TOTAL CHOLESTEROL, GLUCOSE, AND ELECTROLYTES IN PIGLETS SERUM AFTER α-KETOGLUTARATE (AKG) AND DEXAMETHASONE TREATMENT DURING PRENATAL AND NEONATAL LIFE

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Abstract

The aim of this study was to determine the effects of dexamethasone (Dex) and α-ketoglutarate (AKG) on the total cholesterol, glucose, Na+, Cl-, total Ca and K+ levels, in serum for newborn and neonate piglets. The blood samples were collected from newborn non-suckling piglets born by sows administered from the 91st d of pregnancy to the parturition with AKG and/or Dex (AKG-0.4 g/kg b.w./every day, orally; Dex-3 mg/sow, i.m. every second day). The second blood sampling was collected from the same piglets on the 14th d of their life, after further Dex and AKG administration in the same way as in sows. The serum total cholesterol level of control piglets was the highest, and reached the value of 40.6 mg%, whereas the level was the lowest in AKG group (27.9 mg%). The total cholesterol serum level in 14-d-old piglets in AKG+Dex and Dex groups reached 304 mg% and 273 mg%, respectively, in the AKG group only 155 mg%, while in the controls - 197 mg%. The decreased total serum cholesterol level in newborn and neonatal piglets, after maternal and neonatal administration of AKG, indicated the same lowering effect. AKG given to sows during the last weeks of pregnancy decreased serum Na+, Cl- and K+ levels in newborns, but did not influence after further administration to piglets during their 14 d of neonatal life. Increased serum K+ and total Ca levels in 14-day-old piglets from both groups treated with Dex and AKG+Dex, and Cl- loss in Dex group were observed.

Key words: pigs, α-ketoglutarate, dexamethasone, electrolytes, cholesterol, glucose.

Dexamethasone (Dex) as a synthetic glucocorticoid effectively crosses the placenta and normalises lung morphology inducing structural maturity, which decreases mortality in preterm human infants (10, 33). Prenatal corticosteroid therapy is used to treat foetuses (vix the mother), not only in preparing the human foetus for premature delivery; but also for congenital adrenal hyperplasia and full-term neonates for cardiopulmonary disorders (6, 33, 36, 48). Foetal exposure to excess of glucocorticoids has a long-term developmental consequences in the adult, which depends upon the timing of gestational exposure (1, 5, 7-9, 11, 20, 23, 29, 30, 36, 37). The treatment with Dex during pregnancy, reduced intracellular mineral and glucocorticoid receptors in the hippocampus; but also programmed hyperglycaemia and higher total serum cholesterol level, elevated blood pressure and insulin resistance in offspring, or in later life in rats, camels, sheep, rabbits, monkeys, and humans (3, 4, 7, 13, 18, 19, 22, 27, 33, 46). Alpha-ketoglutarate (AKG) is the source of energy for cells, and is the precursor of glutamine, which reaches about 50% of free amino acid pool in bloodstream, and its rapid decrease is associated with glucocorticoid therapy during postnatal time (5, 16). Investigations performed in growing pigs up to 10 weeks of life; showed beneficial effects of AKG on skeletal system development (24, 25). Other studies on lambs; showed that AKG given during the first two weeks of neonatal life positively influences skeletal development with long-term consequences in later life (14). Our earlier studies showed protective effects of AKG administered simultaneously with Dex, during prenatal or neonatal time from reducing the action of Dex on bone mineral density (40-43). Dex reduced insulin-like growth factor I, and growth hormone levels in piglets after administration during the last weeks of prenatal life and 30th d of postnatal life. Moreover, the levels of growth hormone and insulin-like growth factor I; increased in piglets administered with AKG, which indicated that AKG might have protective effects on hormonal regulation, hindered by glucocorticoid therapy (44). Considering the negative effects of glucocorticoid treatment, especially during prenatal and early neonatal development, when individual sensitivity to hormonal and metabolic factors are very high, it is reasonable to investigate the influence of AKG, and...
most frequently used glucocorticoids like Dex on the total cholesterol level as well as other parameters of blood.

The lack of knowledge of the influence of AKG on the cholesterol metabolism during foetal and neonatal development under glucocorticoid treatment; motivated us to undertake this study. The purpose of this study was to determine whether AKG and Dex should be administered separately or simultaneously to pregnant sows; during the last 24th d before delivery, influences the serum of the total cholesterol, glucose, Na⁺, K⁺, Ca and Cl⁻ levels in newborn piglets assessed just after their birth, and after further treatment with Dex or/and AKG, according to their mothers treatment, through 14th d of neonatal life.

**Material and Methods**

This study was approved by the Local Ethic Committee on Animal Experimentations of Agricultural University of Lublin, Poland.

**Experimentation with pregnant sows.** The study was carried out on 12 sows of Large Polish White breed, housed under standard rearing conditions (temperature and humidity); with unrestricted access to fresh water, and fed twice daily with standard commercial diets for pregnant sows. The experimental procedure was performed in the morning. The experiment lasted up to 14th d of the piglets’ neonatal life. Total time of AKG and/or Dex treatment during prenatal and neonatal period was 38 d.

**Blood sample collection.** Just after the piglets’ birth, blood samples were collected from the subclavian vein of non-suckled animals. The second collection of blood samples was performed on the 14th d after their birth. All the 14-day-old piglets were not fasted before blood collection. The blood samples were centrifuged (3000 g for 15 min), and the obtained serum samples were stored at -25°C until analysis. Total cholesterol was determined by DST colourimetric method, using an enzymatic kit (Alpha Diagnostics, USA), with absorbance measured at 550 nm. A glucose level in serum was determined by the oxide method, using a kit provided by Alpha Diagnostics (San Antonio, USA), with absorbance measured at 460-560 nm. The analytic procedures for the determination of Cl⁻, total Ca, Na⁺ and K⁺ levels, were performed using the automatic analyser AVL 9180 (USA) and ROCHE kits (ISE Snap Pack, USA).

**Statistical analysis.** All the data is presented as means ± SEM. Differences between means were tested for statistical significances, with the use of one-way ANOVA and *post hoc* Duncan test, with the aid of STATISTICA 6.0 software. The data were found to be normally distributed, and had an equal variance. The level of statistic significance was set at *P*<0.05 for all comparisons.

**Results**

Glucose levels of newborn piglets was the highest in the AKG+Dex groups, and similar in the controls, while the lowest was in piglets born by sows treated with Dex during pregnancy (Table 1). The total serum cholesterol levels of newborn piglets from controls was the highest, whereas the lowest in was in the AKG group. Na⁺ levels reached similar values in the controls and the AKG+Dex groups; lower levels were found in the Dex group, and the lowest was in the AKG group. The highest value of Cl⁻ was in the control, followed by the AKG+Dex and the Dex group, and the lowest in the AKG group. K⁺ and total Ca levels were the lowest in the AKG group of newborn piglets (Table 1).

Total cholesterol level of piglets at the 14th d of age was the highest in the AKG+Dex groups and the Dex group, but the lowest in the AKG group, although the significant differences were between the AKG+Dex groups and both the control and AKG groups. Na⁺ level reached the highest value in the AKG+Dex group. Cl⁻ level was similar in the control and the AKG+Dex group, and the lowest level was observed in the Dex group. The highest level of K⁺ was in the Dexamethasone group and the AKG+Dex groups, whereas the lowest in the control and AKG groups. Total Ca levels were the highest in the Dex and AKG+Dex groups, the lowest was in the AKG and control groups (Table 2).
Table 1
Mean serum glucose, total cholesterol, Na+, Cl−, K+, and total Ca levels in the control and experimental groups of non-suckled newborns

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 12)</th>
<th>AKG (n = 12)</th>
<th>Dex (n = 12)</th>
<th>AKG+Dex (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg%</td>
<td>58.7±1.9 a</td>
<td>55.5±2.9 a</td>
<td>36.9±3.3 b</td>
<td>58.8±6.3 a</td>
</tr>
<tr>
<td>Total cholesterol, mg%</td>
<td>40.6±2.1 a</td>
<td>27.9±2.3 b</td>
<td>35±1.2 a</td>
<td>34.1±2.7 a</td>
</tr>
<tr>
<td>Na+, mmol/l</td>
<td>134.5±1.94 ac</td>
<td>128.2±0.86 b</td>
<td>131.3±0.55 a</td>
<td>134.3±0.8 a</td>
</tr>
<tr>
<td>Cl−, mmol/l</td>
<td>103.0±0.63 a</td>
<td>90.4±1.32 b</td>
<td>95.5±0.42 c</td>
<td>99.8±0.32 d</td>
</tr>
<tr>
<td>K+, mmol/l</td>
<td>4.83±0.15 a</td>
<td>3.52±0.26 b</td>
<td>4.91±0.05 a</td>
<td>5.26±0.34 a</td>
</tr>
<tr>
<td>Total Ca, mg%</td>
<td>10.1±0.2 ab</td>
<td>9.7±0.18 b</td>
<td>9.96±0.4 ab</td>
<td>10.8±0.22 a</td>
</tr>
</tbody>
</table>

Non-suckled newborns were born by mothers treated with saline (control), AKG, Dex or AKG+Dex. Statistically significant differences between all the investigated groups are marked with different letters a, b, c (P ≤ 0.05). ± SEM

Table 2
Mean serum glucose, total cholesterol, Na+, Cl−, K+, and total Ca levels in control and experimental piglets at 14th d of age

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 12)</th>
<th>AKG (n = 12)</th>
<th>Dex (n = 12)</th>
<th>AKG+Dex (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg%</td>
<td>146.3±11.4 a</td>
<td>143.8±7.4 a</td>
<td>142.2±7.6 a</td>
<td>143.7±6.3 a</td>
</tr>
<tr>
<td>Total cholesterol, mg%</td>
<td>197±34 ab</td>
<td>155±17.1 a</td>
<td>273±7.1 bc</td>
<td>304±16 c</td>
</tr>
<tr>
<td>Na+, mmol/l</td>
<td>135.5±1.08 a</td>
<td>133±1.21 a</td>
<td>132.2±0.23 a</td>
<td>138.8±0.34 b</td>
</tr>
<tr>
<td>Cl−, mmol/l</td>
<td>101.6±0.55 a</td>
<td>99.3±1.02 ab</td>
<td>98.2±0.59 b</td>
<td>101.7±0.59 a</td>
</tr>
<tr>
<td>K+, mmol/l</td>
<td>4.86±0.3 a</td>
<td>4.86±0.3 a</td>
<td>7.68±0.41 b</td>
<td>7.02±0.51 b</td>
</tr>
<tr>
<td>Total Ca, mg%</td>
<td>10.32±0.56 ab</td>
<td>10.7±0.29 a</td>
<td>13.1±0.34 b</td>
<td>12.28±0.18 b</td>
</tr>
</tbody>
</table>

Piglets received respectively to their mother’s saline (control), AKG, Dex or AKG+Dex. Statistically significant differences between all the investigated groups are marked with different letters a, b, c (P ≤ 0.05); ± SEM

Discussion

Total cholesterol. The total cholesterol serum level of newborn piglets was the highest in controls and lower by 30% in the AKG group. Among newborn piglets, the values between the controls and the AKG+Dex, and Dex groups, although it was lower in the experimental groups, and it did not differ significantly. Maternal administration of AKG decreased the total serum cholesterol level in piglets when assessed just after their birth. Moreover, a decreased level of total cholesterol was observed after further administration of AKG to neonatal piglets, in comparison with the other two experimental groups. Hypocholesterolaemic effects of AKG were described in Mongolian Gerbil fed diet, supplemented with the compound (2). The higher total of cholesterol levels in the control piglets, in comparison with the same parameter in non-suckled control newborns, was related with its increased metabolism and supply of nutrients, during this neonatal period (34). In the late-gestation only 10% - 20% of foetal cholesterol comes from the mother, crossing the placental barrier. Foetal tissues originate de novo synthesis of cholesterol, which is an integral component of the developing organism described in the human infant as an adaptive mechanism that regulates cholesterol homeostasis (12, 15, 28). Compared with the prenatal period, the rate of cholesterol synthesis decreased in the liver of the guinea pig, while the cholesterol content in serum increased after the birth (28). At birth, the average concentration of cholesterol is similar too different organs of human infants. At 26 weeks of postnatal life, the pool of cholesterol becomes constant. The concentration of cholesterol is similar in cells from different tissues and de novo synthesis decreases, because there is supply of exogenous cholesterol with the progression of suckling (1, 3, 13). The effect of Dex on lipid metabolism was established. Injections of Dex, at the dosage of 10 mg/kg b.w. at the 8th d to rats, elevated plasma levels of cholesterol (19). Our experiment showed that the total cholesterol serum levels of the 14-day-old piglets was the highest in the AKG+Dex groups, whereas in the Dex group, the tendency to a higher level was observed. Moreover, the tendency to a lower value of total cholesterol was observed in the AKG group of the 14-d-old piglets. The total cholesterol level in present study in the AKG group was lower by 44% and 50%, compared with the Dex group and the AKG+Dex groups, respectively. Cholesterol is a precursor to all steroid hormones, including mineralcorticoids, glucocorticoids, and sex hormones. Cholesterol synthesis begins with the synthesis of acetocetoyl-CoA from two acetyl-CoA molecules. Acetol-CoA is evolved into tricarboxylic acid cycle when it reacts with the oxaloacetate to form citrate and to release coenzyme A (CoA-SH). Then isocitrate is formed from citrate; and loses a molecule of carbon dioxide, it then undergoes oxidation to form AKG. AKG is a precursor of glutamate and glutamine. At the end of a TCA cycle it is malate, which may cause further reductions in CO2, and may be converted into pyruvate. Acetol-CoA is a product of the pyruvate dehydrogenase reaction. Mechanisms which lead to a decreased level of total cholesterol in serum after oral
AKG administration to their mothers, is still unknown and needs further research.

**Glucose.** The main source of energy in the foetus is glucose. It crosses the placenta by facilitated diffusion dependent on the correlation between foetal and maternal serum glucose levels. Many studies in human and animals; showed the presence of gluconeogenic and glycogenolytic enzymes in early foetal development. Nonetheless, the foetus during prenatal life under physiological conditions, does not produce glucose (17). In the present study, glucose levels of newborn piglets were the highest in the AKG+Dex groups, and similar in the control, while the lowest was in newborns, born by sows treated with Dex during pregnancy. This was probably connected with mechanisms dependent on hormonal regulation, which stimulates the induction of certain regulatory enzymes involved in gluconeogenesis (17). Our earlier study revealed that maternal administration of Dex influenced the serum level of cortisol, growth hormone, insulin-like growth factor, and insulin (39). The higher serum glucose level in all the 14-day-old piglets, in comparison with glucose in non-suckled newborns, was related with an increased metabolism and supply of nutrients, during this neonatal period in piglets (38). The influence of AKG and Dex on the concentration of glucose in serum after 38th d of piglets’ treatment, was not observed. AKG as a precursor of glutamate and glutamine in the diet during pregnancy, influences the liver metabolism in the foetus. In relation to the total amino acids supplied to the foetus through the mother’s placenta, glutamine represents predominant amino acid. This transport is possible by the unique flux of glutamine-glutamate from the placenta to the foetal liver (16, 32, 47). Glutamine plays a crucial role in nitrogen inter-organ flow, not only from skeletal muscles to the gut and kidneys; but in particular through the placenta to the foetal liver, where it is transferred to glutamate. Glutamine-glutamate is a unique way, which supplies carbon for the whole biochemical metabolic pathways, and finally participates in ammoniagenesis and gluconeogenesis in the foetus (16, 47).

**Electrolytes.** AKG given to sows during the last weeks of pregnancy decreased Na⁺, Cl⁻ and K⁺ levels in serum newborns; however it did not have an significant influence after further administration to piglets during their 14th d of neonatal life. The mechanisms of this prenatal action are unclear and need additional investigations. Na⁺ and Cl⁻ concentrations are labile in the cells with maintenance of the high intracellular K⁺ levels; and depend on glucose and amino acid uptake, furthermore may influence the absorption of the other. The concentration gradient is maintained by the sodium and potassium-activated adenosine triphosphatase (Na⁺/K⁺-ATPase) pump. Physiological control mechanism is regulated by rennin, vasopressin, and aldosterone (45). The renin-angiotensin system stimulates the aldosterone release. Increased potassium stimulates aldosterone production, and decreased potassium inhibits the production. Chronic adrenocorticotropic hormone (ACTH) deficiency, which may inhibit the production of aldosterone. Its deficiency results in sodium loss, hyperkalaemia, and acidosis. Glucocorticoids may lead to thrombocytosis, and additionally causes pseudohyperkalaemia. Increased serum K⁺ and total Ca levels in 14-day-old piglets from both groups treated with Dex (Dex and AKG+Dex groups), and Cl⁻ losses in the Dex group were observed in the present study. As much as 50-60% of total serum Ca⁺ is ionized calcium, the essential element for nerve and muscle conduction. Ca⁺ activates and stabilises some enzymes. Vitamin D₃, the calcium binding proteins, calmodulin, and osteopontin, modulate its concentration in cells. The changes of Ca⁺ level, leads to deformation and fractures of bones, posterior paralysis in pigs, changes in immune response and blood clotting. Other studies performed with rats treated with Dex, showed increased sodium and calcium levels without an influence on potassium levels (21).

**Conclusions.** This study is a unique one, and for the first time presents the results indicating that AKG administered to pregnant sows, lowered the total cholesterol level in non-suckled piglets born by these sows. This lowering effect was again observed when AKG was administered during the 14th d to neonatal piglets. We present these pigs as an animal model, which is used in many investigations with glucocorticoid therapy, because they are closer to the humans than rat models, in prenatal and neonatal programming (26, 31).

The pig is known as a model for cardiovascular research for two of the important similarities for humans, in anatomy and the biochemistry of the circulatory system (35). The serum lipoprotein pattern of pig’s serum is similar to that of human serum. The pig becomes a widely used animal model for those studying cholesterol metabolisms, especially during foetal, neonatal, and growing periods (35). It is unknown whether the higher cholesterol content observed in 14-day-old pigs treated with dexamethasone; during the last weeks of prenatal life and first 14th d of neonatal life, persists to adulthood and to later life. It is worth underlining that the maternal gut- oetal bone axis between pregnant sows and foetuses is a unique and attractive item for investigations of metabolic problems. It is very important for humans, and should not be ignored, and may serve as a model of prenatal treatment (31).

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**References**


