure on sows was conducted from 70th day of pregnancy to the parturition (the last 45 days of pregnancy). The time of pregnancy was calculated from the day of mating. The sows were divided into 3 groups. The first group consisted of 6 control sows (Cont group) which received *i.m.* and *per os* physiological saline; the second group (DEX group, n=6) was injected *i.m.* every second day with synthetic glucocorticoid-dexamethasone; and the third group (Dex+AKG, n=6) received dexamethasone simultaneously with AKG.

Dexamethasone treatment. Sows from Dex and Dex+AKG groups were injected *i.m.* with dexamethasone (Dexamethasone *pro inj.* 0.2%, Eurovet Animal Health, The Netherlands) at the dosage of 3 mg/sow. Dexamethasone was administered at constant dose of 100% in the morning for the last 45 days of pregnancy. The total amount of dexamethasone administered during pregnancy to each sow from the Dex and Dex+AKG groups was 69 mg. The control group of sows was injected *i.m.* with the same volume of physiological saline.

Alpha-ketoglutarate treatment. Powdered alpha-ketoglutaric acid (AKG; Protista AB, Sweden) with a purity of 99% was mixed with distilled water to make solutions. The pH of these stock solutions was buffered by the addition of NaOH to a final pH of 7.3.

During the last 45 days of pregnancy the AKG-treated sows from the Dex+AKG group received orally every morning 0.4 g·kg^{-1} BW of AKG as a solution. The control group of sows was given the same oral dose of distilled water.

Procedure with newborns. The piglets were weighted just after their birth. The study was carried out on 36 piglets. Each group consisted of clinically healthy male piglets. All had not congenital infections. 2 male piglets were chosen randomly just after the birth from every control litter of sows receiving saline during the last 45 days of pregnancy. 2 male piglets were also chosen randomly from each litter of sows from the Dex and Dex+AKG groups.

Blood sample collection. During the parturition, blood samples were collected from the aural vein of every control and experimental sow. Blood samples were collected before colostrum feeding within 1 hour after the birth. Blood samples were collected from the subclavian vein. The blood samples obtained from sows and newborns were centrifuged at 3,000 x g for 15 min. The obtained blood serum samples were stored at -25°C until analysis. The concentrations of glucose, urea, total cholesterol, triacylglycerol, total protein and albumin, and the activity of AlAT (alanine transaminase), AspAT (aspartate aminotransferase) were determined in blood serum in sows and newborns. The relationship between AspAT and AlAT as the de Ritis coefficient was determined. Total cholesterol was analyzed by enzymatic colorimetric test provided by Alpha Diagnostics (San Antonio, TX, USA). Glucose concentrations were measured by the glucose oxidase method using the glucose kit from Alpha Diagnostics (San Antonio, TX, USA). Urea, triacylglycerol, AspAT and AlAT, total protein, and albumin were determined using kits from Cormay, Warsaw, Poland.

Statistical analysis. All the data are presented as means ± SEM. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) and the Tukey test as correction for multiple comparisons using STATISTICA 6.0 software. In cases of lack of normal distribution or unequal variance, the U-Mann Whitney test was used. $P < 0.05$ was considered statistically significant.

RESULTS

Body Weight. The exposure to maternal dexamethasone excess *in utero* resulted in newborns with reduced body weight. The mean body weight of newborns from the Dex group amounted to 1,197 g ± 142, which was significantly lower when compared with the mean BW of the control newborns which reached the value of 1,616 g ± 143 (P=0.001 *vs* controls). The lowest body weight of newborn piglets from the Dex group amounted to 880 g and the highest 1,420 g. The smallest control newborn piglets weighed 1,340 g and the highest 1,900 g. Simultaneous administration of dexamethasone and AKG resulted in a higher BW of newborns when compared with the Dex group, and reached the mean value of 1,397 g ± 153 (P=0.01). Newborns from this group had lower body weight at the birth when compared with the control group (P=0.006). The lowest BW in Dex+AKG group amounted 1,100 g and the highest 1,840 g.

Biochemical analysis of blood serum of sows. The highest concentration of glucose occurred in sows treated with dexamethasone during pregnancy. In both experimental groups of sows, the urea concentration was similar but higher then in control animals. The highest serum total cholesterol was in the Dex group. Comparable concentrations of total cholesterol were in the control and the Dex+AKG groups (P=0.003 *vs* Dex group). The highest concentration of triacylglycerol was in the Dex group compared with the controls and with the Dex+AKG group (P=0.002 for Dex group *vs* Dex+AKG group). In Dex+AKG group was observed a tendency to decrease of the activity of AlAT comparing with the control group. The

highest level of AP was in the Dex group of sows compared with the control group and Dex+AKG group (P=0.0003 for Dex group *vs* Dex+AKG group). Results of AP and biochemical analyses of sows blood serum are presented in Table 1.

Biochemical analyses in blood serum in newborns. Higher concentration of glucose was in Dex and Dex+AKG groups comparing with the control group. Maternal dexamethasone excess resulted in newborns with decreased urea concentration and decreased activity of AP. Urea concentration of newborns from Dex+AKG group was the lowest comparing with the controls and Dex group (P = 0.03 *vs* Dex group). Chronic influence of dexamethasone during the last 45 days of prenatal life resulted in higher serum total cholesterol in both experimental groups (Dex and Dex+AKG), although significantly higher triacylglycerol concentration was only in Dex group. The lowest concentration of total protein was in Dex+AKG group (P = 0.002 for Dex+AKG group *vs* Dex group) but the lowest albumin concentration was in Dex group. Significantly higher activities of AlAT were in both groups of newborns prenatally exposed to dexamethasone. The activities of AP in blood serum in newborns from Dex and Dex+AKG groups were decreased comparing with control group of newborns. Furthermore, other serum parameters in newborn piglets are presented in Table 2.

DISCUSSION

The metabolic effects of glucocorticoids are mediated by mechanisms related to liver and peripheral insulin resistance leading to obesity, hyperglycemia, and abnormalities such as the metabolic syndrome connected with cardiovascular disease [16, 17, 18, 19]. During development, glucocorticoids prepare different organs for the metabolic adaptations necessary for autonomous life after birth [20]. Prenatal glucocorticoid exposure is associated with postnatal alterations in glucose homeostasis and hypothalamic-pituitary-adrenal axis function [1, 16, 21, 22]. Near to the time of birth, the liver loses its hoematopoesis role and starts to play a metabolic function. In human infants, *de novo* synthesis of cholesterol has been described as an adaptive mechanism regulating postnatal cholesterol homeostasis [2, 5, 6, 7, 14, 23, 24, 25, 26]. Considering clinical findings, the lipogenic effect of glucocorticoid is the accumulation of triglyceride in the liver [27, 28, 29, 30, 31, 32, 33]. In the present study, total cholesterol concentrations in sows and their newborns in the Dex groups were higher by 81% and 79%, respectively, compared with the control group. Triacylglycerol serum concentrations were higher by 54% in sows from the Dex group, and 58% in the newborn piglets, indicating a change in foetal homeostatic mechanisms which may persist or occur later in life. Serum total cholesterol and triacylglycerol concentrations in the blood of sows from the Dex+AKG group were comparable with the control group, which indicate the protective influence of AKG on high cholesterol concentration. This effect was evident in the sows. Although serum total cholesterol concentration in the blood of newborns from the Dex+AKG group was higher compared with the control group, triacylglycerol concentration, however, was not enhanced. Earlier experiments showed that maternal administration of AKG decreased total serum cholesterol level in both piglets and the mother [32]. The hypocholesterolemic effect of AKG supplement to the diet has been noted in Mongolian Gerbils [3].

Table 1 Biochemical analyses in blood serum of sows from the control group and treated with dexamethasone (Dex group) alone or simultaneously with AKG (Dex+AKG group) during pregnancy.

Parameters	Control n=6	Dex n=6		Dex+AKG n=6	
Glucose [mmol/L]	5.08 ± 0.03	6.16 ± 0.2*	*(P=0.001)*	5.64 ± 0.24	
Urea [mmol/L]	3.84 ± 0.04	5.29 ± 0.29*	*(P=0.003)*	5.47 ± 0.23*	*(P=0.0008)*
Total cholesterol [mmol/L]	1.38 ± 0.03	2.53 ± 0.08*	*(P=0.0008)*	1.38 ± 0.04	
Triacylglycerol [mmol/L]	0.42 ± 0.01	0.64 ± 0.02*	*(P=0.0008)*	0.43 ± 0.04	
Total protein [g/L]	66.1 ± 2.2	65.2 ± 1.5		63.4 ± 1.3	
Albumin [g/L]	36.2 ± 0.57	34.9 ± 0.74		30.6 ± 1.86	
AspAT [U/L]	43.9 ± 0.86	56.1 ± 9.02		38.3 ± 3.59	
AlAT [U/L]	48.7 ± 2.33	46.4 ± 2.04		42.1 ± 1.03	
AP [U/L]	16.3 ± 1.54	53.6 ± 8.42*	*(P=0.0008)*	15.6 ± 2.40	

Dex – group treated with dexamethasone: Dex+AKG – group treated simultaneously with dexamethasone and AKG. * – experimental group vs controls (values are means ±SEM).

Table 2 Biochemical analysis in blood serum of newborns born by sows from the control, Dex and Dex+AKG groups.

Parameters	Control n=12	Dex n=12		Dex+AKG n=12	
Glucose [mmol/L]	1.14 ± 0.01	2.77 ± 0.14*	*(P=0.0008)*	2.31 ± 0.19*	*(P=0.01)*
Urea [mmol/L]	6.17 ± 0.19	5.07 ± 0.04*	*(P=0.04)*	4.17 ± 0.07*	*(P=0.001)*
Total cholesterol [mmol/L]	0.23 ± 0.04	0.92 ± 0.09*	*(P=0.02)*	1.05 ± 0.06*	*(P=0.001)*
Triacylglycerol [mmol/L]	0.18 ± 0.009	0.29 ± 0.01*	*(P=0.0008)*	0.24 ± 0.02	
Total protein [g/L]	26.2 ± 0.2	26.4 ± 0.8		22.3 ± 0.5*	*(P=0.001)*
Albumin [g/L]	8.7 ± 0.11	7.2 ± 0.40*	*(P=0.05)*	8.2 ± 0.52	
AspAT [U/L]	35.2 ± 2.97	45.9 ± 4.31		44.0 ± 5.25	
AlAT [U/L]	10.6 ± 0.59	17.9 ± 1.18*	*(P=0.0008)*	16.6 ± 0.76*	*(P=0.001)*
AP [U/L]	2153.7 ±145.03	1042.8 ± 66.07*	*(P=0.0008)*	865.8 ± 181.00*	*(P=0.002)*

Dex – group prenatally exposed to dexamethasone; Dex+AKG – group prenatally exposed to dexamethasone with AKG. * – experimental group *vs* controls (values are means ±SEM).

Developmental changes during foetal life determine the activity of synthesized enzymes in the human liver as well as in the livers of other species. Dexamethasone administered during pregnancy may accelerate these changes [21]. In the present study, the activity of AspAT in sows and their newborns from both experimental groups was unchanged although the activity of ALAT had a tendency to decrease in sows. Moreover, the increase of ALAT activity in newborns from both of these groups was observed. If a liver cell is damaged, this enzyme leaks into the blood; however, in this study the de Ritis coefficient for both groups did not decrease below 0.9.

The foetal liver is metabolically interdependent on the placenta, which is related to the flow of glutamine-glutamate [14, 35, 36, 37]. The adult liver changes excess of amino acids into glucose, but the foetus during prenatal life under physiological conditions is unable to produce glucose, the main source of energy [17, 18, 37, 38, 39]. Gluconeogenic and glycogenolytic enzymes are present in early foetal development [17, 18, 38]. Glucose concentration depends on the correlation between foetal and maternal serum glucose levels [20, 39]. In human pregnancy, gluconeogenesis decreases before term and glucocorticoids influence glucose metabolism [11, 17, 18, 38]. In the present study, the serum glucose concentration in newborns after dexamethasone treatment throughout the last 45 days of prenatal life was 143% higher in comparison with the control. Serum glucose concentration in sows during parturition also increased after dexamthasone treatment. Although the serum glucose concentration was not enhanced in sows after simultaneous administration of dexamethasone with AKG, in newborns, however, a 2-fold increase was observed.

Glutamine supplies carbon for all the biochemical metabolic pathways, and finally participates not only in gluconeogenesis in foetus liver [40, 41]. Ureagenesis is linked with gluconeogenesis from amino acids, which suggests that the ratio of both is similar, while in humans the decrease of urea synthesis is observed at the end of pregnancy [3, 17, 18, 38]. Maternal adaptation in nitrogen metabolism results as a decrease of nitrogen acceptors such as alpha-ketoglutarate and lead to the decrease of ureagenesis [17, 18, 38]. An earlier study showed that urea synthesis increased after infusion with glutamine in a premature infant [42]. In this study, serum urea concentration in sows during parturition was increased by about 37% after administration of dexamethasone, and by about 42% in the Dex+AKG group, compared with the control pregnant sows. Moreover, the decrease of serum urea concentrations was observed in newborns from these groups of sows, together with a decrease of total protein concentration. This decreased total protein concentration might be an effect of increased serum urea concentration in the mothers. Prenatal treatment with dexamethasone during the last 45 days of pregnancy decreased albumin concentration in the serum of newborns.

Enhanced activity of alkaline phosphatase is typical during the normal activity of pregnancy and in bone growth. Dexamethasone induced the increase of the activity of serum alkaline phosphatese [43, 44]. In the present study, exposure to an excess of dexamethasone during pregnancy resulted in increased activity of alkaline phosphatese in the sows; this was not observed in sows from the Dex+AKG group. An adsverse effect was observed in newborn piglets under prenatal influence of dexamethasone. Regarding these data, it is advisable to investigate other sources of phosphatese, such as bones.

Maternal dexamethasone treatment leads to low birth weight, enhanced serum total cholesterol and triacylglycerol concentrations in newborns, which might be linked not only with more frequent metabolic syndrome, but also to coronary heart and cardiovascular diseases [1]. Simultaneous administration of dexamethasone with AKG to sows during the last 45 days of pregnancy did not change the triacylglycerol concentration in the serum of the piglets, but increased their birth weight. AKG might protect the developing organism during the prenatal period from neonatal metabolic changes caused by glucocorticoids maternal treatment that may occur in later life [45].

CONCLUSION

This study shows that maternal administration of AKG to sows during the last 45 days of pregnancy protects partially offspring from metabolic disturbances induced by dexamethasone acting at this prenatal time of developmental programming of metabolic processes which may persist or appear in postnatal period or later in life.

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