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ORIGINAL ARTICLE

Intrauterine programming of bone. Part 2: Alteration of skeletal structure

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Abstract
Summary Osteoporosis is believed to be partly programmed in utero. Rat dams were given a low protein diet during pregnancy, and offspring were studied at different ages. Old aged rats showed site-specific strength differences. In utero nutrition has consequences in later life.
Introduction Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that age-related decrease in bone mass has, in part, a fetal origin and investigated this using a rat model of maternal protein insufficiency.
Methods Dams received either 18% w/w (control) or w/w 9% (low protein) diet during pregnancy, and the offspring were studied at selected time points (4, 8, 12, 16, 20, 47, 75 weeks).
Results Using micro-CT, we found that at 75 weeks of age female offspring from mothers fed a restricted protein diet during pregnancy had femoral heads with thinner, less dense trabeculae, femoral necks with closer packed trabeculae, vertebrae with thicker, denser trabeculae and midshaft tibiae with denser cortical bone. Mechanical testing showed the femoral heads and midshaft tibiae to be structurally weaker, whereas the femoral necks and vertebrae were structurally stronger.
Conclusions Offspring from mothers fed a restricted protein diet during pregnancy displayed significant differences in bone structure and density at various sites. These differences result in altered bone characteristics indicative of significantly altered bone turnover. These results further support the need to understand the key role of the nutritional environment in early development on programming of skeletal development and consequences in later life.

Keywords Density · DEXA · In utero · Micro computed tomography · Programming · Structure

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Introduction

Osteoporosis is a multifactorial skeletal disorder characterised by low bone mass and microarchitectural deterioration of bony tissue, with a consequent increase in the risk of fracture [1]. The bone mass of an individual in later life depends upon the peak bone mass obtained during skeletal growth, and the subsequent rate of bone loss. Current evidence suggests that peak bone mass is partly inherited, although available genetic markers are only able to explain a small proportion of the variance in individual bone mass or fracture risk [2]. In addition, recent epidemiological studies indicate that poor growth during fetal life, infancy and childhood is associated with decreased bone mass in

adulthood and an increased risk of fracture [3–6]. However, there is little understanding, to date, of the cellular and molecular mechanisms, whereby environmental modulation in utero [7, 8] may lead to an altered skeletal growth trajectory and propensity to later osteoporotic fracture.

So far there has been no direct evidence that maternal nutrient restriction can cause osteoporosis in the offspring. Previously, we showed that in rats at the time of natural death, maternal protein restriction resulted in a reduction in bone area and BMC, but not BMD, among the offspring in late adulthood, as well as a widened epiphyseal growth plate in the protein-restricted offspring [9]. We have now extended this observation in a larger study in which we have also assessed bone micro-architecture using high resolution micro-computed tomography and mechanical testing.

Methods and materials

Animal and experimental design

All animal experimentation was performed under license from the Home Office in accordance with the Animals (Scientific Procedures) Act (1986). All rats were raised within the University of Southampton Biomedical Research Facility and were housed in appropriate environments in rooms maintained at 22°C with a 12 h:12 h light: dark cycle. A total of 26 virgin adult female Wistar rats, weighing approximately 200 g each, were housed individually with one of five available adult male Wistar rats for 1 to 5 days. A semen plug on the floor of the mating cage confirmed successful mating, and was taken as day zero of gestation. At this time, pregnant dams were singly housed and allocated to either a normal protein diet containing 18% (w/w) casein (n=13) or a low protein diet containing 9% (w/w) casein (n=13), as previously described [10]. The diets were manufactured from purified ingredients within the University of Southampton facility, and were balanced in energy content through the addition of carbohydrate to the low protein diet; the full dietary composition has been published elsewhere [11]. Pregnant dams were fed throughout the 21 days of gestation ad libitum. Total food intake was not recorded. At birth, the mass of all pups was recorded, and litters were culled to a maximum of eight pups per litter. Litters were culled to eight to prevent possible variation in neonatal growth related to the unavailability of milk during suckling. *All rats* were transferred to a standard laboratory non-purified chow diet (CRME, Special Diet Services Ltd, Witham, Essex, UK) throughout the suckling period. Pups were weaned onto the chow diet at 4 weeks and maintained on the CRME diet throughout the period of the study. Offspring, from a range of litters, from normal and low protein groups were harvested at 4, 8, 12, 16, 20 and 47 week time points.

Collection and preparation of bone specimens

All bones were removed from animals after death. Femora and tibiae were removed from all animals and their lengths were measured using digital calipers (Mitutoyo, Andover, Hampshire, UK). For DEXA analysis, both femora and tibiae were removed, together with the skull and vertebrae.

Bone mineral measurement

Sixty-seven offspring (32 from the maternal low protein group and 35 born to control dams; 14 males and 18 males in the low protein group and 18 males and 17 females in the control group) underwent assessment of bone mineral by DEXA (PIXImus, GE Lunar, Madison, Wisconsin, USA). Whole body BMC, area, and BMD (g/cm^2) were evaluated using the high resolution mode (0.18 mm resolution).

3D Computed tomography

Femora, calvaria and vertebrae from 75-week-old female rats (five control and five in the maternal low protein group) were scanned using an Xtek Benchtop 160Xi scanner (Xtek Systems Ltd, Tring, Hertfordshire, UK) equipped with a Hamamatsu C7943 x-ray flat panel sensor (Hamamatsu Photonics, Welwyn Garden City, Hertfordshire, UK). All scans were taken at 100 kV, 60 μA using a molybdenum target with an exposure time of 534 ms and 4× digital gain. Reconstructed volume images were analysed using VGStudio Max 1.2.1 software (Volume Graphics GmbH, Heidelberg, Germany). All the voxels which formed the structure were automatically assigned Hounsfield units.

Mechanical bone strength testing

All testing was performed on a Bose Electroforce 3200 electromagnetic test instrument (Bose Corporation, Eden Prairie, Minnesota, USA). The midshaft strength of tibia and femur was tested using a three-point bend test. They were placed anterior surface down on two supports equidistant from the ends and 10 mm apart. They were centrally loaded at a constant rate (6 mm/min) up to fracture. Load-displacement curves were used to calculate maximum load, maximum deflection, stiffness, energy, and stress. Stiffness was calculated as the slope of the linear portion of the load-displacement curve. Energy was determined as the area under the curve. Stress was determined as the maximum load divided by the cross sectional area as determined by computed tomography. For femoral neck testing, the femur was mounted vertically using Technovit 3040 bone cement (Heraeus Kulzer GmbH, Hanau, Wehrheim, Germany) and the femoral head loaded at a constant rate (6 mm/min) until the neck fractured. For

femoral head testing the head was placed upright with the femoral neck horizontally and again loaded at a constant rate (6 mm/min) until failure. The vertebral body was placed between two small plates so only the body would be tested and loaded at a constant rate (6 mm/min) until failure.

Statistics

T-tests and Wilcoxon-Mann-Whitney statistical analysis was performed using the SPSS for Windows program version 14 (SPSS UK, Woking, Surrey, United Kingdom).

Results

Femur and tibia lengths

Figure 1a shows the results of femur lengths from both male and female offspring from mothers fed either the control diet (18% w/w protein) or restricted diet (9% w/w protein). Males in the restricted diet group had significantly longer femora compared to controls at 47 weeks of age (p-value 0.04). Males in the restricted diet group had significantly longer femora per unit mass

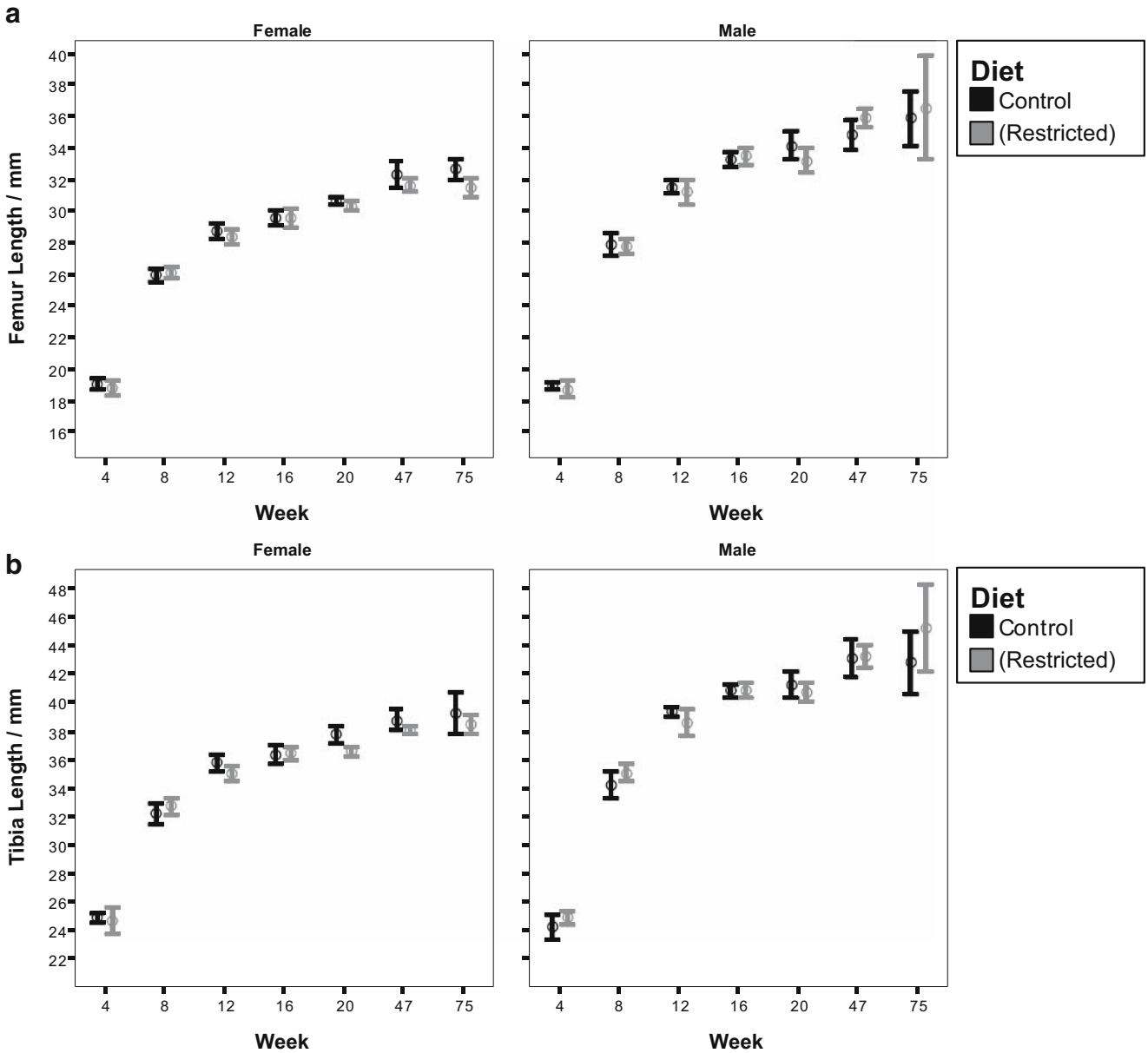

Fig. 1 Femur and tibia lengths. **a**. Variation in femur length in mm from offspring of mothers fed either control protein (18%) or low protein restricted (9%) diet. Each point represents mean and 95% confidence limits with $n=10$. **b**. Variation in tibia length in mm from offspring of mothers fed either control protein (18%) or low protein restricted (9%) diet. Each point represents mean and 95% confidence limits with $n=10$

compared to controls at 12 weeks of age when normalised to mass (p-value 0.03), but significantly shorter per unit mass compared to controls at 20 weeks of age (p-value 0.03). Females in the restricted diet group had significantly shorter femora compared to controls at 75 weeks of age (p-value 0.009). However, when normalised to mass the only difference found was that the restricted diet females showed longer femora per unit mass at 12 weeks of age than the control female group (p-value 0.01).

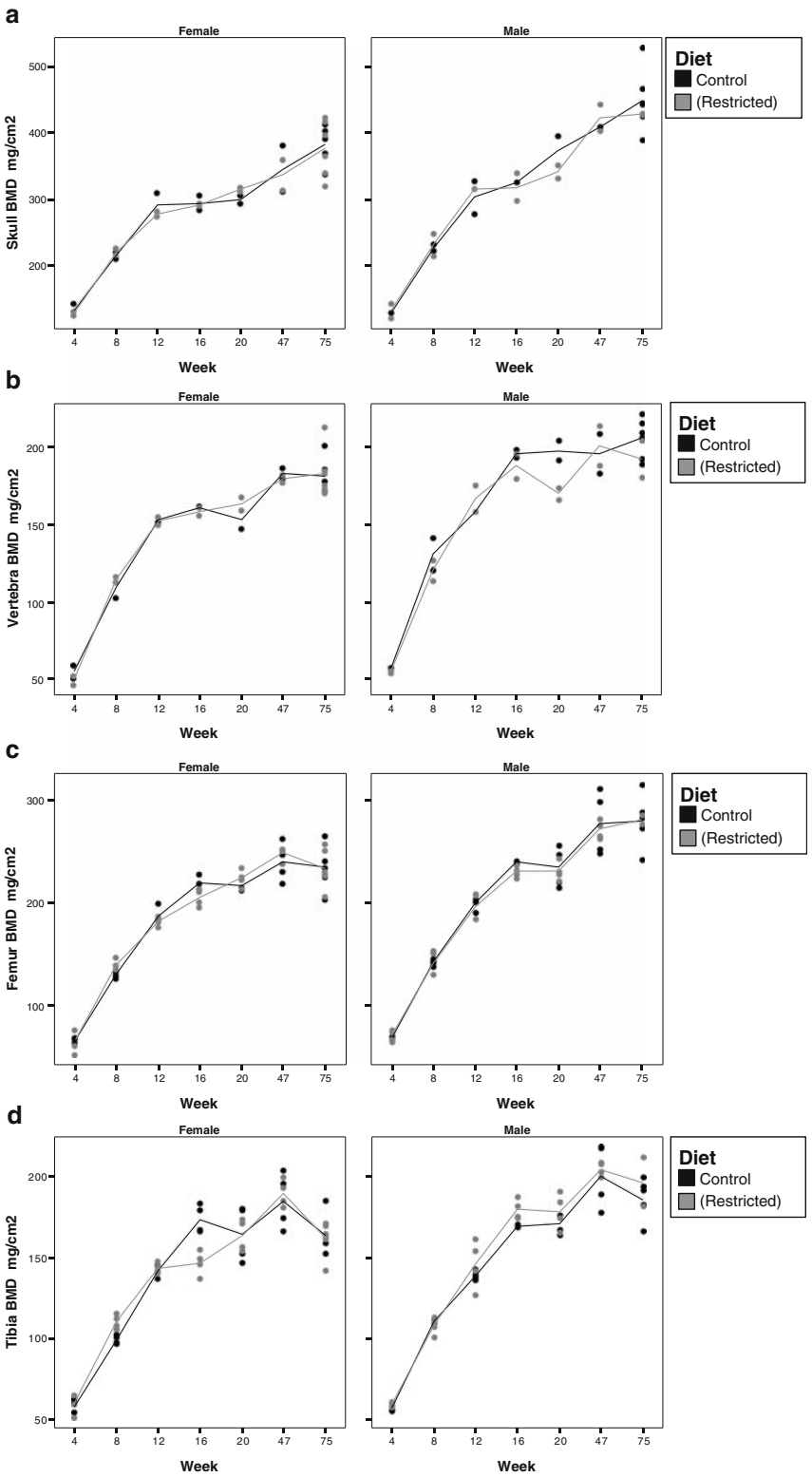

Fig. 2 Dexa analysis of skull, vertebra, femur, and tibia. **a**. Variation in skull bone mineral density (BMD) in offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=2 (n=5 for 75-week-old females, n=6 for 75-week-old control males and n=2 for 75-week-old restricted males). **b**. Variation in vertebra bone mineral density (BMD) in offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=2 (n=5 for 75-week-old females, n=6 for 75-week-old control males and n=2 for 75-week-old restricted males). **c**. Variation in femur bone mineral density (BMD) in offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=4 (n=10 for 75-week-old females, n=12 for 75-week-old control males and n=4 for 75-week-old restricted males). **d**. Variation in tibia bone mineral density (BMD) in offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=4 (n=10 for 75-week-old females, n=12 for 75-week-old control males and n=4 for 75-week-old restricted males)

Figure 1b shows the results of tibia lengths from both male and female offspring from mothers fed either the control diet (18% protein) or restricted diet (9% protein). No differences were found in either tibia length or length per unit mass in males between the two diet groups. Females in the restricted diet group had significantly shorter tibiae compared to controls at 12 weeks (p-value 0.03) and 20 weeks of age (p-value 0.002), as well as significantly longer tibiae per unit mass compared to controls at 12 weeks of age (p-value 0.04).

Dual energy x-ray analysis

Figure 2 shows the results of the DEXA scanning of different parts of the rat body. No significant differences could be found between animals in the different diet groups, although, the resolution of the system was only 180 μm.

3D Computed tomography (CT) analysis of femoral head

Different regions of the femur were analysed at 8 μm resolution. Figure 3a shows the results of plotting the mean femoral head trabecular density of female offspring of mothers fed either the control or low protein (restricted) diet group. In the restricted samples there is a decreased proportion of high density bone, in particular above 2500 Hounsfield units. The reduction in density of the femoral head in the restricted diet group was statistically significant and Fig. 3b shows representative CT images of the femoral head from each of the diet groups.

In order to determine if there were differences in the trabecular structure within the femoral head, purely trabecular bone was electronically extracted from the femoral head. No differences were found in the mean spacing of the trabeculae between the two diet groups; however, the mean trabecular thickness was significantly lower (0.094 mm versus 0.075 mm, p=0.009) and the mean trabecular number per mm was increased (9.43 versus 11.66, p=0.009) in the offspring from mothers fed the low protein diet compared to that from offspring from control mothers (Table 1). In addition, the BS/BV volume was significantly higher (21.31 versus 26.77, p=0.009) in the restricted diet gro,up indicating a more rod-like trabecular structure in this group.

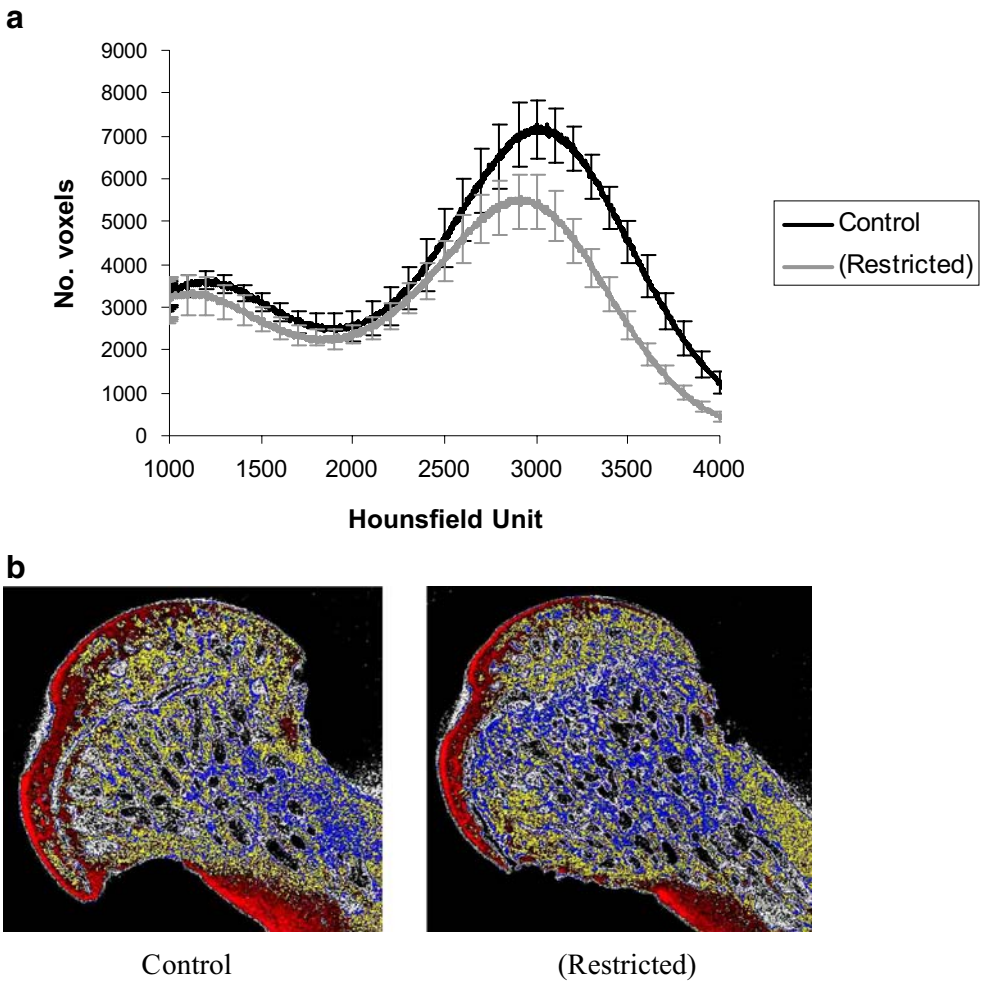

Fig. 3 CT Analysis of femoral head. **a**. Plot of mean femoral head trabecular density for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=5. Error bars represent SEM. **b**. False colour representative CT sections for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. Images show variation in voxel density through femoral head. Hounsfield units 1000–1999 are shown in white, Hounsfield units 2000–2750 are shown in blue, Hounsfield units 2751–3500 are shown in yellow, and Hounsfield units over 3500 are shown in red

Table 1 Bone structure data from different anatomical sites

	Control mean (SD)	Test mean (SD)	*p*-value (control vs. test)
Femoral head			
BS/BV	21.31 (1.55)	26.79 (2.58)	0.009
BV/TV	0.88 (0.04)	0.87 (0.02)	
Thickness	0.094 (0.007)	0.075 (0.007)	0.009
Number	9.42 (0.79)	11.67 (0.99)	0.009
Spacing	0.012 (0.005)	0.011 (0.001)	
Femoral neck			
BS/BV	7.54 (1.17)	7.51 (1.00)	
BV/TV	0.14 (0.03)	0.20 (0.03)	0.016
Thickness	0.27 (0.04)	0.27 (0.03)	
Number	0.52 (0.10)	0.76 (0.19)	0.009
Spacing	1.68 (0.39)	1.11 (0.26)	0.009
Cortical thickness	0.51 (0.05)	0.47 (0.04)	
Lumen area	0.75 (0.13)	1.21 (0.25)	0.009
Cross-sectional area	3.13 (0.41)	3.72 (0.31)	0.009
Femoral midshaft			
Cortical thickness	0.68 (0.04)	0.68 (0.05)	
Diameter	3.32 (0.11)	3.31 (0.17)	
Proximal tibia			
BS/BV	25.59 (3.52)	22.70 (3.06)	
BV/TV	0.50 (0.08)	0.56 (0.05)	
Thickness	0.079 (0.011)	0.089 (0.012	
Number	6.25 (0.45)	6.25 (0.34)	
Spacing	0.081 (0.017)	0.071 (0.007)	
Tibial midshaft			
Cortical thickness	0.66 (0.01)	0.68 (0.06)	
Diameter	2.74 (0.10)	2.66 (0.07)	
Vertebra			
BS/BV	23.21 (5.56)	15.35 (2.27)	0.009
BV/TV	.471 (.051)	.578 (0.055)	0.03
Thickness	.089 (.017)	0.133 (0.019)	0.009
Number	5.57 (2.02)	4.41 (0.50)	
Spacing	0.105 (0.035)	0.097 (0.018)	
Calvaria			
Thickness	0.75 (0.07)	0.68 (0.03)	0.06

Structural data shown are for bone surface to bone volume ratio (BS/BV), bone volume to total volume ratio (BV/TV), mean trabecular thickness (mm), mean trabecular number per mm, mean trabecular spacing (mm), mean cortical thickness (mm), mean diameter (mm), lumen area (mm^2), and cross-sectional area (mm^2). All values shown are mean with standard deviation in brackets

Mechanical testing of femoral head

Table 2 shows the results of the mechanical testing of the femoral head. Although there were no significant differences in the maximum displacement, stiffness, energy, or maximum stress

3D Computed tomography analysis of femoral neck

A similar analysis performed on the femoral neck of samples from control and restricted offspring found no significant differences in density. Animals in the restricted diet group had a larger cross-sectional area of the neck compared to controls (mean of 3.7 mm^2 compared to 3.1 mm^2, p=0.009, Table 1). In addition, the area of the lumen was larger in the restricted group (mean of 1.2 mm^2 compared to 0.75 mm^2, p=0.009, Table 1). However, there was no difference in the cortical thickness of the femoral neck between the two diet groups, although the restricted group had a higher BV/TV ratio (mean of 0.20 versus 0.14, p=0.016) and smaller spacing between trabeculae (mean of 1.11 mm versus 1.68 mm, p=0.009), and more trabeculae per mm (0.76 versus 0.52, p=0.009).

Table 2 Mechanical strength testing of femoral head

	Control ± SD	(Restricted) ± SD	*p*-value
Maximum load / N	149±24	110±22	0.01
Maximum displacement / mm	0.44±0.09	0.47±0.09	n/s
Stiffness	367±19	311±84	n/s
Absorbed energy	33.6±13.3	26.7±4.8	n/s

Strength testing results for femoral head. Femoral heads from 75 weeks old offspring from mothers fed either a control (18% protein) or a restricted (9% protein) diet during pregnancy were loaded until failure. n=5. Results are mean plus standard deviation

Mechanical testing of femoral neck

Table 3 shows the results of the mechanical testing of the femoral neck. Although there were no significant differences in the results between the two diet groups, there was a trend that necks from the restricted diet group showed a higher load before failure (127 *n* versus 96 *n*, *p*-value 0.08).

3D Computed tomography analysis of femoral midshaft

Density analysis of the femoral midshaft was performed at 8 μm resolution. No significant differences were found in the density range between the two diet groups. In addition, no differences were found in the mean cortical thickness or mean midshaft diameter (Table 1).

Table 3 Mechanical strength testing of femoral neck

	Control ± SD	(Restricted) ± SD	*p*-value
Maximum load/N	95.6±24	127.1±24	0.08
Maximum displacement/mm	0.80±0.18	0.73±0.22	n/s
Stiffness	226±133	255±81	n/s
Absorbed energy	47.5±13.6	51±14.2	n/s
Maximum stress	29.2±6.5	31.9±4.7	n/s

Strength testing results for femoral neck. Femoral necks from 75 weeks old offspring from mothers fed either a control (18% protein) or a restricted (9% protein) diet during pregnancy were loaded until failure. n=5. Results are mean plus standard deviation

Mechanical testing of femoral midshaft

No differences were found in any of the mechanical testing results between the two diet groups.

3D Computed tomography analysis of proximal tibia

Density analysis of the proximal tibia was performed at 8 μm resolution. No significant differences were found in the density range between the two diet groups. In addition, no differences were found in the trabecular structure or arrangement (Table 1).

3D Computed tomography analysis of tibial midshaft

Density analysis of the tibial midshaft was performed at 8 μm resolution. No significant differences were found in the mean cortical thickness or mean shaft diameter (Table 1). Figure 4a shows the mean cortical density of the tibial midshaft for the animals tested. In contrast to the femoral head, which showed a reduction in high density bone, the tibial shaft from females in the restricted diet group showed an increase in the high density bone and a reduction in the proportion of low density cortical bone. Figure 5b shows representative CT images of the tibial midshaft from each of the diet groups, indicating an altered distribution of cortical bone density.

Mechanical testing of tibial midshaft

Table 4 shows the results of the mechanical testing of the tibial midshaft. Although there were no significant differences in the maximum displacement, stiffness, energy, or maximum stress between the two diet groups, there was a significant difference in the maximum load with the control diet group displaying a higher load before failure (69.2 N versus 58.5 N, p-value 0.05).

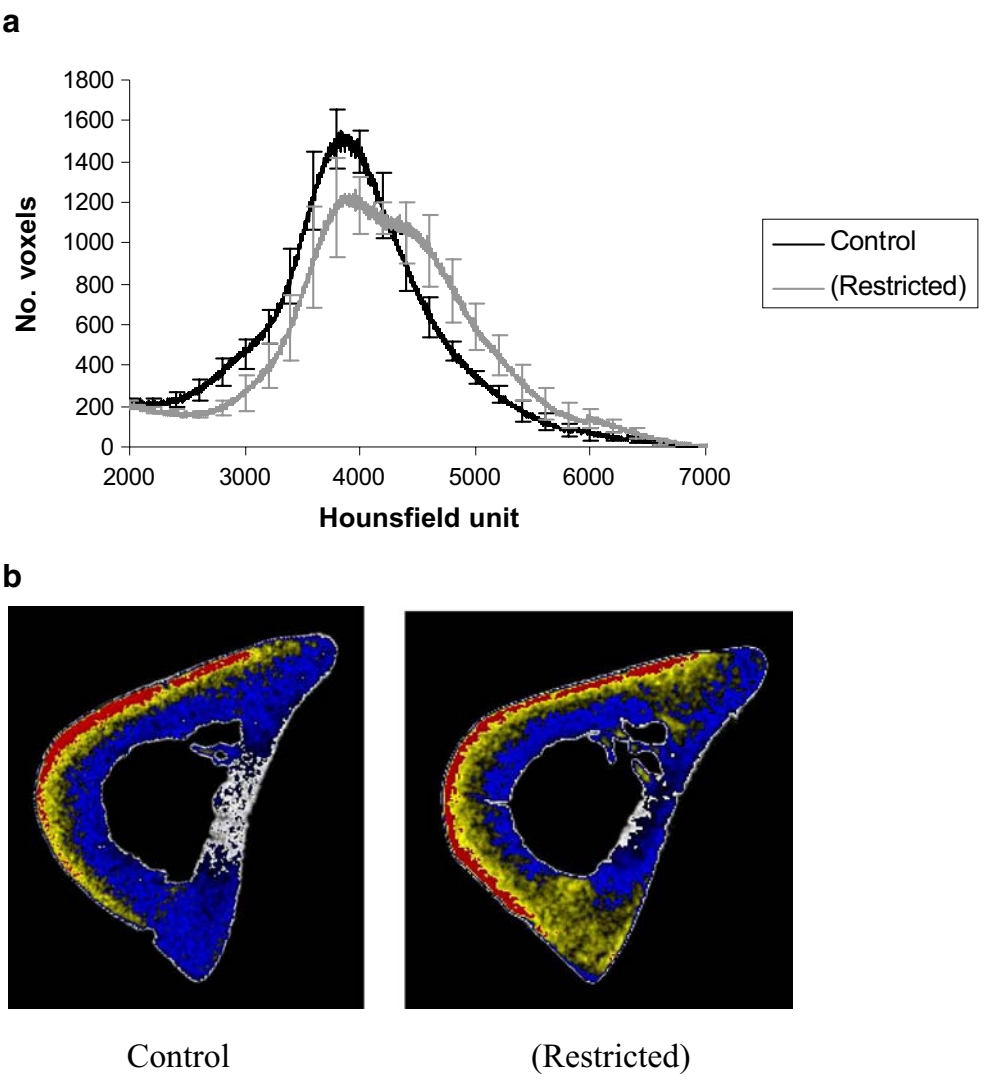

Fig. 4 CT Analysis of tibial midshaft. **a**. Plot of mean tibial midshaft cortical bone density for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. n=5. Error bars represent SEM. **b**. False colour representative CT sections for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. Images show variation in voxel density through tibial midshaft. Hounsfield units 2000–2999 are shown in white, Hounsfield units 3000–3999 are shown in blue, Hounsfield units 4000–4999 are shown in yellow, and Hounsfield units over 5000 are shown in red

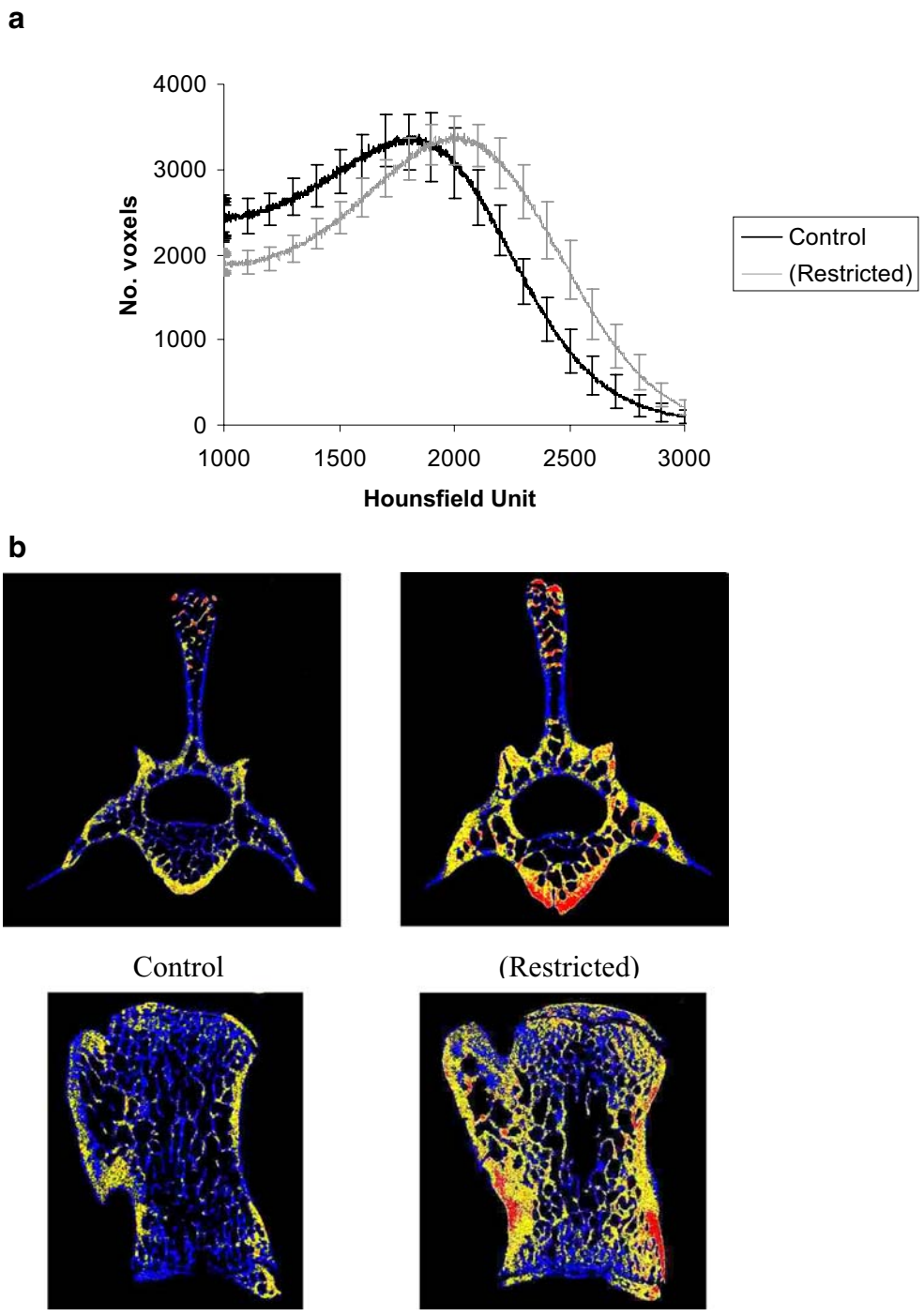

Fig. 5 CT Analysis of 4th lumbar vertebra. **a**. Plot of mean lumbar vertebra trabecular density for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. $n=5$. Error bars represent SEM. **b**. False colour representative CT sections for female offspring of mothers fed either control protein diet (18%) or low protein (8%) diet. Images show variation in voxel density through 4th lumbar vertebra. Hounsfield units 1000–1750 are shown in blue, Hounsfield units 1751–2500 are shown in yellow, and Hounsfield units over 2500 are shown in red. Axial view is shown at the top and sagittal view is shown at the bottom

3D Computed tomography analysis of calvaria

Density analysis of the calvaria was performed at 17 μm resolution. No significant differences were found in the density range between the two diet groups. There was a trend that the animals in the restricted diet group had thinner calvaria compared to the controls ($p=0.06$, Table 1).

3D Computed tomography analysis of vertebra

CT analysis at 17 μm resolution was performed on the 4th lumbar vertebra of all female offspring aged 75 weeks. Figure 5a shows the mean trabecular density of the vertebral body for the animals tested. In contrast to the femoral head, which showed a reduction in high density bone, the 4th lumbar vertebra from females in the restricted

Table 4 Mechanical strength testing of tibial midshaft

	Control ± SD	(Restricted) ± SD	*p*-value
Maximum load/N	69.2±8.4	58.5±5.3	0.05
Maximum displacement/mm	0.97±0.11	0.95±0.22	n/s
Stiffness	96±20	90±9	n/s
Absorbed energy	29.8±5.8	27.1±10.3	n/s
Maximum stress	11.7±0.8	10.6±1.3	0.08

Strength testing results for tibial midshaft. Tibia from 75 weeks old offspring from mothers fed either a control (18% protein) or a restricted (9% protein) diet during pregnancy were loaded until failure. $n=5$. Results are mean plus standard deviation.

diet group showed an increase in the amount of medium and most dense bone and a reduction in the proportion of low density bone in the trabeculae. Figure 5b shows representative CT images of the 4th lumbar vertebra from each of the diet groups, indicating an altered distribution of bone density spread throughout the vertebra with great variation in cortical bone density.

In addition to differences in the density of the bone in the vertebra, there were also differences in the structure of the vertebral bone. Significant differences in the BS/BV ratio, with the restricted group having a lower ratio (mean 23.21 versus 15.35, $p=0.009$), were observed (Table 1) indicating a more plate-like trabecular structure. There were also significant differences in the BV/TV ratio with the restricted group being higher (0.47 versus 0.58, $p=0.03$) indicating more bone in the restricted group vertebrae. This is in agreement with the data indicating the restricted group vertebrae also had trabeculae thicker in structure (0.089 mm versus 0.133 mm, $p=0.009$). Although not significant, the restricted group showed reduced spacing between trabeculae (0.115 mm versus 0.097 mm).

Mechanical testing of vertebral body

Table 5 shows the results of the mechanical testing of the vertebral body. Although there were no significant differences in the maximum displacement, stiffness, energy, or maximum stress between the two diet groups, there was a significant difference in the maximum load with the restricted diet group displaying a higher load before failure (111 N versus 71 N, *p*-value 0.05).

Table 5 Mechanical strength testing of vertebral body

	Control ± SD	(Restricted) ± SD	*p*-value
Maximum load/N	71.4±11.0	110.5±33.5	0.05
Maximum displacement/mm	0.733±0.220	0.969±0.308	n/s
Stiffness	157.9±41.8	165.0±81.8	n/s
Absorbed energy	29.9±15.2	61.8±37.9	0.08

Strength testing results for vertebral body. Vertebral body from 75 weeks old offspring from mothers fed either a control (18% protein) or a restricted (9% protein) diet during pregnancy were loaded until failure. $n=4$ for controls and $n=5$ for restricted group. Results are mean plus standard deviation.

Discussion

This study demonstrates site-specific differences in the femur, tibia and the vertebra in offspring from mothers fed a low protein diet. Differences were observed in structure, as well as in the density of the bone at the different sites. This is the first study, to our knowledge, showing differences in bone structure and strength in offspring from mothers fed a low protein diet during pregnancy.

Interestingly, the differences in bone structure found in the female offspring from mothers fed either a normal 18% protein diet or a restricted 9% protein diet were not uniform. In the femoral head the trabeculae were found to be thinner in the restricted diet offspring, although no significant difference was observed in the mean spacing of the trabeculae. In contrast, in the femoral neck the trabeculae were similar in size to controls, but were more closely packed. However, in the vertebra the trabeculae were thicker in the restricted diet group, although no significant difference was found in the mean spacing. Furthermore, the trabecular density within the femoral head was reduced in the restricted group, no difference in density in the femoral neck, whereas the trabeculae in the vertebra had increased density in the restricted group. Thus, offspring from mothers fed a restricted protein diet during pregnancy only, had femoral heads with thinner, less dense trabeculae, femoral necks with more closely packed trabeculae, vertebrae with thicker, more dense trabeculae, and a tibial midshaft with higher density bone. Mechanical testing of the bone confirms the differences found with CT. In the restricted diet group, the femoral heads failed at a lower load, the femoral necks tended to fail at a higher load, the vertebral body failed at a higher load, and the tibial midshaft failed at a lower load. It appears that although the tibial midshaft in the restricted group has higher density bone than controls, the bone fails at a lower load possibly due to the bone being more brittle, especially as this area is cortical bone with no trabecular structure.

Using a comparative dietary regime, Musha et al. [12] found that when fed to pregnant mothers it resulted in increased blood pressure and vascular changes in the offspring similar to that seen in ovariectomized rats. Similarly, Franco et al. [13] found that a 50% total nutrient restriction fed to mothers resulted in serum estrogen levels in the offspring that were less than half that of controls. In addition, Sun et al. [14] discovered that low levels of oestrogen

are associated with higher levels of follicle stimulating hormone (FSH) and that FSH was shown to stimulate the formation and function of osteoclasts. Hence, there appears to be the potential for increased osteoclast activity in the female offspring of mothers fed a low protein diet.

It is possible that the low protein diet has affected calcium homeostasis in the offspring, for example, via parathyroid hormone (PTH) or PTH related peptide (PTHrP) pathway [15]. It is known that the low protein diet has a direct effect on the animal as a low protein diet fed to 8-month-old male rats induced cortical and trabecular thinning [16]. However, the effect of the diet on the offspring appears to be slightly different to that seen on directly fed animals. Here we show trabecular thinning in the femoral head, but trabecular thickening in the vertebra. It may be possible that the differences are due to a predictive adaptive response to a low protein diet and the requirement to best utilise the (potentially) low levels of calcium in order to maximise survival and chances of reproduction. The predictive adaptive response suggests pathology may occur when there is discordance between the expected nutritional environment as seen in utero and the actual postnatal nutritional environment [17]. One scenario is that in order to maintain a healthy, sexually viable phenotype, the restricted diet group has increased bone deposition in the femoral neck and vertebrae at the expense of the femoral head. However, with the postnatal normal diet, this adaptation results in excess bone in the neck and vertebrae. This may make the bone stronger, but also potentially more brittle. The loss of bone in the femoral head may only become a problem when the animal is large. However, the rat can reach sexual maturity as early as 5 weeks, when it is only about 30% of its final mass. The thinner trabecular seen in the femoral head may have sufficient strength to behave normally in the lighter animal.

We have analysed the femur, tibia and vertebra as these areas are susceptible to fracture due to osteoporosis in aged individuals. In addition, in this study we have only performed CT analysis on female offspring due to the low number of restricted males available at 75 weeks of age. Future studies will determine if the differences seen here are also seen at a much earlier age and also if they also occur in male offspring.

The data suggest that at 12 weeks the tibiae and femora of animals from mothers fed a restricted diet during pregnancy grow faster than control animals as the length of these bones per unit mass of the animal is longer, despite the bones physically being slightly shorter. In other words, despite the restricted diet animals being lighter in mass, they are still maintaining the length of these bones comparable to the heavier control animals.

Although no differences were seen by DEXA analysis, this is probably due to the relatively low resolution of the DEXA equipment (180 μm). No differences in density were seen using CT when femora were scanned at a higher resolution of 64 μm (data not shown). Differences only became apparent when scanned on CT using 25 μm resolution or lower, particularly as differences were mainly in trabecular structure and density rather than in cortical bone.

In conclusion, we have shown that a maternal low protein diet affects bone structure in female offspring in old age, as assessed using micro-CT and mechanical testing. This indicates a key role of the nutritional environment in early development on programming of skeletal development with implicit consequences in later life. Current studies are centred on elucidating the mechanisms involved at the cellular level.

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Conflict of interest statement All authors have no conflicts of interest.

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