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Osteoporosis and Bone Functional Adaptation

Osteoporosis and bone functional adaptation: Mechanobiological regulation of bone architecture in growing and adult bone, a review

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Abstract—During life, bone is continually optimized for its load-bearing role by a process of functionally adaptive (re)modelling. This process, which is more active in growing bone, is dominated by high-magnitude, high-rate strains, presented in an unusual distribution. Adaptation occurs at an organ level, involving changes in whole bone architecture and bone mass. The repetitive coordinated bone loading associated with habitual activity may have little role in the preservation of bone mass, and may even reduce the osteogenic potential of an otherwise highly osteogenic stimulus. Cells of the osteocyte/osteoblast network are best placed to appreciate mechanical strain. Among the strain-related responses they show, is a reduced rate of apoptosis. This may serve to regulate and target osteoclast activity. A more complete understanding of the stimuli and pathways involved in both the physiology and pathology of this structural homeostatic mechanism will allow the design of more appropriate exercise regimens and targeted pharmacological interventions to limit morbidity and mortality by reducing bone fragility.

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INTRODUCTION

The skeleton performs a variety of functions, the relative importance of which will change depending on environmental circumstances. All are important, and the weight-bearing role of the skeleton cannot be viewed in isolation from these other functions. The relative dominance of each of these functions also varies with site in the body. Calcium homeostasis, for instance, occurs primarily in cancellous bone, the anatomy of which provides a large surface area, well suited to rapid mineral exchange. Those bones that serve in a primarily protective capacity, such as the bones making up the vault of the cranium, are only subjected to small loads throughout most of life (1). Despite this, they must remain well mineralized to fulfil their role. The skeleton cannot therefore be regarded as a single entity, governed by a universal set of rules, but rather as a series of individually specialized structures. The demands and requirements that each bone

serves will govern the influences to which it primarily responds (2,3).

Clearly though, the skeleton has evolved primarily to serve mechanical needs, particularly when the load-bearing structures of the appendicular skeleton and vertebral column are considered. The mechanism that matches bone mass and architecture to functional demand is known as functional adaptation. This process occurs continually throughout life as the skeleton adapts to changes in mechanical use, maintaining the inherent safety factor that keeps fracture risk at an acceptable biological level (4,5). Osteoporosis is a disease of increased bone fragility. The ability of the skeleton to bear load depends on its having sufficient bone mass, with adequate material properties, suitably arranged in space to resist all loading possibilities without fracture. The catastrophic failure of this mechanism in women following estrogen withdrawal at biological or surgical menopause accelerates bone loss despite ongoing loading of bones. As this bone loss continues, so fracture risk increases. This increased fracture risk may be subtle initially, affecting, for instance, individual trabeculae in cancellous bone, but resulting in progressive vertebral collapse through repeated episodes of moderate loading. Alternatively, fracture may be more dramatic, following often minimal or mild trauma at sites such as the femoral neck or distal forearm.

Homeostatic Control of Fracture Risk

Genomic factors regulate the early architectural patterning of bones, such that in the absence of any mechanical influence each bone will develop to be anatomically recognizable, with joints, muscular attachments, and ligaments in the correct locations (6). However, the subsequent epigenetic modifications to which the bone is subjected determine its suitability for its primary load bearing function (7). The ability of the skeleton to respond fully or appropriately to mechanical influences may be constrained or enabled by other non-weight-bearing influences, such as nutritional plane, dietary calcium intake, and endocrine factors (8).

For an effective homeostatic control mechanism to be established, a suitable and relevant feedback variable must exist (9,10). In long bones, this must reflect the bone mass and architecture, and the diverse range of modes in which a bone may be loaded. These three influences resolve at the level of the individual bone cell as a mechanical strain or deformation in the matrix. This strain is the only feedback variable that confers the necessary information to allow relation of the current architecture to the prevailing loads (2). It seems likely that it is this strain, or one of its direct consequences, such as cannalicular fluid flow (11,12) that closes the feedback loop, and passes information to the effector cells, the osteoblasts and osteoclasts at the bone surface. These effector cells are then able to bring about the appropriate change in bone mass, material, and architecture to return matrix strain to some predetermined optimum level, at each location within the skeleton (13).

The Strain Stimulus to Adaptive (Re)Modelling

Two areas require a specific expansion of our knowledge. The first is to define the characteristics of the applied strain patterns that bring about adaptive changes in bone mass in a relevant and strategic manner. This would facilitate the design of safe exercise regimens aimed at increasing bone mass to reduce lifetime fracture risk. The second is to establish the series of cellular and molecular events that occur *in vivo* following the application of a suitable stimulus, and in particular the intra- and intercellular signalling pathways involved. A deeper understanding of this complex series of events may permit strategic pharmacological intervention to enhance or maximize the osteogenic effect of exercise.

The structural objectives of skeletal remodelling are still unknown. Direct measurements of bone strain taken from a variety of bones across several species show the peak dynamic strain range for many limb bones to be similar, lying in the 2,000–4,000 microstrain range (14). The aim of skeletal design does not therefore appear to be the production of minimal strain levels, since to achieve this would involve the relatively simple, although biologically costly, task of increasing bone mass. Rather, the majority of weight-bearing bones have a degree of longitudinal curvature that promotes bending, and therefore increases strain levels for any given load (7,13,15). During natural loading, one cortex is placed in tension and the other in compression.

One series of experiments, in which tibiotarsal strains were determined in chickens, found that at anatomically (and therefore functionally) equivalent sites on the skeleton during the period of rapid post-hatch growth, the strain magnitude, orientation, and distribution all remained constant (16). This suggests that during growth, bones may model to maintain a similar pattern and level of dynamic strain specific to each site. When the strain levels were then increased, by exercising these chicks while they carried a weight to increase skeletal loading, there was a change in the tibiotarsal modelling patterns that brought about a change in bone mass and geometry (17,18). This adaptive

change was sufficient to restore bone strain to control levels, although this effect was lost during subsequent growth.

In the design of controlled loading experiments, it is vital that the strain pattern for the bone being manipulated should be known during both the natural situation and the application of the artificial loading stimulus. The lack of this information has been a drawback to interpretation of the results from many of the human and animal exercise intervention studies designed to increase bone mass. Such studies are often based on exercise regimens better suited to the improvement of cardiovascular fitness. Only a handful of studies have used exercise regimens that are specifically designed to load the skeleton, and even fewer have attempted to quantify the change in skeletal loading caused by the exercise program (19–22). Without this information, it is not possible to relate the results of such studies to the change in skeletal loading patterns.

Adaptive (re)modelling is, by definition, a long-term, continual process that occurs over a period of months, if not years, as functional demands on the skeleton alter with changing activity levels and lifestyles. At present, *in vitro* studies have only a limited, although important, contribution to make in terms of helping to understand the relevant stimulus to, and ultimate aims of, the adaptive change. Cell culture systems are, of course, well suited to examine the immediate and short-term responses of cells to mechanical stimuli. Since cells in monolayer culture are deprived of the normal attachments to their underlying substrate, they are unlikely to accurately reflect the responses of the same cell population *in vivo*. Obviously, changes in whole bone mass and architecture by which adaptation occurs are not reflected in such culture systems. Organ culture systems, in which cells remain *in situ* in a relatively normal environment, represent a halfway house between cell culture and *in vivo* systems, and controlled strains can be applied to the bone matrix. Unfortunately, at present these culture systems have a limited life span that is insufficient to allow anything but the very early stages of the adaptive process to be studied. *In vivo* models therefore have been, and are likely to remain, an essential tool to understanding functionally adaptive (re)modelling.

Controlled Loading Experiments in an Adult Bone Model

Our present understanding of the loading related stimulus has come primarily from *in vivo* models, in which precisely controlled strains can be produced in the bone matrix by carefully applied loads. One such model is the functionally isolated, externally loadable, avian ulna preparation (23), which has made an enormous contribution to our understanding of the loading related stimulus to adaptation. This model allows controlled strains to be applied to a substantial segment of bone, and removes the confounding influence of background activity between controlled loading episodes. Prolonged and repeated loading over a course of weeks is also possible.

From a series of well-designed experiments performed using this model, several fundamental concepts emerged:

- As expected, disuse resulted in a loss of bone mass, primarily the result of endocortical resorption and an increase in intracortical porosity. There was a positive linear relationship between the applied strain magnitude and the change in bone mass, such that strains of approximately 1,000 microstrain stopped the bone loss and maintained bone mass. Increasing strain magnitudes above this level produced a proportional increase in bone area (24). This was achieved by production of woven bone at the periosteal and endocortical bone envelopes. Over time, this woven bone remodelled to produce a more regular compact bone (25).

- Only dynamic strain had this protective or osteogenic effect; strains of similar magnitude applied in a static manner not only failed to cause an osteogenic response, but were no different from disuse, both resulting in substantial bone loss (26).

- If an osteogenic stimulus was applied to the isolated ulna segment on a daily basis, as few as four cycles each day were sufficient to stop the bone loss associated with disuse. Increasing the number of loading cycles to 36 each day resulted in a considerable increase in bone mass. However, increasing the number of cycles beyond this did not increase the bone gain any further (23).

These experiments demonstrate that mechanical stimuli arrest bone resorption and enhance bone formation. Clearly, they also demonstrate that a short exposure is all that is necessary to trigger a substantial osteogenic response, provided that the stimulus is adequate.

This paradigm was developed further with the concept of the strain error distribution hypothesis (24). In all these loading experiments, the strains applied never exceeded those that the bird was able to apply to its ulna during normal physiological activity. Despite this, the controlled loading stimulus resulted in a substantial increase in bone mass. This was explained by the difference in the distribution of strain across the section between the normal and artificial

loading situations, which was rotated through approximately 90°. This mismatch in the strain distribution throughout the bone was hypothesized to be a vital influence on the adaptive response.

Further evidence to support this hypothesis was provided by a physiological loading model in which the sheep calcaneus was mechanically isolated by the application of a transarticular external skeletal fixator. When these animals were allowed a period of controlled walking exercise each day, the calcaneus lost bone in a similar way to those animals that were not exercised at all (27,28). The strains associated with controlled walking exercise were of low to moderate magnitude and presented in a normal distribution. High-rate, high-magnitude transient loading events, associated with a strain distribution error, were attenuated by the presence of the external fixator bar. This further supported the concept that it is these components of the strain history that maintain bone mass and maximize the adaptive response.

This series of experiments indicates that bones do not adapt to the predominant activity, which in this case was disuse, but rather to the predominant stimulus, which came from the brief daily loading period. Therefore, in terms of the adaptive response, bones may not be concerned with the entire strain history, but only with a small component of these strains. This subset is dominated by high magnitude strains applied in unusual distributions. Since such strains occur infrequently, it is not surprising that relatively few cycles are needed to maximize the osteogenic adaptive response. This concept also explains why in these and previous experiments in which bones had been overloaded, the primary areas of new bone deposition had not necessarily been related to the sites of greatest overstrain (24,29,30). It further explains why many of the human and animal exercise studies may have failed to demonstrate any beneficial effect on bone mass, since physiological exercise tends to load the skeleton repeatedly with strain cycles of moderate magnitude and low strain rate, presented in a normal distribution (31–33). The exercise studies that consistently show a beneficial effect have been those involving impact loading which is error rich and involves strain delivered with high rates and magnitudes (34–37).

The principle that a change in the strain distribution is vital to the maintenance of bone mass and production of an adaptive response raises the idea that the resident cell population of bone must be able to detect matrix strain. Furthermore, it must also be able to form a three-dimensional (3-D) appreciation of the strain distribution, compare this with the expected strain distribution and magnitude, and integrate this information, before passing on regulatory information to the effector cells at the bone surfaces. Clearly, the bone cells best placed to do this are the osteocytes and osteoblasts. These cells form a 3-D network throughout the bone, cover all surfaces, and communicate with each other via the extensive cannalicular network (38–40).

The Osteocyte as the Strain Sensitive Cell

It is probably fair to say that the idea of the osteocyte network as the strain sensor within bones originated from the lack of any other clear purpose for such a highly connected cell population. A sizeable body of evidence now supports this hypothesis, much of which is the subject of several extensive reviews, showing these entombed cells to be highly responsive to changes in their strain environment (11,41–44). It has been repeatedly demonstrated that both osteocytes and the surface osteoblasts from which they are derived respond to changes in the strain environment with an almost immediate release of prostacyclin (PGI_2; 45). In addition, surface osteoblasts and lining cells also release prostaglandin E_2 (PGE_2; 45). Within five minutes after a period of strain either *in vivo* or *in vitro* there is a well documented increase in osteocyte glucose-6-phosphate dehydrogenase activity (46,47), which increases in a local strain magnitude dependent manner (48,49), and is followed within 24 hours by an increase in RNA synthesis in the osteocytes (46,50). What this RNA may code for is not yet known, although it has been assumed that it may be some growth factor, and an upregulation of IGF-I expression has been demonstrated by *in situ* hybridization (51). Loading of cortical or cancellous bone explants has been shown to increase expression of IGF-II mRNA (52). Molecular techniques, such as differential display, reverse-transcription polymerase chain reaction, have been used, with limited success to date, to try to detect candidate genes that are differentially regulated as a result of mechanical strain (53).

It also appears that there is a regional sensitivity in the response of osteocytes to mechanical strain, since *in vitro*, the osteocyte population of the calvarium appears less responsive to mechanical strain than that of the appendicular skeleton (1). This may provide an explanation for the fact that the bone mass of the cranial vault is preserved, despite the habitually low level of strain to which it is subjected. If the long bones of the limbs are subjected to similar low strain levels, during disuse for instance, the result is a marked loss of bone mass. This difference in osteocyte responsiveness seems likely to reflect a difference in bone

function, since the primarily protective purpose of the bones in the cranial vault is clearly different from that of the limb bones (2,3,54).

In vitro experiments have demonstrated that exogenously applied, either PGE_2 or PGI_2 is able to replicate the increase in G6PD activity in both osteoblasts and osteocytes, but that only PGI_2 replicates the increase in RNA synthetic activity and causes increased IGF-II release (55). Following mechanical loading there is a rapid release of the messenger molecule nitric oxide (NO; 56,57). This originates from both the osteoblasts and osteocytes, although on a per-cell basis, production by the osteocytes is greater than that by the osteoblasts. One of the known stimuli to NO release is wall shear stress exerted on cell membranes by fluid flow (58). Strain gradient driven fluid flow through the cannalicular network is one of the putative mechanisms by which osteocytes may detect strain (12). By use of specific inhibitors *in vivo*, it can be shown that both NO and PG production are essential for the development of a subsequent adaptive osteogenic response (59,60), providing evidence for a direct link between these strain-related responses in osteocytes, and the adaptive process.

Controlled Loading Experiments in a Juvenile Bone Model

Although the functionally isolated avian ulna preparation advanced our knowledge of the stimulus to adaptive bone remodelling, as with all *in vivo* models, there were several limitations. One important drawback was that this was a surgical model. To minimize the potential interference of the inflammatory or reparative processes with the interpretation of the results, the analysis was confined to a small central region of the bone equidistant from both osteotomy sites. A more recent model that avoids this complication is the functional rat ulna-loading model (61,62). In this system, loads are applied through the skin and soft tissues at the carpus and olecranon to load the intact ulna between its ends. Thus, there is no need for prior surgical preparation of this model. The longitudinal curvature (medial side concave) of this bone imposes a consistent strain distribution throughout any section of the ulna in response to axial loading. By adjusting the loading magnitude, frequency, or rate, the pattern of strain change, as verified by strain-gauged calibration specimens, can easily be precisely controlled and easily adjusted. Between loading episodes, normal cage activity is allowed, and this model therefore more closely resembles the normal situation in which a short period of adaptive loading is superimposed on a more prolonged background of habitual activity. Since osteotomy is not necessary with the preparation of this model, and the sampling site along the midshaft is anatomically remote from the site of load, it has proved possible to analyze the response to loading along a considerable length of the diaphysis.

The marked longitudinal curvature of the rat ulna is maintained during longitudinal growth by a consistent medial to lateral modelling drift (61–64). This drift involves resorption of bone from the medial bone surface, and active formation on the lateral surface (**Figure 1, A**).

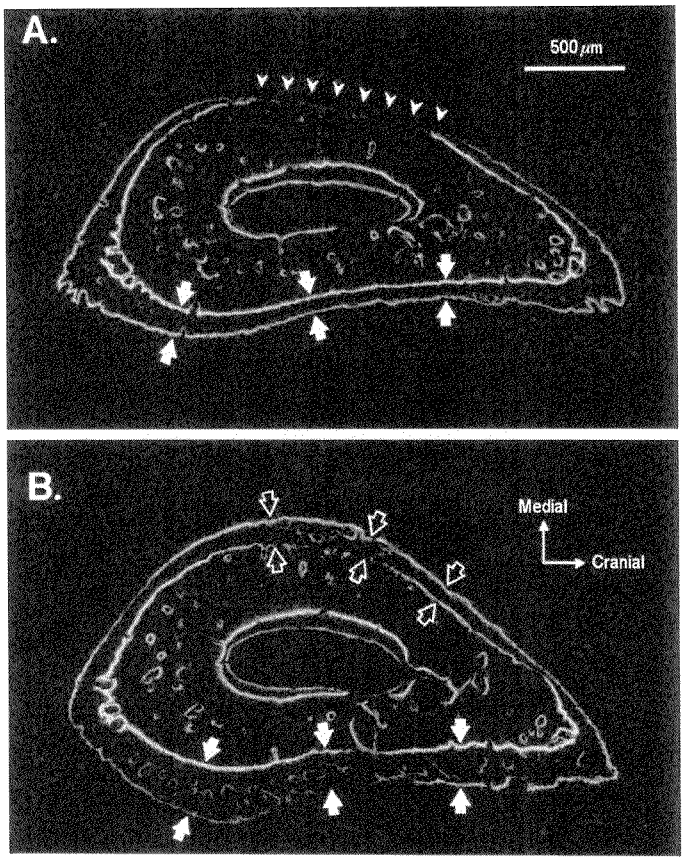

Figure 1.
Confocal fluorescence images (488 nm excitation, <515 nm emission) of paired cross sections through the ulna of a male Sprague-Dawley rat, taken 2 mm distal to the midshaft. **A.** *Non-loaded control ulna.* There is an extensive area of periosteal resorption on the medial face (arrowheads). Laterally there is deposition of lamellar bone (solid arrows), with formation of primary osteons at the cranial and caudal bone edges. **B.** [es]Contralateral loaded ulna. This ulna was loaded to give peak midshaft dynamic strain levels of -4000 microstrain. Note the complete reversal of resorption to active formation on the medial face (open arrows), and the increased mineral apposition rate on the lateral face (solid arrows). Scale bar represents 500 μm. Reprinted from Mosley JR, March BM, Lynch J, Lanyon LE. Strain magnitude related changes in whole bone architecture in growing rats. Bone 1997;20:191-8, with permission from Elsevier Science.

It has been proposed that growing bones such as this are more responsive to mechanical load since it should be easier to modify the ongoing modelling process of a growing bone, than to initiate a *de novo* remodelling response (33,54). There is a body of evidence in both animal and human studies to support this idea (65–67). The relationship between peak strain magnitude and the adaptive response in this model system proves to be complex (62). When loads are applied to engender strains of -2,000 microstrain at the ulna midshaft, a reduction in the amount of bone deposited at the periosteal surface is seen (**Figure 2**). At first sight this is counterintuitive, since strains of -2,000 microstrain are at the top end of the normal range of strains recorded from the ulna midshaft during natural activities. Since these physiologic strains were provided in addition to natural loads associated with normal activity, a reduction in new bone production might be unexpected. However, when the distribution of this change is studied in more detail, it can be seen that it results from a reduction in mineral apposition rate laterally, and a slowing of resorption on the medial surface. The combined result of these two changes is a coordinated slowing in the rate of modelling drift, which therefore fails to maintain curvature as the bone continues to grow in length. The result is a measurably straighter bone that is better able to resist axial loading, although at the expense of developing a normal curvature. This response shows adaptation of long bones as complete structures that adapt primarily by architectural modification (and in so doing save on the expense of *de novo* bone synthesis).

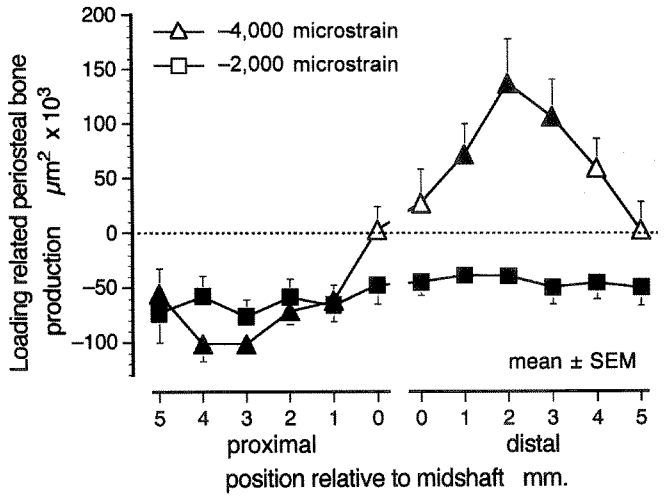

Figure 2.
Mean (±SEM) difference in periosteal new bone production between ulnae loaded to a peak dynamic strain magnitude of either -2000 or -4000 microstrain, and their respective non-loaded controls. Data are shown over the length of the diaphysis. A statistically significant difference between loaded and control limbs is indicated by solid plot symbols ($p<0.05$ by paired, two-tailed, Student's *t*-test). At moderate strain levels (-2000 microstrain), the adaptive response to axial loading involves a uniform suppression of new bone deposition along the diaphysis. At higher strain levels (-4000 microstrain), the suppression in the proximal diaphysis remains, but is replace in the distal diaphysis by a marked osteogenic response. Reprinted from Mosley JR, March BM, Lynch J, Lanyon LE. Strain magnitude related changes in whole bone architecture in growing rats. Bone 1997;20:191-8, with permission from Elsevier Science.

Longitudinal curvature, which is a feature of many bones, is believed to have evolved as a mechanism to confer predictability of strain distribution in response to the varied loading patterns which a bone may experience during natural loading events (7,13,15). A curved bone is forced to bend predictably in response to most loading configurations. Although bending increases strains significantly, the strain increases in a predictable manner with increasing loads. This predictability allows bones to increase their mass proactively if the safety factors are inadequate (4,13,15). This is in contrast to a straight column, which although able to withstand a greater axial load, may fail suddenly by buckling without warning (3,15).

As strains are increased above -2,000 microstrain, an osteogenic response to load is seen which, as for the avian ulna, involves an increased rate of osteogenesis on the previously forming lateral bone surface. Osteoclast activity on the medial surface is rapidly halted, with an associated cessation of resorption. Osteoblast recruitment and activation is seen at this site, and organized bone formation ensues (62,63; **Figure 1, B**). Taken together, the result of loading at these higher strain levels is a more massive bone, in which the mass is also better distributed to resist axial loading.

When the whole bone is considered, the adaptive response is clearly more complex than the findings from the single sampling site of the avian ulna experiments might suggest. The architectural response seen at moderate strain levels involved a net reduction in new bone formation. The many studies that have been conducted to investigate the effects of exercise on the skeleton often rely heavily on estimates of bone mass alone, and seldom assess changes in bone geometry. Clearly however, a lack of osteogenesis does not always indicate the absence of an adaptive response, and the findings documented in this experiment would have been incorrectly interpreted as a deleterious effect of mechanical load on the skeleton. The advent of newer imaging modalities that assess true vol-

umetric density, and allow 3-D reconstruction, may reveal a deeper understanding of such geometric changes.

The dual response we report contrasts with the purely osteogenic reaction in the avian ulna, which was linear from disuse to -4,000 microstrain (24). This discrepancy may be explained by a number of differences between the two models. In the avian ulna, an unusual strain distribution was imposed on a bone that was otherwise isolated from any loading stimulus. This strain distribution error was probably sufficient to produce an osteogenic response even at low strains, and this stimulus was undiluted by normal activity. In the rat ulna experiment, a short period of strain was applied in a distribution similar to that normally encountered during ordinary weight-bearing activity. This extrinsic stimulus was superimposed on the normal strain patterns produced by locomotory loading. This situation closely approximates the normal situation during load bearing exercise. In the absence of a strain distribution error, it may be necessary to increase strain magnitude to produce a similar increase in bone mass.

A similarly complex adaptive response involving architectural modification can be demonstrated when the rate at which load is applied to and released from the rat ulna is changed (68). As strain rates are increased across the physiological range, the magnitude of the peak osteogenic response does not increase, but rather bone mass is increased overall as the region showing a net osteogenic response extends further proximally along the ulna diaphysis.

To investigate the relevance of normal background loading to the development of the strain related stimulus, the functional rat ulna model was modified to allow loading in the presence or absence of background activity (69). Immobilization between loading episodes was achieved by a combination of neurectomy and external support. Strain gauge recordings verified that immobilization of the forelimb in this manner reduced peak ulna strain at the midshaft, from the -800 to -2,000 microstrain levels associated with normal activities to trivial levels (<10 microstrain). In each of these two groups (immobilized or normal activity) half the animals were subjected to daily loading, and the remainder served as controls to evaluate the effect of the immobilization procedure on normal growth. Since the primary interest was in the effect of background activity on the osteogenic response, the loads applied to the ulna were designed to produce the well-characterized osteogenic response, which was evaluated at the site of maximal response, just distal to the midshaft.

The immobilized animals did not grow as rapidly as the animals allowed normal activity. Comparison of the magnitude of the osteogenic response between the two groups revealed a significant interaction between the magnitude of the response to the application of a controlled strain stimulus and the presence or absence of background loading (**Figure 3**). This effect was present whether or not the data were corrected for the slowed growth rate in the immobilized group. Thus, the clear effect of background loading in this experiment was to reduce the osteogenic effect of an otherwise highly osteogenic stimulus.

The results of this experiment provide the first evidence

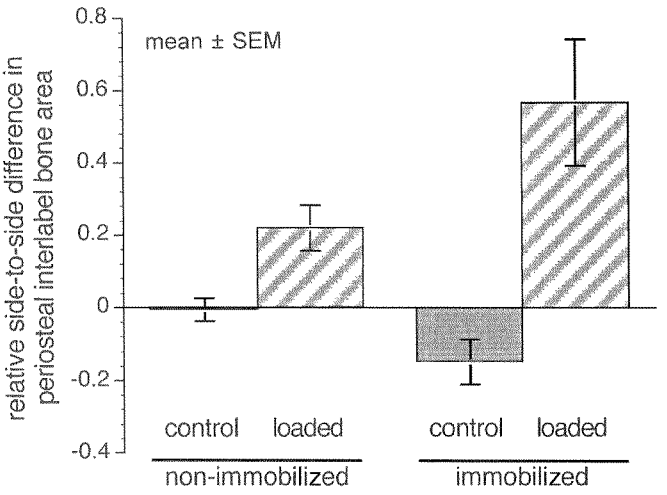

Figure 3.
Mean (±SEM) relative difference in periosteal new bone production for immobilised and loaded animals at the site of maximum osteogenesis (2 mm distal to the midshaft). Periosteal interlabel area differences between test and control limbs were calculated. These were then normalised to periosteal growth rate on an individual animal basis before calculating group means. In non-immobilised non-loaded (control) animals, there was no difference in periosteal bone deposition between limbs. In non-immobilised loaded animals, there was a considerable increase in periosteal bone production (22 percent above baseline growth) consistent with the previously documented osteogenic response at this site. Immobilised non-loaded animals demonstrated a reduction in periosteal bone deposition (15 percent below baseline growth). Animals in which the immobilized limb was subjected to a period of daily loading showed a large osteogenic periosteal response (57 percent above baseline growth). Within the two loaded groups, those animals which were immobilised showed a 48 percent larger osteogenic response than those which were allowed normal activity between loading episodes. ANOVA revealed that the magnitude of the relative loading-related response is highly significant ($p<0.001$), and that there is a significant interaction between load and the presence or absence of background activity ($p<0.02$). Background activity appeared to degrade the osteogenic potential of an otherwise highly osteogenic strain stimulus.

from an *in vivo* controlled loading model, that bone adapts to a time-averaged strain stimulus. Numerous low magnitude, low rate strains associated with normal coordinated activity, although not osteogenic in their own right, appear to degrade the effect of a single daily exposure to a highly osteogenic, high-amplitude, high-rate, strain stimulus.

This finding has important implications for the design of osteogenic exercise regimens. Such programs are aimed at reducing the risk of osteoporotic fracture by maximizing peak bone mass, or slowing the rate of postmenopausal and senile bone loss. Relatively short periods of vigorous activity interspersed with sedentary or low energy activity might be more efficient in promoting an increase in bone mass. It had been generally assumed that the effect of each loading cycle was cumulative in terms of production of the adaptive response, and algorithms for the relative contribution of each subset of loading events to the osteogenic response have been developed (70,71). These algorithms have assumed an increased importance with the spiralling use of computer simulation in adaptive modeling studies. How this time averaging of the strain-related stimulus is achieved at the level of the osteocyte network raises some interesting possibilities. It is worth commenting that in the avian ulna, a reorientation of matrix proteoglycans was detected following a period of controlled osteogenic loading. This reorientation persisted for up to 24 hours in the absence of further loading episodes, and was postulated as a "strain memory" (72,73). If this is the case then such a mechanism would provide a means to capture and time-average loading related events.

A Targeting Role for Osteocyte Apoptosis

Osteocyte apoptosis is known to occur in growing bones, as well as those undergoing remodelling (74,75). Following estrogen withdrawal in the rat (76) and human (77), there is an increase in the rate of both apoptosis and remodelling that can be prevented by the administration of estradiol (**Figure 4**). These data have led to the hypothesis that osteocyte apoptosis may play a role in the regulation and targeting of osteoclast activity. Using the functional rat ulna model, which has discrete and well characterized resorbing and forming surfaces, the distribution of apoptotic osteocytes has been mapped in relation to the arrest of bone resorption seen during the adaptive response. In normal rat ulnae, the distribution of apoptotic osteocytes is strongly related to the local strain magnitude, with the majority of apoptotic osteocytes clustered at the neutral axis, and in bone adjacent to the resorbing medial periosteal surface. In those bones loaded to produce an adaptive osteogenic response, the incidence of osteocyte apoptosis was reduced by almost 50 percent, associated with a change from resorption to formation on the overlying medial bone surface (78).

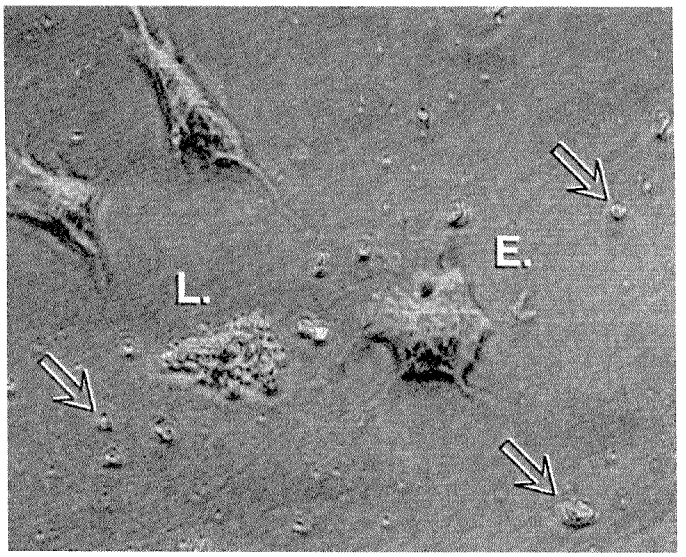

Figure 4.
Osteocytes (MLO-Y4 cell line) in cell culture showing morphological evidence of apoptosis. One cell (**L**) is in the late stages of apoptosis and an adjacent cell (**E**) is in the earlier stages. Numerous apoptotic bodies derived from these cells can be seen scattered throughout the field (arrows). Immunostaining reveals these apoptotic bodies to be strongly positive for osteopontin. This may be one mechanism by which osteocyte apoptosis is able to influence osteoclast function in vivo. (Original magnification × 400. Image shown courtesy of Dr. B.S. Noble).

Similarly, overloading bones to produce controlled matrix microdamage results in a large increase in the number of apoptotic osteocytes, which is followed by targeted osteoclast invasion, and remodelling of the damaged bone (79). These spatial and temporal relationships suggest the possibility that osteocyte apoptosis acts as a signalling system during bone growth to assist with the coordination of bone modelling. If this is the case and strain functions as a survival factor for osteocytes *in vivo*, then the documented association with a reduced (or in this case abolished) osteoclast activity might be expected.

Microscopic studies have revealed that apoptotic bodies appear to be able to travel through the lacunar/canalicular system to the bone surface, where they could exert an influence on the effector cells (B.S. Noble: personal communication). Whether this is a passive movement, associated with the outward fluid-flow that occurs within this system of canals, is open to debate, since this

might be argued to be too random a process to coordinate the highly targeted process of adaptive (re)modelling. It is tempting to speculate that the flow of these apoptotic bodies may be directed by the changes in cannalicular flow that occur with bone matrix strain, as fluid is forced along pressure gradients.

Postmenopausal Osteoporosis

The increase in bone resorption that is seen following estrogen withdrawal at the menopause results in increased skeletal fragility. At this time, there is clearly a failure of homeostatic adaptation to functional load bearing that suggests a critical role for estrogen in the adaptive process. The use of exercise regimens postmenopausally to prevent or slow the rate of bone loss has been advocated and studied (21,22,80–82). The characteristic loading patterns required to enhance bone mass significantly are those that are, particularly in a prematurely fragile skeleton, most likely to be dangerous in terms of fracture risk. Recently it has been demonstrated that mechanical strain exerts its action at least in part through the estrogen receptor (83). The down regulation of the estrogen receptor following estrogen withdrawal may well have a profound influence on the adaptive potential of bone. A logical therapeutic approach to restoring appropriate bone mass following the menopause would therefore be to enhance the adaptive process (84). This strategy would allow targeted bone deposition at sites of increasing strain to restore normal safety margins to failure. Although theoretically promising, a much greater knowledge of the signal transduction pathways which govern the adaptive response will be needed before this therapeutic option is likely to become a reality.

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