The bone-muscle ratio of fetal lambs is affected more by maternal nutrition during pregnancy than by maternal size


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The bone-muscle ratio of fetal lambs is affected more by maternal nutrition during pregnancy than by maternal size. Am J Physiol Regul Integr Comp Physiol 294: R1890–R1894, 2008. First published April 2, 2008; doi:10.1152/ajpregu.00805.2007.—Bone formation and loss are regulated by various factors, mainly endocrine. The threshold or set point above and below which bone mass is accrued or lost is determined at day 140 of pregnancy in 8 groups of ewes, which were of either large or small body size, on either high (ad libitum) or maintenance pasture intake from day 21 of pregnancy, or carrying either singletons or twins. BMC and LM (using DXA scanning) of fetal hindquarters/spine were corrected to leg length. BMC and LM were less in twin than singleton groups (P < 0.001). Large ewes on high intake produced single fetuses with a (group mean) BMC/LM ratio that was higher (P < 0.002) than that in fetuses of large ewes with singletons on maintenance intake or twins on either high or maintenance intakes, the ratios of which were not different. In single fetuses from small ewes on high intake, the BMC/LM ratio was higher than those from small ewes with singletons on maintenance intake or twins on either high or maintenance intakes, the ratios of which were not different. The ratio was not different in singleton fetuses of ewes on high intake, whether they were large or small. Different fetal environments resulted in a given amount of muscle being associated with a higher or lower bone mass. Dietary intake during pregnancy was more important than maternal size in affecting the ratio. We conclude that intrauterine environmental factors may be important in determining bone mass postnatally, and possibly later in life. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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EPIDEMIOLOGICAL AND EXPERIMENTAL studies have highlighted a relationship between the periconceptual, fetal, and early infant phases of life and the subsequent development of adult disease. The “developmental origins of health and disease” (DOHaD) model refers to this relationship and speculates that the fetus adapts to environmental cues in utero, with permanent readjustments in homeostatic systems to aid survival. However, these adaptations, known as predictive adaptive responses (6, 7), may ultimately be disadvantageous in postnatal life and may lead to an increased risk of disease in adulthood. Most research has been directed to the relationship of an adverse prenatal environment and enhanced risk of some of the major chronic diseases of later life, such as coronary heart disease, hypertension, type 2 diabetes (1), and obesity (16). Less attention has been given to the possible impact of fetal and infant developmental programming on skeletal growth, health, and disease, but there is epidemiological evidence that adult bone size is related to body size at one year of age, and adult hip fracture is related to maternal height and to growth rates during childhood (3). One of the environmental effects that could be involved is maternal nutrition during either pregnancy and/or very early life. Maternal undernutrition processes in the fetus can have an effect on fetal cell division, and if it occurs at a time that organ growth is rapid, would result in measurable differences in neonatal tissues, including bone mass (9).

In postnatal life, the mechanism by which bone adapts to the changing environment to which it is exposed, is referred to as the mechanostat, in which the bone responds to the strain (deformation) that is induced by the muscles acting upon it (5). The major forces that bone experiences come from the action of muscles, and it is suggested that an acceptable surrogate for force applied by the muscle is muscle mass, or lean mass (LM). If sufficient force is applied to bone, a certain threshold strain in the bone is reached, and bone is synthesized; if muscle force is below a certain set point, if, for instance, muscles are immobilized or paralyzed, bone tissue is lost. This allows the bone’s resistance to forces to be as great as needed to counteract predictable forces. The relationship between bone mineral content (BMC) and LM is consistent in normal children and young adults and predictably altered in those with clinical disease affecting primarily bone or primarily muscle (12, 15) and undertaking specific sport exercise (4). The threshold or set point above and below which bone mass is accrued or lost is regulated by various factors, mainly endocrine.

If it is true that strong muscle activity makes strong bones, and chronically weak muscles make weak ones in postnatal life, a valid question is whether the model is applicable in utero (13), during which the fetus moves actively by application of forces by its developing muscles. If the mechanostat thresholds or the ratio of bone mass to muscle mass present in utero were to be maintained into adult life, as has been shown for the characteristics of metabolic pathways of other body systems (1), such differences in utero might have significance in explaining the onset of osteoporosis later in life. Our intention in the present study was to examine whether there indeed were differences in the bone/muscle ratio in the fetuses of dams exposed to different maternal environments.

As part of a large experiment on the effects of dam size and nutritional intake on lambs, we studied effects on the fetal musculo-skeletal system, namely, the bone and muscle com-
position of the hindquarters. The sheep is used widely as an experimental model in DOHaD studies for several reasons, among which are the similarity with human birthweight, the very small variation in gestational length (which reduces the confounding effect of prematurity), and the possibility of studying the effects of uterine environment on single and twin fetuses.

We hypothesised that there would be no gender difference in BMC and LM of 140-day gestation lamb fetuses, but that dam size, nutrition, and twinning and the interactions between them would have significant effects on the ratio of BMC to LM.

### MATERIALS AND METHODS

The study was approved by the Animal Ethics Committee of Massey University.

Large (L) and small (S) ewes were selected from the extremes in a commercial flock of 2,900 Romney ewes, on the basis of size, as determined by weight and condition scoring (the latter is an estimate of fatness in animals, usually undertaken by palpation of various parts of the body and scored semi-quantitatively between 1 and 5). The respective mean ± SE body weight and condition score of these two groups of ewes were 60.8 ± 0.18/3.02 ± 0.03 kg and 42.5 ± 0.17/1.97 ± 0.03 kg. Estrus of the ewes was synchronized using a controlled internal drug release device which contains 0.3 g exogenous progesterone and following removal of which ovulation occurs within 24–48 h. Nine hundred ewes were bred using semen from one of four Suffolk rams of similar size (~100 kg), and 561 ewes were determined to have either single or twin pregnancy at 50 days post-insemination by transabdominal ultrasonographic examination.

From day 21 post-insemination, the ewes were randomly allocated to either high (H) or maintenance (M) nutritional regimens until 140 days of pregnancy. Pasture herbage was the only nutritional source. By the skilful use of known stocking rates of ewes on a known pasture acreage, the dry matter production of which can be accurately estimated, it is possible to consistently manipulate the average daily dry matter intake of the ewes and thus affect average production indices. H ewes were offered ad libitum pastoral feeding conditions, and M ewes were grazed such that total increase in maternal body weight during pregnancy would be approximately equal to the expected conceptus mass at term, i.e., the ewes were, on average, on maintenance intake of herbage. Thus, from days 21–140 of pregnancy, there were eight treatment groups, of combinations of single/twin fetuses from L or S ewes allowed a H or M pasture intake (n = 10 fetuses lambs/group), as outlined in Table 1.

At day 140 of pregnancy, 5 twin-bearing ewes and 1 singleton-bearing ewes from each size by feeding treatment group (total 60 ewes) were euthanized. A range of fetal organs and tissues were collected for further analysis (to be reported elsewhere), and the hindquarter/spine region of each fetus was frozen at 20°C until dual energy X-ray absorptiometry (DXA). Maternal and fetal measurements were taken, including measurement of fetal hindlimb length (HL), for which the limb was extended maximally, the coxo-femoral joint space palpated with the fingernail of the thumb, and the distance to the end of the digit measured using a steel tape graduated in millimeters. Such procedures have been shown to have low margin of error in even small subjects (2).

A dedicated small animal program (LUNAR Prodigy, General Electric, Madison, WI) provided BMC and LM in a region of interest, which included the hindquarters, tail, and spine caudal to a line passing through the junction of the 3rd and 4th vertebral cranial to the lumbosacral junction. The specimens were scanned in both dorsoventral and ventrodorsal projections, and the parameter used for analysis was the mean of the two values; two preparations were scanned three times, and the CV was 0.54% and 1.02% for BMC and LM, respectively. The BMC and LM values used for analysis were standardized to fetal size by dividing each individual value by the lamb’s HL.

As LM and BMC were standardized to HL, we first used linear regression to test for differences in the relationship of LM to HL and BMC to LM between the eight groups.

The effect of the treatment groups on standardized BMC and LM and on the ratio of BMC/LM was examined using a full factorial general linear model. In the model, pregnancy status (single vs. twin), ewe body size (L vs. S), and nutritional treatment (H vs. M) were treated as fixed factors. For multiple comparisons, data were examined using the least significant difference correction. All data were analyzed within SPSS v13 (SPSS, Chicago, IL) with the significance level set at P < 0.05. Data are presented as means ± SE unless otherwise stated.

### RESULTS

That there was an effect of environment on the groups was shown by the difference in ewe body weight at term and in lamb birthweight in that part of the cohort studied only after birth; at term, L-H (groups 1 and 2), S-H (groups 5 and 6), L-M (groups 3 and 4), and S-M (groups 7 and 8) ewes weighed 85.86 ± 0.52, 70.88 ± 0.53, 70.94 ± 0.50, and 58.13 ± 0.51 kg, respectively. Lambs born to the heavier ewes tended (P = 0.09) to be heavier at birth than those born to the light ewes (5.49 ± 0.05 vs. 5.34 ± 0.06 kg, respectively). There was a twinning × feeding interaction on lamb birth weight such that feeding regimen had no effect on singleton birth weights (5.98 ± 0.09 vs. 5.93 ± 0.10 kg for lambs born to H and M fed ewes, respectively), but twin lambs born to ewes on H nutritional intake were significantly (P < 0.05) heavier than those born to M ewes (5.11 ± 0.07 vs. 4.62 ± 0.07 kg, respectively).

For the cohort reported in this paper, the mean body weight of the 8 groups of ewes and the mean fetal weights at 140 days gestation for the harvested cohort are presented in Table 2.

There was a consistent positive relationship between LM (R² = 0.754, P < 0.001) and BMC (R² = 0.58, P < 0.001) and HL in all eight groups; only in group 8 was the regression coefficient significantly different from those of the other groups (~140 ± 35, P < 0.05 and ~9.56 ± 2, P < 0.05) for LM and BMC, respectively.

There was a strong positive relationship (R² = 0.752, P < 0.001 for all fetuses) between fetal body weight and BMC. Singletons had a greater BMC/kg fetal weight [3.18 g ± 0.049 ± 0.006 BMC (g), R² = 0.47, P < 0.001] than twins [2.365 g ± 0.054 ± 0.004 BMC (g), R² = 0.55, P < 0.001]. For each fetal weight, standardized BMC, or standardized LM, there was no significant difference between pooled values of male and female fetuses.
For BMC, there was a significant interaction between twinning and feeding ($P < 0.017$). Singletons, irrespective of feeding regimen, had significantly greater standardized BMC than did twins on either high or maintenance diets. However, the effect of the maintenance diet on standardized BMC was greater in the fetuses from singleton ewes.

In single fetuses from small ewes on maintenance intake (group 7), BMC was significantly ($P < 0.05$) less than in other groups of singletons (1, 3, and 5). There was no significant difference between the BMC of groups 1 and 3, both of which were significantly different from group 2 ($P < 0.001$). In twins from large ewes on high intake (group 2), BMC was significantly ($P < 0.01$) greater than in groups 4, 6, and 8; BMC in group 4 was significantly greater than in group 8 but was not significantly different to group 6.

For LM, there was a significant interaction between twinning, ewe size, and intake ($P < 0.004$) (Fig. 1). LM in group 2 was significantly ($P < 0.01$) greater than that in the other twins groups 4, 6, and 8, the differences between which were not significant. The LM of group 7 was significantly less than that of groups 3 ($P < 0.02$) and 5 ($P < 0.001$) but was not significantly less than group 1.

There were significant between-group differences in BMC/LM ratio. Large ewes on high intake (group 1) produced

<table>
<thead>
<tr>
<th>Group</th>
<th>Ewe Body Weight at 140-Day Gestation, kg</th>
<th>140 Day Fetal Body Weight, kg</th>
<th>Weight Gain of Ewe Including Conceptus, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 single/L/H</td>
<td>86.25±1.23a</td>
<td>6.29±0.17a</td>
<td>25.5</td>
</tr>
<tr>
<td>2 twin/L/H</td>
<td>87.50±1.16a</td>
<td>5.34±0.16b</td>
<td>26.7</td>
</tr>
<tr>
<td>3 single/L/M</td>
<td>69.90±1.16b,c</td>
<td>6.33±0.16a</td>
<td>9.1</td>
</tr>
<tr>
<td>4 twin/L/M</td>
<td>71.90±1.16b,c</td>
<td>4.65±0.16c</td>
<td>11.1</td>
</tr>
<tr>
<td>5 single/S/H</td>
<td>68.65±1.16b</td>
<td>6.63±0.16a</td>
<td>26.2</td>
</tr>
<tr>
<td>6 twin/S/H</td>
<td>72.25±1.35b</td>
<td>4.96±0.18a</td>
<td>29.8</td>
</tr>
<tr>
<td>7 single/S/M</td>
<td>56.75±1.17d</td>
<td>5.78±0.16b</td>
<td>14.3</td>
</tr>
<tr>
<td>8 twin/S/M</td>
<td>59.83±1.06d</td>
<td>4.44±0.15a</td>
<td>17.3</td>
</tr>
</tbody>
</table>

Mean ewe body weight fetal body weight, and weight gain (ewe body weight plus conceptus, kg) at 140 days gestation. a,b,c,dDifferent superscripted letters indicate significant difference ($P < 0.05$).

Mean body weight of the eight groups of ewes and the mean fetal weights at 140 days gestation

Mean body weight of the eight groups of ewes and the mean fetal weights at 140 days gestation.

For BMC, there was a significant interaction between twinning and feeding ($P = 0.017$). Singletons, irrespective of feeding regimen, had significantly greater standardized BMC than did twins on either high or maintenance diets. However,

![Fig. 1. Mean ± SE bone mineral content (BMC), lean mass (LM) standardised to hindlimb length, and ratio of BMC/LM of fetuses of 8 groups of ewes, as shown in Table 1. L = Large ewe, S = small ewe, H = high dietary intake, M = maintenance intake. Different letters reveal significant difference ($P < 0.05$).](image-url)
single fetuses with a ratio that was greater than that in fetuses from other large ewe groups (2, 3, and 4) \((P < 0.002)\); the ratio in group 1 was not significantly different \((P = 0.154)\) from that in small ewes on high intake (group 5). There were no significant differences between the ratio of groups 2, 3, and 4.

The ratio in single fetuses of small ewes on high intake (group 5) was higher than that in other groups of small ewes (6, 7, and 8) \((P < 0.001)\); the differences in the ratio of groups 6, 7, and 8 were not significant.

**DISCUSSION**

Environmental effects, which might influence tissue development in utero could do so either directly, for instance by alteration of energy, protein, vitamin, or macro/micromineral delivery to the fetus, or indirectly, by affecting uterine or placental size or function, or by regulation of various endocrinological axes or receptors, which promote and control growth. We quantified the development of both bone and muscle, because it seems unlikely that either the direct or indirect effects would influence only one tissue, not least because skeletal patterning is controlled (at least postnatally) predominantly by the functional strains (deformations) sustained by bones as a result of the muscle forces applied to them (5).

Data on large numbers of animals to define what is an exactly “normal” bone/muscle ratio are sparse, and have been obtained using different methods. Therefore, we could determine only the differences between groups within this study and did not attempt to relate these findings to the whole ovine population. Study of whole body composition was not possible due to evisceration for other studies, and we thus measured BMC and LM in the hindquarters and lower spine only. This limitation precluded study of the relationship of whole body composition to metabolism but may have been an advantage, since exclusion of the viscera may have revealed the differences in LM that might not have been evident on whole body DXA scanning.

DXA measurement of LM, or any other index of muscle size (e.g., weight, cross-sectional area, or volume), is only a surrogate for the force exerted by the muscle(s) concerned, as BMC is only an indicator of bone “strength.” Accepting this possible limitation, which in young normal healthy individuals is probably justified, the BMC/LM ratio indicates the association between the amount of bone (measured by BMC) and of muscle (LM) in the region scanned and at the particular sampling time. The ratio in boys and girls (14) and rats (17) changes as they go through puberty, after which for a given amount of LM, females have a higher BMC, although males have higher BMC and LM due to their average larger size (18).

The relationship between BMC and LM is consistent in normal children and young adults and predictably altered in those with clinical disease affecting primarily bone or primarily muscle (12, 15) and in those undertaking specific sport exercise (4). In our study, the basis for the use of the ratio was sound, and the imaging method was reproducible and sensitive in these specimens from fetal lambs. We therefore applied the BMC/LM ratio to determine whether differences could be detected between offspring exposed to different maternal environments, even though we expected such differences to be much more subtle than those resulting from exercise or clinical disease in children.

Although data pooled across groups revealed no male-female difference in body weight, standardized BMC and LM were significantly less in twins than in single fetuses. More importantly, there were highly significant differences between several of the eight groups of fetuses. The differences between groups were not identical for BMC and LM, indicating that the relationship between the two parameters differed between groups.

Development of fetal muscle is initiated before that of bone. LM was not significantly different in groups 1, 3, and 5, but the BMC of fetuses of ewes on high nutrition was significantly greater (groups 1 and 5) than that of fetuses from ewes fed only maintenance rations during pregnancy. The higher nutrition plane produced fetuses with the highest BMC/LM ratio whether the dam was small or large. Expressed differently, for a given amount of LM, these two groups produced significantly more bone than did the singleton groups, whose dams had been fed only maintenance rations (groups 3 and 7). The data suggest that processes particular to groups 3 and 7 precluded development of sufficient muscle force, or through metabolic, hormonal, or morphological effects elevated the force threshold required to induce formation of the same relative amount of bone. The same applied, although less explicitly, to twin-bearing ewes, but in group 8, the BMC/LM ratio was clearly lower, possibly because of the combined effects of twinning, maintenance rations during pregnancy, and small body size. Although the ratios themselves appear small, there is around 15% difference between the lowest and highest ratio; this gains additional importance in terms of bone health when the data show that the highest mean BMC (group 5) is 46% greater than the lowest BMC (group 8).

For a given LM in the hindquarters and lower spine, some groups of lamb fetuses produced significantly more BMC than others. Some of the differences are intuitive or even expected. For instance, in ewes of a given size, either large or small, it would be expected that twins would have lower bone and muscle masses, because of the effects of competition for placental and nutritional resource, but this does not explain why the relative amount of bone produced by the LM, standardized to body size, is different. It is logical that in smaller ewes, space is at a greater premium for twins than for single offspring and that fetal bone and muscle mass would be less than in larger ewes. Some of the differences were highly significant, and some of the trends support the expectations suggested. But the exact reasons for the differences are not explicable by the data that we collected and require other more mechanistically directed studies. For instance, if bone development in utero is highly dependent on forces applied to bones by fetal muscle movement, then the effect of fetal size itself requires further study, since young adults born with very low birth weight have lower LM (even after standardization for height) than do those of normal size at birth (8).

The present study has shown that the environmental changes were associated with different effects on bone and lean masses in the fetuses, and that of these influences, dietary intake during pregnancy was the most important in determining the highest LM and BMC, and that a high LM in ewes fed ad libitum during pregnancy resulted in much higher BMC than in other groups. It cannot be stated that the difference in BMC is solely due to the higher LM, since ad libitum nutrition may have had positive effects and/or maintenance rations may have had a
negative effect. Further studies are required to elucidate the mechanisms by which the differences in the responsiveness of osteogenesis occurred. It is equally important to determine that the clear differences resulting in higher BMC in some groups will be maintained through the life of the siblings of the fetuses examined in this paradigm.

**Perspectives and Significance**

Epidemiological and experimental studies have highlighted a relationship between the periconceptual, fetal, and early infant phases of life and the subsequent development of adult obesity and type 2 diabetes. The DOHaD model speculates that the fetus makes predictive adaptations (known as predictive adaptive responses) in response to adverse environmental cues in utero, resulting in permanent readjustments in homeostatic systems to ensure immediate survival and improve success in an adverse postnatal environment. However, when there is a mismatch between the prenatal predictions and actual postnatal environment, these adaptations may ultimately be disadvantageous, leading to an increased risk of chronic noncommunicable disease in adulthood and/or the inheritance of risk factors and a cycle of disease transmission across generations. We show that the bone-muscle ratio of the fetus can be significantly affected by the maternal environment. It is unclear whether this variation represents a predictive adaptive response, since we do not know whether the effect persists into adult life, and if it did, what the fitness and reproductive advantages of the change in bone-muscle ratio specifically would be. The prospect is intriguing, however, since the bone-muscle ratio may indicate a change in the sensitivity of the response of bone to the muscle forces applied to it and/or to the capability of muscle to apply force. Were either of these two effects abnormal in human individuals from birth, it could explain part of the natural variation in bone mass attainment and maintenance, and also contribute to the reduced maintenance of bone strength in postmenopausal osteoporosis be-

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