INFLUENCE OF ALPHA-KETOGLUTARATE ON BONE MINERAL DENSITY OF THE FEMUR IN PIGLETS

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Abstract

The aim of this study was to determine the influence of daily oral administration of alpha-ketoglutarate (AKG) on bone mineral density of the femur and concentration of 17-β-oestradiol in blood plasma during 70 d of postnatal life in piglets. All the animals were kept under standard rearing conditions. AKG was administered orally from the 1st d of life, while the control piglets were treated in the same way and time with physiological saline. The experimental and control groups were assigned to 6 age subgroups: 3, 14, 21, 35, 56 and 70 d of life. The animals from both groups were euthanised, then bone samples were collected and frozen at –25°C until further analyses. Using dual-energy x-ray absorptiometry (DEXA method) bone mineral density of the femora was estimated. Additionally, 17-β-oestradiol concentration in blood plasma was assayed using RIA-test. The obtained results indicate positive influence of enteral AKG administration on bone mineral density of the femur in piglets. Moreover, AKG increased the level of 17-β-oestradiol in blood plasma in post-weaned piglets.

Key words: piglets, alpha-ketoglutarate, femur, bone mineral density, 17-β-oestradiol.

The important role of glutamine in the neonatal period of life inclined to undertake experiments on the influence of AKG, as a precursor of glutamine, administered orally on the general development with special direction to the skeletal system in piglets (10, 23). In mammals, glutamate is the only amino acid that can be formed by reductive amination of its ketoacid namely α-ketoglutaric acid (10, 15). Recent studies reported beneficial effects of AKG on bone geometrical and mechanical parameters but the mechanism of this interactions is still unknown (6, 7, 9, 19, 24, 26). It is well documented that AKG serves many functions in the organism. For example, it plays a central role in Krebs cycle, stimulates protein synthesis and inhibits protein degradation. It is also a main source of energy for enterocytes and immune cells (8, 24, 25). Moreover, AKG activates a hydroxylation of proline to hydroxyproline, one of the part of type I collagen, which is the main component (about 95%) of bone matrix (8). The skeletal system development and its mineralisation from experimental point of view is still not explained sufficiently because of lack of experimental animal model. Piglets seem to fulfil this gap. This paper revealed up-to-date findings on AKG influence on bone structural properties and changes of 17-β-oestradiol in blood plasma in pre- and post-weaned piglets.

Material and Methods

Experimental design and sampling procedure. The experiment was performed on 120 piglets of Large Polish White breed. The animals were housed in piggery under standard rearing conditions with constant access to fresh water. After weaning, the piglets were fed standard commercial diets twice a day at 7.00 a.m. and 3.00 p.m. The piglets were divided into two equal
groups: experimental and control. Both groups were divided additionally into 6 age subgroups: 3, 14, 21, 35, 56 and 70 d of life. The experimental procedure was conducted from the 1st d of neonatal life to the 70th d of postnatal life. The experimental piglets were treated per os with buffered AKG solution in a dosage of 0.4 g/kg b.w./d. At the same time, the piglets from the control group were treated per os with physiological saline (PhS) in a dosage of 2.0 ml/kg b.w./d. The body weight of the individual piglets was measured every day. After the set up periods piglets were treated with a single i.m. injection of azaperonum (Stresnil; Janssen Pharmaceutical Ltd, Belgium) at a dose of 4 mg/kg b.w. to induce a sedation and then euthanised with a lethal intravenous dose of pentobarbitalum natrium (Morbital; Biowet Pulaawy, Poland). Their right femora were isolated and stored at –25°C until analyses were performed. During the tests, the bones were kept at room temperature. The length and weight of the femora were determined. The weight of the bone was then compared to the total body weight of the individual piglet. The results of this parallel were presented as a percentage of the body weight and defined as a relative weight of the femur.

**Blood sampling.** Blood samples were collected from jugular vein (v. jugularis externa) of each piglets using 10 ml syringe. Blood was centrifuged (1500 g for 20 min) within 30 min after sampling and serum was harvested. The serum was stored at –25°C until analyses were performed.

**Bone mineral density.** Bone mineral density (BMD) was measured with the use of dual-energy x-ray absorptiometry technic (DEXA) using a DPX-A densitometer (Lunar Radiation Corp.). The measurements were carried out in the distal and proximal epiphyses of the femur. The areas of measurements were 1 cm² each. The mean values between the first and second measurement was expressed as absolute values (ABMD) of the BMD. Bone mineral density was calculated by automatic computation and expressed in g/cm².

**17-β-oestradiol.** Serum concentration of 17-β-oestradiol was measured using commercial radioimmunoassay, containing rabbit antigens DSL-4400 (DSLabs Inc., Webster, Texas, USA) and γ-radiance detector PACKARD-CANNBERA. Before the analyses, the serum samples were allowed to reach room temperature (~20°C) and then were mixed thoroughly by gentle inversion.

**Statistical analysis.** All data are presented as a mean ± standard error (±S.E.). Statistical analyses were performed using CORE programme. The Student’s t-test was used to determine statistical significance of differences in variables between the investigated parameters. The level of statistic significance was set at P≤0.05 for all comparisons.

**Results**

The relative weight of the femur presented similar values in both groups during the whole time of observation with the exception of the 21st d of life. At that time the relative weight of the femur in control group was significantly higher (0.54±0.02%) than in animals of the AKG group (0.43±0.02%) (Table 1). The mean values of the BMD of the proximal and distal epiphyses of the femora were similar in the 3rd d of neonatal life in the experimental and control piglets. The tendency to higher level of these values in experimental group was observed at the age of 14 d of neonatal life in comparison to the control. However, there were no significant differences between these two main groups during the first period of neonatal life (3rd and 14th d). At the age of the 21st d the mean values of BMD of the distal epiphysis in control piglets were significantly higher (0.499±0.01 g/cm²) than those in experimental ones (0.434±0.02 g/cm²) (Table 1). Similarly, the BMD of the proximal epiphysis in control group showed higher values (0.533±0.01 g/cm²) in comparison to the experimental one (0.472±0.02 g/cm²), with significant differences stated at the level of P≤0.01.

In the next analysed period, i.e. on the 35th d of life, the mean values of BMD of the proximal and distal epiphyses decreased (Table 2). In contrast, the BMD of the proximal epiphysis in experimental piglets maintained at the same level of values and the difference between the values was statistically significant (P≤0.05). Moreover, the values of the BMD of the proximal epiphysis in AKG group increased at this time to 0.477±0.02 g/cm². At the age of 56 and 70 d of postnatal life of the piglets, BMD of the proximal and distal epiphyses showed tendency to higher values in group which received AKG in comparison to the control group, however, with no significant differences (Table 2).

The calculations of the absolute bone mineral density (ABMD) in the femur revealed tendency to higher values in experimental piglets at the age of 14, 35, 56, and 70 d (Tables 1 and 2). Moreover, at the age of 35 d the difference between mean values of ABMD of the experimental and control piglets was statistically significant (P≤0.05).

The analysis of the 17-β-oestradiol level showed that oral AKG administration increased the concentration of this hormone in blood plasma in post-weaned piglets with statistical significant differences on the 35th and 56th d of postnatal life (Table 2).
Table 1

Characteristics of the femur in control piglets treated *per os* with PhS and experimental ones treated in the same way with AKG before weaning

<table>
<thead>
<tr>
<th>Day of life</th>
<th>3</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PhS</td>
<td>AKG</td>
<td>PhS</td>
</tr>
<tr>
<td>Number of investigated bones</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Relative weight of femur (%)</td>
<td>0.52 (±0.10)</td>
<td>0.48 (±0.08)</td>
<td>0.49 (±0.04)</td>
</tr>
<tr>
<td>BMD of proximal epiphysis (g/cm²)</td>
<td>0.294 (±0.04)</td>
<td>0.289 (±0.04)</td>
<td>0.437 (±0.03)</td>
</tr>
<tr>
<td>BMD of distal epiphysis (g/cm²)</td>
<td>0.286 (±0.02)</td>
<td>0.304 (±0.02)</td>
<td>0.401 (±0.04)</td>
</tr>
<tr>
<td>ABMD of femur (g/cm²)</td>
<td>0.290 (±0.03)</td>
<td>0.296 (±0.03)</td>
<td>0.419 (±0.03)</td>
</tr>
<tr>
<td>17-β-oestradiol concentration (pg/ml)</td>
<td>24.74 (±1.95)</td>
<td>36.27 (±6.79)</td>
<td>165.02 (±15.65)</td>
</tr>
</tbody>
</table>

* P ≤ 0.05; ** P ≤ 0.01.
Table 2

Characteristics of the femur in control piglets treated per os with PhS and experimental ones treated in the same way with AKG after weaning

<table>
<thead>
<tr>
<th>Day of life</th>
<th>35</th>
<th>56</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>PhS</td>
<td>AKG</td>
<td>PhS</td>
</tr>
<tr>
<td>Number of investigated bones</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Relative weight of femur (%)</td>
<td>0.52 (±0.03)</td>
<td>0.50 (±0.04)</td>
<td>0.43 (±0.02)</td>
</tr>
<tr>
<td>BMD of proximal epiphysis (g/cm²)</td>
<td>0.399 (±0.02)</td>
<td>0.472* (±0.02)</td>
<td>0.509 (±0.03)</td>
</tr>
<tr>
<td>BMD of distal epiphysis (g/cm²)</td>
<td>0.430 (±0.02)</td>
<td>0.477 (±0.02)</td>
<td>0.513 (±0.02)</td>
</tr>
<tr>
<td>(\Delta)BMD of femur (g/cm²)</td>
<td>0.414 (±0.02)</td>
<td>0.474* (±0.02)</td>
<td>0.511 (±0.03)</td>
</tr>
<tr>
<td>17-(\beta)-oestradiol concentration (pg/ml)</td>
<td>25.63 (±2.20)</td>
<td>66.10** (±12.14)</td>
<td>29.83 (±4.80)</td>
</tr>
</tbody>
</table>

* P ≤ 0.05; ** P ≤ 0.01.
Discussion

At the present time it is well documented that the mechanical properties of bones depend on maintaining a delicate balance between bone resorption and bone formation that plays an important role in determining bone strength and integrity (18, 27). Our earlier study proved that the mechanical parameters of bones, such as maximum elastic strength and ultimate strength are also closely correlated to mineral contents of bones (6, 7, 8, 9, 19). Thus, evaluation of bone mineral density (BMD) using DEXA method is suitable for the assessment of the progress of mineralization during the growth not only in humans but in animals as well. Moreover, this method is still the most available and frequently used in the evaluation of skeletal system properties.

Although the molecular action of oestrogens on bone is not fully understood it is generally known that oestrogen loss is associated with elevated bone resorption caused by a rise of osteoclast number and it is the most common cause of osteoporosis in postmenopausal women (11, 14, 17). Results from animal models concerning the oestrogen level and its influence on the skeletal system are still not sufficient.

The obtained results on the model of the femur proved the existence of three different phases of growth and development of this bone during 70 d of postnatal life in the piglet. Additionally, oral administration of AKG influenced positively some of these phases of the development. The 1st phase is characterized by the rapid growth of the body mass, femur weight and its mineralization. This phase lasted up to the 14th d of neonatal life. The 2nd phase was extended up to 35 d of life with stability of these values. The 3rd phase presented again dynamic increase in the analysed parameters and lasted up to the 70th d of life. Analysis of mechanical and geometrical parameters revealed during the 1st phase a significant increase in the values of the femur in piglets during the first two weeks of neonatal life. During the 2nd phase there were no significant changes in the analysed parameters. During the 3rd phase a dynamic increase in these values was observed again with statistical significance between the 21st and 70th d of life (6, 7, 8, 19).

It is interesting that AKG administered orally in piglets did not disturb the existence of these three phases but increased during the 1st and 3rd phases the mean values of the analysed parameters in comparison to the controls. It is worthy of underlining that the femur of the experimental piglets which were treated with AKG did not show the decrease in the level of the analysed values during the period of weaning at the 28th d of life as it was observed in the control piglets when these values were lower. It may be concluded that the weaning period is connected with lower dynamics of bone and whole body weight growth together with essential bone parameters which reflected the process of maturation together with growth of the bone tissue. AKG may be treated as a protecting metabolite when administered orally in piglets, countering negative influences of weaning periods directed to the whole body weight and to the skeleton represented by the femur as a model bone in our research.

Several possible mechanisms may contribute to an increase in the concentration of 17-β-oestradiol in blood plasma in the piglets of both groups in pre-weaning period. It may be presumed that the reason of higher concentration of 17-β-oestradiol in blood plasma of the pre-weaning piglets is the presence of this hormone in colostrum and then in milk of the sows (5, 21). These observations are verified by the measurement of this hormone in our study in post-weaned piglets, when its dramatical decrease was observed just after the weaning. It may be suggested that the cessation of suckling and the change in diet during weaning was connected with abrupt alimentary stoppage of supply of oestradiol in our experiment.

In conclusion, results of this study indicated that there are three different phases of femur mineralization and formation of structural, mechanical and functional properties during 70 d of postnatal life of piglets. AKG administered orally during this period positively influenced the analysed mineral changes in the femur not only during the first and last two weeks of this period but also during the post weaning weeks. These results suggest the possibility to apply AKG in practice as a nutritional factor improving in piglets not only the growth of the whole body but of leg bones as well. Further research is needed to enable gaining new knowledge about the mode of action and other effect of this metabolite of glutamine.

References