Overview of Bone Biology in the Egg-Laying Hen

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ABSTRACT In young pullets, long bones elongate by endochondral growth. Growth plate chondrocytes proliferate, then hypertrophy, and are replaced by osteoblasts that form a network of trabecular bone. This bone is gradually resorbed by osteoclasts as the bone lengthens. Long bones widen, and flat bones are formed, by intramembranous ossification in which cortical bone formation by osteoblasts in the periosteal layer is accompanied by osteoclastic resorption at the inner endosteal surface. Growth of structural trabecular and cortical bone types continues up to the onset of sexual maturity in pullets. At this point, the large surge in estrogen changes the function of osteoblasts to forming medullary bone rather than structural bone. Medullary bone is a woven bone that acts as a labile source of calcium for eggshell formation. It lines structural bone and also occurs as spicules within the marrow cavity. It has little inherent strength but can contribute to fracture resistance. Osteoclasts resorb both medullary and structural bone so that during the period the hen remains in reproductive condition there is a progressive loss of structural bone throughout the skeleton, which is characteristic of osteoporosis. The increasing fragility of the bones makes them more susceptible to fractures. The dynamics of bone loss can be affected by a number of nutritional, environmental, and genetic factors. If the hen goes out of reproductive condition, estrogen levels fall, osteoblasts resume structural bone formation, and skeletal regeneration can take place.

(Key words: bone, laying hen, osteoporosis)

INTRODUCTION

The discovery of calcium- and bone-related disorders that have affected both production and welfare has stimulated interest in the bone biology of the laying hen. Caged layer paralysis, identified soon after the introduction of battery cages (Couch, 1955), was found to be associated with weakened skeletons characterized by osteoporosis (Urist and Deutsch, 1960; Bell and Siller, 1962). Osteoporosis is defined as a progressive decrease in the amount of mineralized structural bone and leads to bone fragility and susceptibility to fracture. The paralysis of hens can sometimes be attributed to spinal degeneration, but it is also probable that transient irregularities in intracellular calcium metabolism leading to inhibition of muscular activity are a major contributory factor. These irregularities can also diminish skeletal calcium reserves and contribute to the severity of osteoporosis. However, it has become evident that, even in the absence of caged layer paralysis, osteoporosis is widespread in laying flocks (Whitehead and Wilson, 1992) and is a major contributory factor in the high incidence (about 30%) of hens experiencing fractures, either during the production period or during depopulation (Gregory and Wilkins, 1989). The severe welfare problem that this represents is the main motivation for the interest in hen bone biology.

This paper reviews the bone biology of the laying hen from the perspective of osteoporosis. It describes the mechanisms of bone formation during rearing and bone turnover and loss during the laying period. It also describes factors that can modify these mechanisms but concentrates on the factors that are most relevant to osteoporosis. Thus nutrient deficiencies that may modify bone growth during rearing, for instance, but do not have any practical implication for osteoporosis are not discussed.

BONE GROWTH DURING REARING

The structural skeleton of the laying hen becomes fully developed during the rearing period. Two processes bring about bone growth. Longitudinal growth in long bones of the appendicular skeleton occurs by endochondral ossification. This is based on the epiphyseal growth plate where some of the resting chondrocytes with fibroblastic phenotype in the germinal layer become committed to differentiate into proliferative
chondrocytes. These cells multiply and form columns of flattish cells closely packed within an extracellular matrix, secreted by the chondrocytes, that contains a high content of type II collagen. This zone of proliferative chondrocytes is nourished by epiphyseal blood capillaries. These cells gradually become more separated within their columns as more matrix is secreted, and then they start to differentiate into a hypertrophic state. They become enlarged and more rounded and start secreting a new matrix component, type X collagen. The hypertrophic zone receives its blood supply from metaphyseal blood vessels so there is a narrow avascular zone of prehypertrophic chondrocytes between the proliferative and hypertrophic zones. Apart from collagen, chondrocytes secrete other matrix components, such as proteoglycans, and growth factors. These matrix components and growth factors in turn regulate further development of the chondrocytes.

Bone formation starts in the lower hypertrophic zone. Chondroclasts resorb matrix, and fully hypertrophied chondrocytes secrete alkaline phosphatase that helps to initiate the initial formation of crystals of hydroxyapatite, the bone mineral. The chondrocytes then experience apoptosis and are resorbed and osteoblasts, bone-forming cells, form from precursor cells in the marrow. Osteoblasts produce the bone matrix of fibrils of type I collagen and the raised concentrations of Ca$^{2+}$ and PO$_{4}^{3-}$ ions that result in the formation of hydroxyapatite crystals within and around this matrix. Bone-resorbing cells, osteoclasts, are also active in this area and bone remodeling, the coupled actions of osteoclastic resorption followed by osteoblastic bone formation, results in the development of a network of trabecular bone. This is a woven bone based on a rather irregular structure of collagen fibrils. As the bone elongates, by continued proliferation of chondrocytes at the head of the growth plate followed by hypertrophy and mineralisation at the rear, the trabecular network is largely resorbed to form the marrow cavity. This developmental process differs from mammalian bone growth in that it does not involve a secondary ossification center. Mammalian growth plates are also much less well vascularized than avian ones.

The long bones widen by a process involving intramembranous ossification. Osteoblasts develop in the perichondrium and produce spicules of bone that merge to produce a network of bone with cavities lined by osteoblasts. These cavities are gradually infilled by osteoblasts continuing to secrete concentric layers of lamellar cortical bone. At the endosteal surface, osteoclasts resorb bone so that the bone widens as an expanding ring with bone formation on the outer surface.
and resorption on the inner. In the early growing period, the ring expands rapidly so that cavities do not become completely filled with bone before endosteal resorption commences, but as growth slows the degree of infilling rises. This is illustrated in the tibia cross sections of pullets at 12 wk of age and during lay (Figure 1). During early growth there is little remodeling of bone but toward the end of the growing period secondary osteons are formed by cutting cones in which osteoclasts cut a tunnel in the bone and are followed by osteoblasts forming new concentric layers of lamellar bone. In the process of formation of primary and secondary osteons, osteoblasts become entombed within bone and differentiate into osteocytes. These cells have a network of interconnections and can regulate bone remodeling in response to biomechanical forces.

**THE LAYING PERIOD**

A dramatic change takes place in the bone biology of the hen at the onset of sexual maturity. The function of osteoblasts changes from forming lamellar cortical bone to producing a woven bone called medullary bone. This bone type, unique to birds and crocodilians, is intended as a labile source of calcium for shell formation and is laid down on the surfaces of structural bone and in spicules within the medullary cavities, especially in the leg bones. The humerus is a pneumatic bone (i.e., the inner cavity is hollow, filled with air, not marrow), but in some hens medullary bone can form on the endosteal surfaces and, indeed, in a few birds can completely fill the cavity (Figure 2). The amount of medullary bone builds up rapidly during the early stages of lay and can continue to accumulate slowly over the remainder of the laying period. The switch from structural to medullary bone formation seems to be virtually total, because fluorescent marker studies give no indication of structural bone formation or bone remodeling to give secondary osteons in hens during lay (Hudson et al., 1993). However, osteoclastic resorption of structural bone continues with the result that structural bone content of the hen declines. Structural bone loss can be alleviated by the use of bisphosphonate, a drug that inhibits osteoclast action (Thorpe et al., 1993). The loss of some structural bone types, especially trabecular bone in the spine and epiphyses of leg bones, can be quite marked during the early laying period, although it is not known whether this is directly linked to the initial accumulation of medullary bone (Whitehead and Fleming, 2000). Loss of cortical bone, as judged by cortical bone thickness, is a more gradual process. Structural bone is lost throughout the skeleton, from long bones of the wings, legs, vertebrae (Figure 3); keel, and other bones, although it may be replaced by medullary bone, as shown in Figure 1. This progressive loss of structural bone during the laying period is characteristic of osteoporosis and results in weakening of the skeleton and increased fracture.

Although structural bone content declines during the laying period, the accumulation of medullary bone means that total bone content may remain constant or even increase over the laying period. Medullary bone is fundamentally weaker than structural bone for 2 reasons. First because it is a form of woven bone based upon a very irregular arrangement of collagen fibrils and, second, because much of it is present in isolated spicules. This does not mean that medullary bone has no overall structural benefit; its covering of structural bone surfaces may help to maintain trabecular connec-
tivity, and its presence in the marrow cavity can increase fracture resistance as shown by the strong positive relationship between humerus strength and medullary bone content (Fleming et al., 1998a). However, the general net effect of the replacement of structural bone, with medullary bone is to weaken the overall strength of the hen’s skeleton and thus increase fracture risk. Medullary bone is as radiodense as structural bone and therefore measurements of radiographic density or bone ash content are not good measures of bone quality when medullary bone is present.

The processes described above are reversed when the hen goes out of lay. Medullary bone gradually disappears and structural bone formation recommences. This can be demonstrated by the appearance of a new layer of structural bone being laid down on top of the layer of medullary bone that previously coated the structural bone surface. This cycle of structural bone loss during egg laying followed by regeneration is normal in a hen laying eggs in clutches followed by incubation and allows the hen to maintain good bone quality over its lifetime. However, selection of the modern hen to remain in a continuously reproductive condition over a prolonged period makes it highly susceptible to osteoporosis.

The mechanism behind these changes is driven by estrogen. Estrogen stimulates osteoblast function and has an inhibitory effect on osteoclast function. It appears that in hens, the considerable rise in circulating estrogen at the onset of maturity has a stimulatory effect on osteoblasts, causing them to produce medullary bone instead of structural bone. This is reversed when hens go out of lay and estrogen levels decline (Whitehead and Fleming, 2000). The mechanism causing laying hen osteoporosis thus contrasts with that in human postmenopausal osteoporosis, in which the decline in estrogen suppresses (structural) bone formation and increases bone resorption.

Although the loss of structural bone is progressive over the life of the in-lay hen, medullary bone is turned over at a much faster rate in relation to the egg-laying cycle. There is increased demand for calcium for shell formation during the period the egg spends in the shell gland. Because this usually occurs during the night when supply of calcium from the digestive system is low, a high proportion of shell calcium comes from resorbed medullary bone. There is thus a surge in osteoclastic resorption during the shell formation period. However, osteoclasts are not specific to medullary bone so resorption can also occur at exposed structural bone surfaces, which explains the osteoporotic structural bone loss. After the egg is laid, osteoclasts replace osteoclasts and regenerate medullary bone. Although medullary bone is important for shell formation, there is not a direct relationship between medullary bone content and shell quality. Shell quality is usually good at the very start of lay when little medullary bone has been formed. Presumably the calcium for these early shells may come largely from structural bone resorption, which may explain the fast rate of trabecular bone resorption in the early laying period. The lack of relationship between medullary bone content and shell quality is further illustrated by the decline in shell quality later in the laying period when medullary bone content is high and the improvement after molting, when much of the medullary bone has been lost. It is probable that ability to mobilize bone, as represented by osteoclast activity, is more important than total medullary bone content in influencing shell quality.

OTHER FACTORS

Activity

Exercise can have a considerable effect on bone quality in hens. Hens housed in conventional battery cages have considerably weaker bones than hens in alternative systems. The improvement in bone strength is related to the degree of biomechanical load experienced by the bone. Thus hens kept in cages fitted with perches or in low-level aviary systems show improved strength in leg bones but relatively little change in wing bone strength, whereas birds able to fly in high-level aviaries show relatively greater improvements in wing bone strength (Knowles and Broom, 1990; Fleming et al., 1994). Effects of exercise as a way of stimulating bone growth during rearing have been studied, but neither housing birds in pens nor giving extra exercise through use of a carousel have improved bone quality at start of lay compared with cage rearing (Whitehead and Wilson, 1992).

There is little information on the mechanism by which exercise improves bone characteristics in the hen. The finding by Newman and Leeson (1998) that tibial strength increased within 20 d of transfer of hens from cages to an aviary suggests that the mechanism may involve stimulation of structural bone formation rather than inhibition of resorption. However, a change could also be explained by an inhibition of egg production induced by the change in environment. More research is needed to establish the precise effects of exercise on bone formation and resorption in laying hens.

Genetics

The effect of genetic factors in influencing bone development has been demonstrated by a study involving divergent selection for skeletal characteristics. Bishop et al. (2000) selected lines of hens on the basis of a bone index (BI) based on measurements in hens at end of lay for tibia and humerus 3-point breaking strengths and keel radiographic density and containing a negative factor for body weight to eliminate the effect of this characteristic. There was a rapid divergence in a range of bone characteristics in females and males over the first 3 generations, and continuation of this selection has resulted in further divergence in bone traits in later generations. Thus after 7 generations of selection, there is now a 2-
fold difference in tibia breaking strength between the lines. Other comparisons between the lines have identified some of the important factors involved in bone development in hens.

Some bone characteristics of the hens in the 2 lines are given in Table 1. Comparisons of tibia cortical thickness at different ages show that the superior thickness in the high BI line at end of lay is attributable to 2 factors, namely a greater amount of bone formation during growth and, more importantly, less bone resorption during the laying period. Consistent with these observations, the high BI hens also contained more medullary bone and fewer osteoclasts, both in relation to the amount of medullary bone and also in absolute numbers in bone sections. Osteoclast number or activity is thus an important factor determining the extent of osteoporosis in hens.

The differences in bone strength between the lines can be explained mostly on the basis of quantities of structural bone. However, the possibility cannot be exclude that there are also differences in structural bone quality. This arises from findings of greater crosslinking, particularly pyrrolic crosslinking, in the collagen matrix that could contribute to the improvement in bone strength in the high BI line (Sparke et al., 2002).

**Nutrition**

Nutritional deficiencies of calcium, phosphorus, or cholecalciferol have been shown to result in bone loss attributable to osteomalacia (Wilson and Duff, 1991) and are likely to lead ultimately to greater severity of osteoporosis. However, there is no evidence that avoidance of osteomalacia can prevent the development of osteoporosis. A study by Rennie et al. (1997) investigated the effects of a number of dietary factors during the laying period on bone structure. None of the factors had any effect on the proportions of cancellous bone in the free thoracic vertebra or proximal tarsometatarsus, but treatments involving feeding a particulate source of calcium (oystershell) increased the proportions of medullary bone. Confirmation of the practical benefits of particulate calcium sources has been provided by Fleming et al. (1998b) who found that feeding limestone particles resulted in improved bone strength in older hens. This finding was associated with a lower loss of cancellous bone, especially during the early laying period, and a greater accumulation of medullary bone. The finding that provision of calcium with improved digestive characteristics can increase the total amount of bone confirms that calcium deficiency is not a primary cause of osteoporosis.

**Relationships with Egg Production**

It is often stated that osteoporosis occurs in hens because they deplete their bones by laying so many eggs. This statement is not totally correct because, of course, hens consume more calcium in their diets than they export in eggs. We have studied relationships between egg production and bone quality in several experiments. In the genetic study (Bishop et al., 2000), we have not observed any significant difference in mean egg production or egg weight between the high and low BI lines over a laying year. Data from birds of the third generation are shown in Table 2 and have been confirmed by observations on later generations. Rennie et al. (1997) reported correlations in a commercial strain between egg production and trabecular bone content of the proximal tarsometatarsus and free thoracic vertebrae of 0.00 and 0.16, respectively. The initial evidence has thus suggested that egg production has little or no effect on bone quality.

We have just completed a more detailed investigation of these relationships in a larger flock (n = 500). The distributions of egg number up to 68 wk plotted against BI are shown in Figure 4. It is apparent that birds laying very few eggs have a very high BI (good bone quality), results in a high correlation between total egg number and BI (r = −0.360; P < 0.001). However, if birds laying less than 230 eggs are excluded, the correlation becomes quite low (r = −0.066; P < 0.001). The correlation declines

### Table 1. Bone characteristics of hens selected for resistance (H) or susceptibility (L) to osteoporosis

<table>
<thead>
<tr>
<th>Trait</th>
<th>Line</th>
<th>Age (wk)</th>
<th>Pooled SEM</th>
<th>Probability (line effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibia cortical width (mm)</td>
<td>H</td>
<td>15</td>
<td>0.465</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>25</td>
<td>0.473</td>
<td>0.001</td>
</tr>
<tr>
<td>Medullary bone content of proximal tarsometatarsus (%)</td>
<td>H</td>
<td>70</td>
<td>0.365</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>7.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Osteoclasts per unit of medullary bone</td>
<td>H</td>
<td></td>
<td>6.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>979</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### Table 2. Egg production and shell characteristics of hens selected for resistance (H) or susceptibility (L) to osteoporosis

<table>
<thead>
<tr>
<th>Trait</th>
<th>Line</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of lay (%)</td>
<td>H</td>
<td>86.9</td>
</tr>
<tr>
<td>Egg mass (g/h/d)</td>
<td>L</td>
<td>87.3</td>
</tr>
<tr>
<td>Feed intake (g/h/d)</td>
<td>H</td>
<td>105.2</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>106.2</td>
</tr>
<tr>
<td>Second grade eggs (%)</td>
<td>H</td>
<td>2.93</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>2.30</td>
</tr>
<tr>
<td>Candling cracks (%)</td>
<td>H</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>2.6</td>
</tr>
<tr>
<td>Shell weight (mg/cm²)</td>
<td>H</td>
<td>79.5</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>80.5</td>
</tr>
</tbody>
</table>

1Data were generated over multiple periods; raw data are unavailable.
2Visibly cracked or unsuitable for public sale.
further if birds laying less than 250 eggs are excluded
\( (r = -0.055; P < 0.001) \). These results can be explained
on the basis that birds laying very few eggs are likely
to have long periods when estrogen levels are low and
structural bone formation can take place. Birds laying
an intermediate number of eggs are also likely to be out
of lay for periods and thus able to regenerate structural
bone at these times. The very low correlation for birds
laying more than 250 eggs is consistent with the theory
that birds that are laying regularly will have continu-
ously high estrogen levels that preclude structural bone
formation. However, within the group of birds with egg
number in the range of 250 to 332 eggs, it is still likely
that some individuals went hormonally out of lay for a
short period during which they were able to regenerate
structural bone. This would account for the statistically
significant positive, although low, correlation between
bone index and egg number observed with this group.
These results thus suggest that the major factor in
determining the degree of osteoporosis is the length of
time that birds are in a continuously reproductive state
not the precise number of eggs laid during that period.

**Relationships with Shell Quality**

A study by Buss and Guyer (1984) on bone characteris-
tics of thick and thin eggshell lines of chickens con-
cluded that skeletal metabolism was not a limiting factor
determining shell thickness. However, the bone mea-
surements (ash and calcium contents) would not have
given any indication of whether there was any relation-
ship between bone structure and shell quality. More
insight into relationships between osteoporosis and
shell quality has come from the lines generated by the
genetic study of Bishop et al. (2000). Comparisons be-
tween the lines have shown decreased shell thickness
and inferior shell quality in the high BI line (Table 2).
The relative line difference in shell quality was much
less than the difference in degree of osteoporosis; never-
theless this finding establishes the principle that birds
more resistant to osteoporosis deposit less calcium in
eggshells.

The possible mechanism relating bone and shell qual-
ity is of interest. Does less production of shell calcium
conserve bone, or does a lower rate of bone resorption
result in less calcium available for shell formation (i.e.,
is the mechanism shell gland or bone-based)? Consider-
ation of the results from the genetic study, in which
effects of selection for bone quality were apparent in
males as well as in females, might suggest that line
differences in bone quality are not dependent upon fe-
male reproductive factors, such as shell gland function.
The theory proposed earlier, that resistance to osteopo-
rosis is associated with less osteoclastic bone resorption,
could imply that birds more resistant to osteoporosis
do not mobilize sufficient bone calcium to fully meet the
needs for shell formation. Nutritional evidence supports
this explanation. It has been shown that provision of a
dietary calcium source in particulate from rather than
powdered form improves both shell and bone quality
(Guinotte and Nys, 1991; Fleming et al., 1998b). Particu-
late calcium sources remain in the digestive system
longer at night and can provide a greater dietary source
of calcium during the period of shell formation, thus
making the birds less dependent upon bone mobiliza-
tion to provide calcium for eggshells. The hypothesis
that the particle size of the calcium source might allevi-
ate the poorer eggshell quality in the osteoporosis-resis-
tant hens is currently being tested.

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