The physiology of habitual bone strains

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CHAPTER 7

GENERAL DISCUSSION
§ 7.1 Habitual bone loading—neither anabolic nor catabolic

Mechanical loading plays a significant role in co-determining the architecture and material properties of bone in the processes of skeletal growth and maintenance. Effectively, the mechanical loading of bone can be divided in three categories: ‘underloading’, which results in bone loss, habitual loading, which causes neither loss nor gain of bone, and ‘overloading’, which results in bone gain. The thresholds that separate these three categories are unclear, not in the least because of a varying mechanosensitivity between bones.

Due to its anabolic effect, and subsequently its use in treating osteoporosis, overloading has been studied the most to pinpoint the mechanical stimuli needed for bone-volume accrual. In-vivo experiments in which specific mechanical-loading regimens were artificially imposed on long bones have demonstrated that strain magnitude, strain rate, the number of strain cycles, strain frequency, strain gradient, and the insertion of pauses between loading bouts all are parameters that influence the amount of bone-volume accrual under overloading. The effects of underloading on bone have been extensively explored through bone-immobilization studies, bed-rest studies, and situations of microgravity. Indeed, the facts that bone loses volume in conditions of underloading and gains volume in conditions of overloading have been underlined more than once in the past decades.

However, in the average healthy situation, skeletal underloading and overloading do not play large roles—habitual loading plays the main role. Although there is bone gain during skeletal growth, mechanical loading in that period may be considered as habitual loading and not overloading as bone growth is, in part, genetically driven and can still increase under conditions of overloading (Raub et al., 1989; Biewener and Bertram, 1994). In adulthood, bone gain under habitual loading is minimal (High et al., 1981; Parfitt, 1994; Horton et al., 2008) except when mechanical overloading thresholds are crossed (Vainionpää et al., 2006). Nevertheless, hardly any attention has gone out to what the characteristics of bone strain are under habitual loading.

To better understand why a specific bone or a specific region within a bone reacts anabolic or catabolic to a mechanical stimulus at all, we first need to know how a bone is loaded regularly, on a day-to-day basis. Outlining this habitual bone-strain history will further clarify the thresholds above and below which bone tissue might start adapting. Following from this ‘Frostean’ view (named after Harold M. Frost, who introduced the term bone mechanostat; Frost, 1987) is that habitual bone strains are effective enough to prevent...
underloading-provoked bone loss. The loss of bone following a decrease in the intensity of
daily loading, which can then be categorized as underloading, confirms this assumption. As
mentioned earlier, prolonged bed rest and muscle paralysis, e.g., cause loss of volume of the
‘disused’ bones (Hulley et al., 1971; Rittweger et al., 2006). In the rabbit this dependence of
bones on habitual loading to sustain their volume has been observed also (Kenner et al.,
1975; Smith et al., 1992). Therefore, the habitual strain history at a given location on a bone’s
surface provides information on the mechanical stimulus for volume maintenance and for
balanced levels of turnover in that bone.

To quantify habitual bone-strain magnitudes, rates, and frequencies, we made
long-term measurements of bone strain *in vivo*, i.e., in the living animal, through the
combination of strain gauges and an implantable transmitter. Extending the length of the
bone-strain measurements up to several days enabled the inclusion of a variety of
behaviours, the loads that accompany those behaviours, and the resulting range of bone-
strain characteristics. The wireless feature enabled the laboratory animals, in this case
rabbits, to behave in a less restricted manner (Chapter 2). To evaluate how the habitual
bone-strain histories of different bones may overlap and deviate we sampled the rabbit
mandible; a non-weight-bearing bone, and the rabbit tibiofibula; a weight-bearing long bone
of the hind leg (Chapters 3 and 4). The shared daily habitual strain history of the two bones
contained, amongst others, 200-300 strain events per hour with amplitudes of 30-40 µε in
tension or compression at the sites of measurement. The number of strain events decreased
exponentially for larger bone-strain amplitudes. Averaging over the recording lengths of up
to 57 hours, strain events with amplitudes of about 100 µε in tension or compression
occurred only 10-20 times per hour, and events with amplitudes of 200 µε a mere 1-5 times
per hour. On the other side of the strain spectrum amplitudes of 10 µε or smaller were
represented very strongly in the habitual strain histories of the two bones. Unidirectional
strain analysis—instead of principal strain analysis—revealed that in the rabbit mandible
strain events with amplitudes smaller than 10 µε take place about two thousand times per
hour, i.e., there is roughly one strain event every two seconds. Also similar between the
mandible and tibiofibula were the exponential drop in occurrence of larger strain rates and
the exponential drop in power of higher strain frequencies. The most striking difference
between the two bones was the strong tendency of mandibular strains to be bound to
specific frequencies and amplitudes—a feature mostly absent in the tibiofibular strain
history.
Chapter 7

Thus, the habitual bone-strain histories point out that the vast majority of the daily strain events bone tissue experiences has much smaller amplitudes compared to the maximum amplitudes so often reported in literature. The modest daily share of more intense loading events is backed up by a host of bone-strain events that have a tremendous variety of amplitudes, frequencies, and rates. This habitual flow of bone-strain events somehow contains or is the mechanical share of the stimulus for bone homeostasis. The receivers of this stimulus are the cells that inhabit bone tissue. They must somehow perceive and react to the strains of the bone matrix that surrounds them.

§ 7.2  Habitual bone strains and bone cells

Bone tissue is equipped with complex cellular machinery that senses and responds to mechanical loads and their concurrent bone-matrix strains. Cells are the actual effectors of the processes of bone loss, maintenance, and gain. The conversion of mechanical stimuli into cellular responses is called mechanotransduction. The results of the mechanotransduction of non-habitual loading are obvious: infra-habitual bone loading starts up a catabolic response and supra-habitual bone loading elicits an anabolic response. Therefore, we may assume that the cells residing in bone perceive not only the presence of mechanical loading, but also its intensity. Under habitual loading, bone loss is prevented, but bone gain is absent. What, then, is the response of the many cells that populate bone under circumstances of habitual loading? Although the exact routes of mechanotransduction in bone are not unequivocally unmasked some discussion on this subject is warranted.

Generally, loss of bone under disuse is characterized by endosteal resorption, which concerns the trabecular, endocortical, and intracortical surfaces (Jaworski et al., 1980). A net loss of bone is achieved either by the origination of new sites of resorption, or bone turnover (Haversian remodelling) leaning over to a net bone loss, or both. In all cases, it is the osteoclasts that execute bone demolition. If habitual loading prevents bone loss, then the stresses as experienced by ‘decision-making’ bone cells must be large enough to inhibit the onset of new sites of endosteal resorption or prevent balanced bone turnover from tipping over to bone loss, or both.
To create a new site of endosteal resorption, new osteoclasts need to be recruited locally. Outside of the basic multicellular units that perform turnover, osteoclasts do not inhabit bone surfaces in anticipation of a resorption stimulus. They need to be formed by fusion of osteoclast precursors (Perez-Amodio et al. 2004; Ishii and Saeki, 2008). Developmentally, these osteoclast precursors belong to the monocyte lineage of common myeloid precursor cells. Osteoclast precursors can be recruited from the blood stream or the bone marrow by chemotactic agents secreted by bone-lining cells (Yu et al., 2003, 2004). Although these bone-lining cells—like many other cells—probably are mechanosensitive and can sense the sudden disappearance of habitual deformations of the surface they inhabit (Rubin et al., 2006), it is generally thought that the osteocyte population of bone functions as the main mechanosensor (Burger et al., 1995; Burger and Klein-Nulend, 1999).

Osteocytes have been reported to enter programmed cell death, or apoptosis, under conditions of disuse, whilst loading increases osteocyte viability (Dodd et al., 1999; Bakker et al., 2004). Moreover, osteocyte death has been linked to a locally increased osteoclastic activity (Bronckers et al., 1996; Noble et al., 1997, 2003; Aguirre et al., 2006; Hedgecock et al., 2007), whereas osteocytes experiencing mechanical stress inhibit the formation of osteoclasts (Tan et al., 2007; Kulkarni et al., 2010). The creation of a new site of endosteal resorption could therefore be prevented by keeping osteocytes from entering apoptosis, but also by obstructing any osteocyte-death-independent recruitment of osteoclast precursors. Thus, habitual bone strains may stimulate the osteocytes to obstruct osteoclast formation. Furthermore, if bone-lining cells can start chemotaxis of osteoclast precursors on the basis of their own mechanosensitive findings, the habitual bone strains must be dynamic enough to prevent such a string of events also.

If unloading causes bone turnover to shift towards a net loss of bone, than either osteoclastic resorption in the basic multicellular units is increased—e.g. by recruitment of more osteoclast precursors—or the deposition of new bone by the osteoblasts is slowed down. Osteoblasts are equipped with mechanosensitivity (Bakker et al., 2001; Kaspar et al., 2002), but it is lower than that of osteocytes (Klein-Nulend et al., 1995). Hypothetically, osteoblasts could be inhibited in laying down osteoid when the bone they reside in is no longer subjected to mechanical stresses. However, should unbalanced turnover play a role in disuse-induced bone loss, it is more likely that this is due to increased resorption rates as the more mechanosensitive osteocytes influence osteoclastic activity. This could be
prevented by the habitual bone strains stimulating osteocytes to not allow over-recruitment of osteoclasts in the basic multicellular units.

As mentioned in subsection § 7.1, larger strain amplitudes and rates are not very common on a daily basis in the rabbit mandible and tibiofibula, whereas the less ‘spectacular’ strain events are abundant. The shared ongoing habitual strain stimulus at the level of the periosteal bone surface contained an exponential decrease in occurrence of larger strain-event amplitudes. Recall that strain events with amplitudes of 10 µε or smaller even take place about two thousand times per hour. These less spectacular strain events—with average strain rates—might play an important role in bone-volume maintenance, as their daily presence is so certain (Fritton et al., 2000). Small bone-strain events, however, will be less easy for cells to perceive. The osteocytes have the advantage over other bone cells because they reside in lacunae. The architecture of lacunae has been shown to sometimes amplify locally the global bone-matrix strain, i.e., the strain measured at the periosteum, three to fifteen times (Nicolella et al., 2005; Bonivtch et al., 2007). Therefore, a strain-gauge measurement of 10 µε could mean a matrix deformation of 150 µε to certain osteocytes. In addition, bone deformation causes flow of bone’s interstitial fluid, including that in the lacuno-canalicular network. The fluid flow-induced shear stresses along the osteocyte’s cytoplasmic processes and the deformation of the lacunar wall around the osteocyte body both are likely candidates in amplifying global strains to elicit osteocyte responses in vivo (Bacabac et al., 2004; Vatsa et al., 2007). But what are the minimal strain and strain-rate amplitudes that will still suffice to prevent osteocyte apoptosis? Or ensure an osteocyte-mediated repression of osteoclast formation? Or prevent bone-lining cells from recruiting osteoclast-precursor cells? The actual minimal magnitudes of bone-matrix strain, strain rate, or bone fluid flow needed in vivo to stimulate the many mechanosensitive bone cells to perform their tasks concerning bone maintenance are unknown, but it is very likely that these magnitudes are included in the habitual bone-strain histories we measured in the rabbit mandible and tibiofibula.

Bone gain under circumstances of overloading is marked by periosteal bone apposition (Torrance et al., 1994; Hsieh et al., 2001). The initiation or amplification of periosteal apposition in response to overloading must therefore be executed through activation of bone-lining cells (the quiescent osteoblasts) or stimulation of present active osteoblasts. Strains associated with clear anabolic responses have large amplitudes (Cullen et al., 2001), high rates (LaMothe et al., 2005), and high frequencies (Hsieh and Turner, 2001).
Such pronounced bone strains may directly affect the bone-lining cells and osteoblasts without the involvement of the osteocytes. Like there is a minimal habitual strain history to prevent bone loss, there also is a maximum habitual strain history above which bone cells will start up an anabolic response.

Assuming homeostasis in the skeletons of the rabbits we sampled, the collected habitual strain histories should fall below the stimulus level that would trigger bone gain and above the minimum stimulus level needed for bone-volume maintenance, however, only in their respective bones. The habitual strain history of the rabbit mandible contained a very strong 5-Hz peak, which was absent in the strain history of the tibiofibula. The possibility exists that the minimal strain-history threshold for bone gain differs between the mandible and the tibiofibula. It is not unlikely that the presence of 5-Hz strains would be anabolic to the rabbit long bone. Conversely, the habitual strain history of the tibiofibula, lacking the explicit presence of rhythmicity, could have catabolic effects on the mandible. We should bear in mind that strains habitual to one bone may fall within the category of underloading of overloading to another bone.

§ 7.3 Loading of the mandible—a muscular matter

Bones and muscles work together. The stiffness of the bones of the skeleton provides the body with a frame from and onto which many skeletal muscles originate and insert. The musculoskeletal system of vertebrate lifeforms serves to maintain body posture and to enable locomotion in all its variations. It also executes all kinds of oral behaviours such as mastication, expression, posture, grooming, gnawing, and the grabbing and retaining of prey. It is not surprising that most of the mechanical loads on bones arise from muscle contractions, either directly or indirectly (Judex and Carlson, 2009). We studied several aspects of the muscle-bone relationship within the context of the rabbit masticatory apparatus. The masticatory system is an intriguing part of the musculoskeletal system as skeletal-muscle contractions seem exclusively responsible for all causes of loading; when they do not load the mandible, little else does.

In Chapter 5 simultaneous recordings of masseter and digastric muscle activity and mandibular bone strain clearly illustrated that the jaw-closing masseter muscle was almost
solely responsible for all daily bone-strain events. The largest strain amplitudes were found during episodes of prolonged cyclic loading, which indicate masticatory behaviours. However, the mechanical muscle-bone link existed not only during these bouts of cyclic activities, but also outside of them. The mandible is sensitive to changes in its habitual loading (Sato et al., 2005; Kingsmill et al., 2010). Therefore, the masticatory muscle contractions and the concomitant reaction loads co-determine mandibular bone maintenance. The facts that masticatory muscles are the main loaders of the mandible and that the most powerful muscles insert only on the mandibular ramus might have consequences to mandibular bone biology as muscles carry traits that set them apart from gravity and other causes of loading.

Several reasons exist to assume that loads of a muscular origin may separate themselves from gravitational and ground-reaction loads in their contribution to the habitual bone-strain history. Although the loads individual muscles impose on the skeleton are not necessarily greater than those caused by gravity and ground-reaction forces (Judex and Carlson, 2009), skeletal muscles will surpass gravitational loading in terms of the sheer amount of load events delivered to bone. Dynamic gravitational loading is mostly confined to behaviours such as walking, running, and jumping; activities that in many vertebrates cover only a small portion of the day. Outside of these behaviours many skeletal muscles participate in numerous other activities, as has been observed for human upper-limb and lower-limb muscles (Kern et al., 2001), and cat ankle muscles (Hensbergen and Kernell, 1997). The rabbit masticatory muscles display activity almost throughout the entire day with all kinds of intensities (Van Wessel et al., 2005). Data presented in Chapter 5 of this thesis revealed that the smallest masseter muscle bursts could still cause bone-strain events.

Not only will muscles load bone more often, muscle forces may also load the bone in a more versatile manner regarding amplitude and direction at the muscle-attachment site. The contraction of muscles is fine-tuned by the separate innervation of motor units, which consist of one somato-motoric neuron and the muscle fibres it innervates. Contraction of the muscle fibres of one motor unit would load the bone very regionally and along one specific line of action (Weijs et al., 1993; Turkawski et al., 1998). In addition, many skeletal muscles are compartmentalised, i.e., they are divided by sheets of fibrous connective tissue called aponeuroses. The muscle fibres of each compartment are oriented differently and insert onto the bone with a different angle. The combined traits of having motor units and
compartmentalisation enable individual muscles and muscle groups to load bones with a multitude of forces and varying directions.

The masseter and pterygoid muscles attach to the lateral and medial sides of the mandibular ramus, respectively. They are the most powerful muscular loaders of the rabbit mandible (Weijs and Dantuma, 1981). Highly compartmentalised (Schumacher and Rehmer, 1960), and with large attachment areas, they are endowed with all the traits to load the mandible dynamically and with diverging directions, rates, frequencies, and amplitudes. Indeed, the habitual strain history of the rabbit mandible as presented in Chapter 3 featured a great variety of strain-events. Moreover, the mandibular strain history included more events than that of the tibiofibula: more compressive principal-strain amplitudes between 200-400 µε, a greater incidence of tensile and compressive strain rates above 2000 µε/s, and a very strong presence of 4.5 Hz and 9 Hz bone strains (Chapter 4). The muscles that load the tibiofibula seem to do so less rhythmically in comparison with the masticatory muscles that load the mandible. Hopping and walking around in a cage cause tibiofibular bone-strain events, but these are not as frequency-bound or as numerous as the mandibular bone-strain events caused by daily bouts of mastication and other behaviours. The loads imposed on the tibiofibula by the hind-limb muscles may also feature less variety in amplitude and direction at the muscle-insertion areas as the compartmentalisation of the hind-limb muscles is not as advanced as that of, e.g., the masseter muscle. In Chapter 5 it was revealed that especially for smaller mandibular strains, the direction of the principal strain could vary greatly. No such an analysis was done for the tibiofibular strains, however.

With this in mind, the heterogeneous mineral density of the rabbit mandible, presented in Chapter 6 is striking. The most obvious feature of the rabbit’s mandibular mineral map is that the ramus has a lower mineral density than the corpus. An attempt was made to relate regional mineral-density variations to predicted regional strain-amplitude variations under simulated biting. However, the bone mineral density also seemed more related to muscle-attachment sites; with lower values where strong muscles insert. There is no clear answer as to what causes or maintains this heterogeneous mineral-density distribution. However, if the masticatory muscles that insert on the ramus were of relevance to the local mineral density, than they must somehow increase the local bone-turnover intensity— which would lower the local mineral density. The high-frequency component of muscle-force transmissions likely will not play a role in this as the highest observed frequency at which the masseter muscle strained the bone was ~15 Hz and this value was
measured anteriorly of the masseter insertion, on the lateral side of the corpus. In other words, high-frequency bone strains in the ramus are transmitted to the corpus. Another explanation could again be the compartmentalisation of the masseter and pterygoid muscles, which is extensive compared to the temporal, digastric, and facial muscles. The diversity in loading amplitudes and directions these two muscles can deliver might stimulate local bone turnover. Alternatively, the corpop-ramal difference in bone-mineral density might be related to paracrine effects of muscle tissue. Paracrine regulation between skeletal-muscle tissue and bone tissue has been observed to work through the expression of various growth factors, such as insulin growth factor I (IGF-I) and sonic hedgehog (SHH) (Alzghoul et al., 2004). Such proteins might influence, amongst others, the intensity of bone turnover. In the future, histology, immunohistochemistry, and other skills may chart Haversian remodelling activity and the expression of paracrine factors throughout the mandible. In addition, activity of skeletal muscles could be artificially altered, e.g., by injecting botulinum toxin, whilst simultaneously made measurements of the habitual bone strain history reveal which strain-stimulus components change the most.

The different relevance of the activity of muscles to the habitual strain histories of the mandible and tibiofibula reminds us that the origins of mechanical loading are not uniform throughout the skeleton. Skeletal muscle activity remains the primary loader of bone, but the differing anatomical functions between bones—including weight-bearing versus non-weight-bearing—ask for very different loading roles by the muscles. Long bones and vertebrae are chiefly weight-bearing bones that deal with combinations of gravitational and muscular loads as well as their reaction loads. In other words, the muscles that load weight-bearing long bones work together with gravitational forces to enable posture and locomotion. Limb muscles, limb bones and gravity form a trinity. Alternatively, ribs and calvarial bones face negligible gravitational forces. The muscles loading those bones do not work together with gravity and have altogether different functions—from lung-ventilating intercostal muscles to expression fine-tuning facial muscles. Like in the masticatory apparatus, such muscles and bones work together, independently of gravity. This will undoubtedly be of consequence to the amplitudes, rates, directions, and frequencies with and at which the muscles load the bones. As mentioned in section § 7.1, although many adult bones depend on habitual loading to maintain their volume (Weinreb et al., 1989; Lee et al., 2009; Rittweger and Felsenberg, 2009), this dependence is diverse between bones. Hence, the relation between the contractions of skeletal muscles and the morphology and
tissue composition of the bone loaded by those muscles will differ greatly from bone to bone.

§ 7.4  Bone strains and tissue engineering

Knowledge of the habitual strain history of a bone might be used in the expanding field of tissue engineering. The application of controlled regimens of mechanical loading has been shown to speed up and improve indirect, i.e., callus-mediated bone repair of a fractured bone (Goodship and Kenwright, 1985). The inclusion of higher strain rates and strain frequencies have appeared beneficial to the healing process (Goodship et al., 1998, 2009). Importantly, the applied mechanical stimuli must not exceed certain dynamics; otherwise the fracture-bridging callus would be continuously damaged by the mechanical stimulator (Kenwright and Goodship, 1989). These are strong indications that applying a more-or-less habitual loading environment with specific load rates and frequencies amplified can improve the quality of fracture repair.

The use of simulated habitual bone-strain histories may also augment the growth of bone tissue \textit{in vitro}. Osteotomies and bone loss due to, e.g., diseases or accidents sometimes call for the placement of a bone graft. Such grafts can be taken from one of the other bones of the patient’s skeleton, but may also be grown \textit{in vitro} using patient-derived mesenchymal stem cells seeded on scaffolds. The differentiation and proliferation of the scaffold-bound stem cells proceeds more effectively if the scaffold is loaded in a habitual-loading fashion; incorporating pauses (Sen et al., 2011) and loading it with habitual, but not supra-habitual strain frequencies (Dumas et al., 2009). These are strong clues that the habitual loading of bone is an important source of information on which strain-stimulus regimens may be based that are more effective in ensuring bone growth and maintenance than amplitudes, rates, and frequencies that simply overload bone.
Chapter 7

§ 7.5 Conclusions

• The strains engendered the rabbit mandible under habitual mechanical loading are highly versatile in amplitude, rate, and frequency.
• The strain events with the smallest amplitudes take place thousands of times per hour.
• In the rabbit, the greatest difference in the habitual strains of the non-weight-bearing mandible and the weight-bearing tibiofibula is not the maximum amplitude of the strains, but the strong rhythmicity of mandibular strains.
• To the rabbit mandible, the jaw-closing masseter muscle is an influential mechanical loader, while the digastric muscle plays a much lesser role.
• When regional bone-mineral density variations are neglected, the predicted strain amplitudes under habitual loading are especially large in bone regions that have a higher mineral density.

A start has been made in increasing our knowledge of what the habitual strain history of bone is composed of. This knowledge will help us in constructing new experiments and ideas to test how habitual bone strains influence the activities of the cells residing in bone. In such experiments it will be important to retain a loading environment that doesn’t deviate too much from the in-vivo situation, i.e., a three-dimensional loading situation would be preferable. Also, the boundaries between habitual loading and underloading and between habitual loading and overloading should be tried to discern. These will differ per bone and, quite possibly, between the regions of one bone.

The combination of anatomy, physiology, cell biology, technology, and curiosity will lead to new insights in the mechanobiology of bone tissue and might help us in improving the in-vitro culture of bone tissue, in improving the quality of bone healing, and in slowing down the loss of bone in diseases such as osteoporosis.
§ 7.6 References


~ 146 ~


~ 148 ~


