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Influence of Simulated Microgravity on Leg Bone Development in Japanese Quail Chicks

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Abstract

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The goal of this study was to assess the influence of simulated microgravity (hypodynamy) on morphological characteristics and mineral content of the long bones of the right leg of female Japanese quail chicks from 3 to 56 days of age. Femur and tibiotarsus were collected at 14, 28, 42 and 56 days; the variables studied were: weight (g), length and width (mm), bone index (ash weight/bone length in mg·mm^{-1}), bone breaking strength (N) and calcium, phosphorus and magnesium content (mg·g^{-1} dry bone).

All variables increased with bird age. However, the effect of hypodynamy on the examined variables was frequently large and significant. The tibiotarsus mean weight was significantly (P < 0.001) reduced by 0.154 g, although the femur mean weight in hypodynamy reared quails was almost identical to that of the age-matched control at 56 days. Similarly, there was significantly decreased tibiotarsus mean length (P < 0.01) by 3.16 mm and tibiotarsus mean width (P < 0.001) by 0.44 mm by 56 days. Similarly, bone index and bone breaking strength of both bones in birds reared under hypodynamy were significantly lower (P < 0.001) than those of control. Moreover, hypodynamy significantly (P < 0.01) decreased the calcium content in femur and tibiotarsus at 42 days; phosphorus content in tibiotarsus was reduced significantly at 14 and 42 days (P < 0.001; P < 0.05, respectively) and magnesium content was decreased at 42 days (P < 0.01). On the contrary, magnesium content in both long bones of test quails was increased significantly (P < 0.01) at 14 days. Other differences in femur and tibiotarsus mineral content between test and control group were not significant. These findings suggest that, although hypodynamy reduce bone growth of test birds, the long bones of the right leg of female Japanese quail were able to develop under conditions of simulated microgravity.

Hypodynamy, femur, tibiotarsus, calcium, phosphorus, magnesium

In humans and other mammals, the microgravity has been observed to have a negative impact on bone homeostasis as a result of reduced calcium absorption and negative calcium balance during extended space flight duration (Daniel et al. 1997). Similarly, the simulated microgravity caused reduction in mineral content of leg bones of adult Japanese quail as compared to control birds (Sviatko et al. 1998). However, it is unknown, how simulated microgravity or long-duration space flight may influence the avian and human postnatal development during the subsequent ontogenetic phases. A partial answer may be provided by the ground-based animal and human models of simulated microgravity (hypodynamy).

During the last three decades, the effects of microgravity on embryogenesis of Japanese quail have been studied. The results show that in some instances quail embryogenesis can be performed in the environment of weightlessness without serious difficulties (Guryeva et al. 1993; Dadasheva et al. 2001). Quail chicks developing to hatch had all external characteristics of a normal development. In flight experiments on board MIR space station, there were successfully hatched 8 (March 1990) and 36 (February 1999) quail chicks from 21 and 56 fertilised eggs (Boďa et al. 1992; Sabo et al. 2001).

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The first studies to examine the effect of hypodynamy were conducted with adult Japanese quail (Juráni et al. 1983). However, there are minimal data on survival and development of Japanese quail chicks in conditions of hypodynamy (Škrobánek et al. 2001; Škrobánek and Hrančová 2003).

The purpose of the present study was to investigate the influence of simulated microgravity (hypodynamy) on long bone (femur and tibiotarsus) development in female Japanese quail chicks reared under hypodynamy from day 3 posthatch to 56 days.

Materials and Methods

Eighty female, newly-hatched Japanese quail chicks (Laying Line 01 Ivanka pri Dunaji), mean body weight 7.29 ± 0.43 g, were used in the present study. On the third day after hatching, forty-one chicks (test group) were exposed to hypodynamy (as described in Škrobánek et al. 2001). Hypodynamy is a simulation of weightlessness where quails are placed in individual slings suspended by a flexible metal device such that their legs cannot touch the floor. However, chicks may move about freely by moving their wings. The size of the slings was enlarged at 7, 14, 21, 28 and 35 day to age (from 4 cm × 3 cm to 5 cm × 4 cm to 6 cm × 5 cm to 7 cm × 6 cm and 8 cm × 7 cm) to accommodate the growth of the quail. At the same time, thirty-nine quail chicks (control group) were placed in a rearing box (1.2 × 0.6 × 0.3 m). The birds of both test and control groups were kept in these conditions until 56 days of age in a windowless poultry room with controlled ventilation and heating by infrared lamps. The temperature was gradually reduced from 35-36 °C for the first few days after hatching to about 20 °C at 4 weeks, remaining at this level until the end of the experiment. A commercial starter mash HYD-13 and water were available *ad libitum.* The granular diet contained 260 g/kg protein and 11.5 MJ metabolisable energy/kg. Lighting in the rearing room was continuous. The care and use of animals were in accordance with laws and regulations of the Slovak Republic and as approved by the ethical committee of the Institute of Animal Biochemistry and Genetics of Ivanka pri Dunaji.

Test and control quail were killed by cervical dislocation at 14, 28, 42 and 56 days. Thirty-two (test) and thirty-five (control) right femur and tibiotarsus samples were removed from the quail, dissected free of muscle, ligaments and tendons. The weight of freshly dissected bones was recorded (g). The length and width of the bones were measured using hand callipers (mm). Breaking strength (N) of the femur and tibiotarsus was measured on a materials testing apparatus (developed by Marcinka and Gažo 1964). The bone index (proposed by Seedor et al. 1991), that represents an index of bone density, was calculated by dividing bone ash weight by bone length ($mg \cdot mm^{-1}$). Bones were defatted by submerging in diethyl ether for 2 hours and placed in an oven at 105 °C for 4 hours and the dry weight measured. Bones were ashed in a furnace at 550 °C for 6 hours and ash weight obtained. The ash was dissolved in 2.5 ml hydrochloric acid (water solution 1:1) and diluted to 50 ml using redistilled water. The content of calcium, inorganic phosphorus and magnesium was determined by colourimetric kit assay (BIO-LA-TEST, Lachema Brno, Czech Republic). The results were expressed as milligrams of calcium, phosphorus and magnesium per gram of dry bone weight (femur, tibiotarsus). Statistical comparisons between the overall mean values for test and control groups were calculated using the Student's t-test.

Results

The effects of hypodynamy on bone weight, length and width are summarised in Table 1. We observed that the femur and tibiotarsus absolute weight gradually increased with age in both groups. However, the bone weight of hypodynamy quail was significantly smaller than that of age-matched controls already by day 14 ($P < 0.001$). The maximum differences in femur and tibiotarsus weight were observed on day 28. By 56 days, the quail reared in the hypodynamy were unable to make up this difference, regardless of the increased bone weight gains. Femur and tibiotarsus of the test group showed reduced weight by 0.05 and 0.154 g ($P < 0.001$) vs. control.

Similarly, tibiotarsus absolute length was affected by hypodynamy ($P < 0.001$), but no significant differences in femur length were found between the experimental and control groups. At day 56, the mean tibiotarsus length of hypodynamy quail was decreased by nearly 3 mm ($P < 0.01$).

The mean long bone width was altered throughout the experimental period. At 56 days, animals exposed to hypodynamy had significantly ($P < 0.001$) decreased absolute tibiotarsus width (by 0.44 mm) and slightly lower femur width (by 0.01 mm) when compared to controls.

Table 1. Weight, length and width of Japanese quail long bones

Age (days)	Femur		Tibiotarsus	
	H	C	H	C
		Weight (g)		
14	0.162 ± 0.015^{+++}	0.215 ± 0.023	0.204 ± 0.026^{+++}	0.285 ± 0.021
28	0.256 ± 0.052^{+++}	0.436 ± 0.025	0.331 ± 0.078^{+++}	0.528 ± 0.057
42	0.355 ± 0.018^{+++}	0.458 ± 0.036	0.394 ± 0.044^{+++}	0.537 ± 0.055
56	0.461 ± 0.061	0.511 ± 0.051	0.445 ± 0.048^{+++}	0.599 ± 0.053
		Length (mm)		
14	24.69 ± 1.54	25.99 ± 1.21	29.60 ± 1.80^{+}	32.45 ± 1.01
28	32.50 ± 3.41	35.10 ± 0.99	38.50 ± 3.52^{+++}	43.20 ± 1.07
42	36.47 ± 1.02	36.30 ± 0.70	44.06 ± 1.20^{+++}	46.90 ± 0.94
56	36.67 ± 0.27	37.35 ± 0.75	44.30 ± 1.60^{++}	47.46 ± 1.10
		Width (mm)		
14	1.60 ± 0.25^{+++}	1.90 ± 0.20	1.50 ± 0.13^{+++}	1.70 ± 0.17
28	2.10 ± 0.21^{+++}	2.35 ± 0.08	1.80 ± 0.12^{+++}	2.15 ± 0.10
42	2.30 ± 0.18^{+++}	2.60 ± 0.08	2.05 ± 0.15^{+++}	2.45 ± 0.07
56	2.61 ± 0.17	2.62 ± 0.21	2.00 ± 0.18^{+++}	2.44 ± 0.09

$^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$
H - hypodynamy, C - control

Table 2. Effect of hypodynamy on bone index (ash weight / bone length) and breaking strength of Japanese quails

Age (days)	Femur		Tibiotarsus	
	H	C	H	C
		Bone index (mg/mm)		
14	0.976 ± 0.092^{++}	1.117 ± 0.072	1.001 ± 0.114	1.170 ± 0.160
28	1.462 ± 0.301	1.592 ± 0.186	1.031 ± 0.078^{++}	1.144 ± 0.073
42	1.258 ± 0.058^{+}	1.366 ± 0.090	1.056 ± 0.055^{++}	1.158 ± 0.056
56	1.311 ± 0.199	1.427 ± 0.251	1.081 ± 0.102	1.192 ± 0.224
		Breaking strength (N)		
14	25.166 ± 1.000^{+++}	29.160 ± 1.054	20.313 ± 1.100^{+++}	24.111 ± 0.740
42	31.333 ± 1.960^{+++}	46.214 ± 1.046	24.063 ± 1.321^{+++}	37.875 ± 3.640

$^{++}P < 0.01$; $^{+++}P < 0.001$
H - hypodynamy, C - control

The effect of hypodynamy on bone index and bone breaking strength are shown in Table 2.

The bone index (ash weight/bone length) in the experimental group was lower than in the control group. The femur index for quail reared in hypodynamy decreased significantly at 14 and 42 days ($P < 0.05$) and the tibiotarsus index at 28 and 42 days ($P < 0.01$).

The bone breaking strength increased markedly between 14 and 42 days in both groups, although hypodynamy significantly reduced the femur and tibiotarsus breaking strength ($P < 0.001$).

The results of the measurements of calcium in the long bones of quail chicks exposed to hypodynamy vs. controls are presented on Fig.1 and 2. Femur and tibiotarsus calcium contents were similar throughout the entire experiment. Femur and tibiotarsus calcium significantly declined between 14 to 28 days and then increased between 28 to 42 days ($P < 0.01$). At 42 and 56 days, there was no significant difference in calcium content. Femur

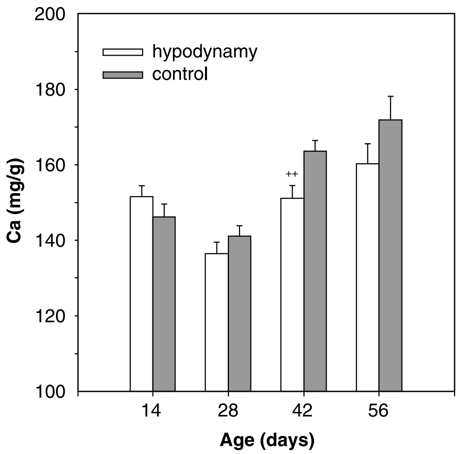

Fig. 1. Calcium content in the femur (mg/g dry bone) (**$P < 0.01$)

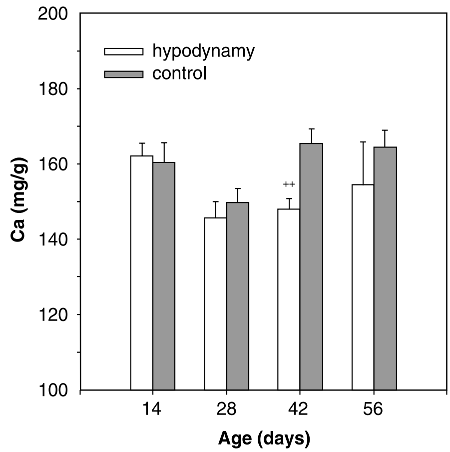

Fig. 2. Calcium content in the tiobiotarsus (mg/g dry bone) (**$P < 0.01$)

and tibiotarsus calcium content of hypodynamy quail was lower than control throughout the experiment, except 14 days; a significant difference was only observed at 42 days ($P < 0.01$). The maximum femur and tibiotarsus calcium content in the hypodynamy group was about 160 mg·g^{-1} at 56 days and 162 mg·g^{-1} at 14 days, respectively.

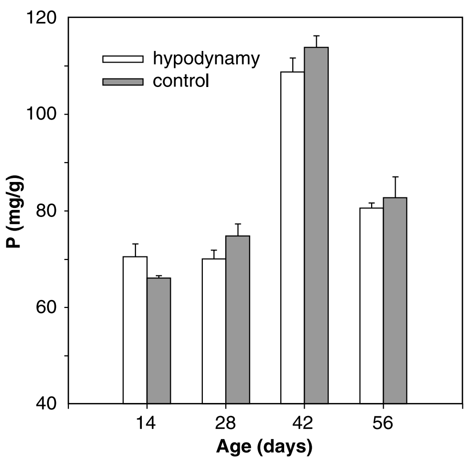

Fig. 3. Phosphorus content in the femur (mg/g dry bone)

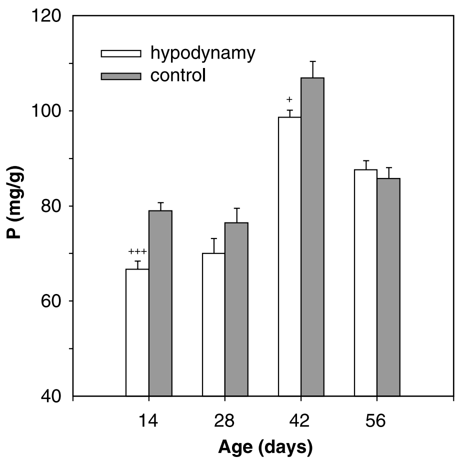

Fig. 4. Phosphorus content in the tibiotarsus (mg/g dry bone) (***$P < 0.001$, *$P < 0.05$)

The mean femur phosphorus content of hypodynamy group was similar at 14 and 28 days (Fig. 3), but increased significantly at 42 days and had declined again significantly by the end of the experiment ($P < 0.001$). Phosphorus content of the femur in control birds increased from 14 to 42 days and then decreased at 56 days ($P < 0.001$). Similarly, tibiotarsus phosphorus content in the experimental and control group increased to 42 days and then decreased at the end of experiment (Fig. 4). Except for the tibiotarsus phosphorus content at 14 days ($P < 0.001$) and 42 days ($P < 0.05$), the differences between hypodynamy and control group were not significant.

The femur magnesium content of test and control quail increased from 14 to 42 days ($P < 0.01$) and decreased significantly at 56 days ($P < 0.001$). Similarly, tibiotarsus magnesium content varied in both groups (Fig. 5 and 6). However, the femur and tibiotarsus magnesium content of test group was non-significantly lower (with the exception in tibiotarsus at 42 days; $P < 0.01$) than the control group throughout the experiment, except 14 days ($P < 0.001$ and $P < 0.01$, respectively).

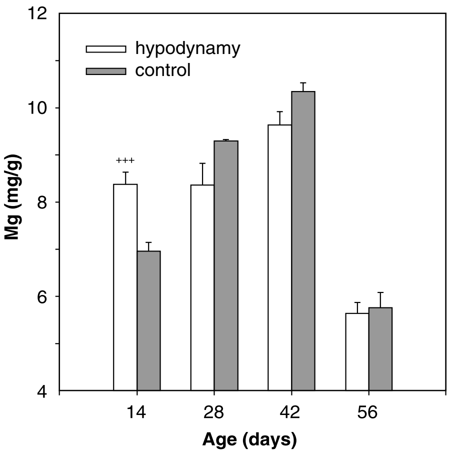

Fig. 5. Magnesium content in the femur (mg/g dry bone) (***$P < 0.001$)

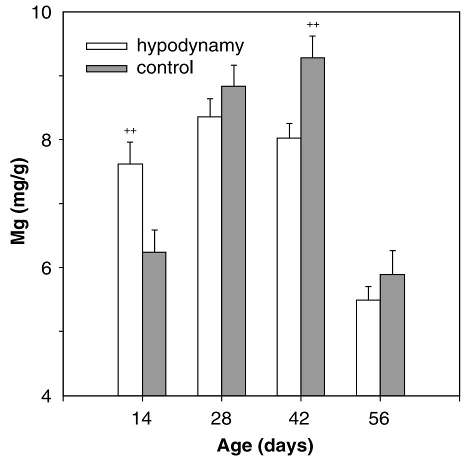

Fig. 2. Magnesium content in the tibiotarsus (mg/g dry bone) (**$P < 0.01$)

Discussion

Growth is the most studied factor to estimate postnatal development of an individual (Ricklefs 1979; Blom and Lilja 2004). There are many studies that characterise the growth and development in Japanese quail under standard earth conditions (Lilja and Marks 1991; Aggrey 2003; Hyánková et al. 2001). Several studies investigated the rate of long bone development (Starck 1996), however, only a few studies examined the effects of altered gravity (microgravity) on quail postnatal development (Boďa et al. 1992; Sabo et al. 2001).

The purpose of the present study was to evaluate the effect of simulated microgravity (hypodynamy) on the morphology and composition of femur and tibiotarsus of developing Japanese quail females from 3 to 56 days of age. The results demonstrated that hypodynamy had a significant negative effect on most of the morphological characteristics of long bones of the leg for the duration of the experiment. Significant differences were also observed between test and control quail for bone index and breaking strength. Similarly, calcium, phosphorus and magnesium contents of femur and tibiotarsus varied significantly in several intervals of measurement.

This variation was not entirely unexpected, since hypodynamy and microgravity are known to be stressful factors (Juráni et al. 1991). Our previous experiments demonstrate the mean body weight, food consumption and conversion and size of the right leg of female quail chicks, when exposed to simulated weightlessness conditions from hatching to maturity, were significantly reduced as compared with the age-matched controls at 56 days (Škrobánek et al. 2003). Similarly, it was reported that periosteal bone formation and tubular bone growth gradually decreased during the 33-day hypodynamy in legs of adult

Japanese quail (Guryeva et al. 1998). The hypodynamy treatment also evoked different changes in the calcium and phosphorus content in Japanese quail males and females. The calcium content in the marrow of femur was only slightly changed in hens, while in cockerels it was significantly decreased in the upper part and marrow of the tibiotarsus. Furthermore, changes in the phosphorus content were observed in the male tibiotarsus (Antalíková et al. 2001). In another study, the 84-days hypodynamy decreased calcium, copper, manganese, zinc, iron and increased phosphorus, magnesium, potassium and natrium content in the femur and tibiotarsus of quail (Sviatko et al. 1998). On the contrary, the distribution of phosphorus in the skeleton of Japanese quail exposed to hypodynamy did not change in the experiments by Jankela et al. (1998). On the basis of these observations it appears that the absence of moving activities in the adult Japanese quail exposed to hypodynamy will be influenced especially the mineral metabolism in the long leg bones than that in the skeleton.

In summary, this report is the first study to examine the effects of simulated microgravity on the leg bone development in Japanese quail from day 3 after hatching to 56 days. The present results indicate that simulated microgravity has a significant impact on the growth and mineral deposition of leg long bones of quail chicks. Hence, this data may be useful for understanding quail ontogeny during exposure to altered gravitation during space flight. However, for appropriate validation of these results, it is necessary to conduct a similar experiment in the weightlessness environment of space.

Vplyv simulovanej mikrogravitácie na vývoj kostí nohy u kurčiat prepelice japonskej

V práci bol skúmaný vplyv simulovanej mikrogravitácie (hypodynamie) na morfologickú charakteristiku a ukladanie minerálnych látok v dlhých kostiach nohy prepelice japonskej od 3 do 56 dní veku. Za týmto účelom bola vo veku 14, 28, 42 a 56 dní zisťovaná hmotnosť (g), dĺžka (mm), šírka (mm), index kosti (hmotnosť popola/dĺžka kosti; $mg \cdot mm^{-1}$) a pevnosť (N) femuru a tibiotarsu, ako aj obsah vápnika, fosforu a horčíka ($mg \cdot g^{-1}$ suchej kosti).

Hodnoty všetkých sledovaných ukazovateľov sa zvyšovali s vekom zvierat, pričom vplyv hypodynamie bol významný. Priemerná hmotnosť tibiotarsu prepelíc pokusnej skupiny bola vo veku prepelíc 56 dní v porovnaní s kontrolou nižšia o 0,154 g ($P < 0{,}001$), hoci priemerná hmotnosť femuru bola takmer rovnaká. Obdobne, na konci experimentu bola signifikantne znížená priemerná dĺžka tibiotarsu o 3,16 mm ($P < 0{,}01$) a jeho priemerná šírka o 0,44 mm ($P < 0{,}001$). Redukované boli tiež hodnoty indexu kosti a pevnosti obidvoch kostí pravej nohy zvierat chovaných v hypodynamii ($P < 0{,}001$). Hypodynamia taktiež signifikantne ($P < 0{,}01$) znižovala obsah vápnika vo femure a tibiotarse v 42 dňoch; v tibiotarse bol obsah fosforu signifikantne znížený v 14 a 42 dňoch ($P < 0{,}001$; $P < 0{,}05$) a obsah horčíka v 42 dňoch veku zvierat ($P < 0{,}01$). Naopak, obsah horčíka v obidvoch dlhých kostiach pokusných zvierat významne narástol v 14 dňoch veku ($P < 0{,}01$). Ostatné rozdiely v obsahu minerálnych látok vo femure a tibiotarse neboli štatisticky významné.

Na základe dosiahnutých výsledkov možno konštatovať, že hoci hypodynamia redukovala rast dlhých kostí pokusných zvierat, dlhé kosti pravej nohy kurčiat prepelice japonskej samičieho pohlavia boli schopné vyvíjať sa aj v podmienkach simulovanej mikrogravitácie.

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