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Abstracts from the 3th International Workshop on Musculoskeletal and Neuronal Interactions

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Program Chairmen: J.A. Gasser, D. Thompson and T. Nakamura

The presentation of the Workshop on Musculoskeletal and Neuronal Interactions abstracts in two parts: Oral Presentations (OR) and Posters (P).
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OR-1

FRAIL BONES; WHAT *DO* WE MEAN?

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The clinically important end-result of frailty is fracture. Many factors contribute to whether a bone will fracture or not, including severity of fall, musculoskeletal integrity, energy absorption by overlying tissues and the mechanical properties of the skeleton. This talk deals only with the last factor. The mechanical properties of a bone result from a combination of the bone material properties and the bone's architecture.

Correlation between sites

First there is the question of whether knowledge of mechanical behaviour in one bone, or site within a bone is much use for determining what goes on in another bone, or site. Although the statistical relations are often quite strong, the actual power of the correlations is not usually large. For instance Eckstein et al.[1] found the following relationships (amongst others):

Fracture Load ⇒	T10	L3	Femur all	Trochanter
L3 BMD	42	52	38	31
Neck BMD	37	42	62	72
Trochanter BMD	24	27	55	67
Femur BMD	26	34	62	72

Table 1. Percentage variation 'explained' by the correlation (r^2) at various sites between in situ BMD values and strength.

	Vertebral load	Femoral load
Femoral load	26	-
Radial load	19	15

Table 2. Percentage variation 'explained' by the correlation (r^2) between three pairs of properties.

DEXA measurements often have a reasonable correlation, giving probably a useful amount of information, but the strength properties of one site are often very poor predictors for other sites. Of course, using DEXA measurements from one site to predict failure load at another will be even more dubious.

(Although I have used r^2 above, and r^2 is a popular method of assessing relatedness, it is important to remember that, from the point of view of prediction, often s (the mean square error about the regression line) is more useful and is not subject to distortion by outliers.)

Material properties

Material properties are difficult to measure non-invasively. Stiffness (Young's modulus), strength, fatigue resistance, toughness, fracture mechanics properties and so on vary with degree of mineralisation, remodelling, the integrity of the organic component, the degree of structural anisotropy in relation to the main load, and homogeneity. Some relationships between these properties and age, and also their effect on mechanical properties, are suggested in Table 3.

Bone becomes more completely remodelled with age, the integrity of its

organic material declines, and it also become less homogeneous, in particular, it tends to develop regions of very high mineralisation. Unfortunately, also, the most desirable outcomes are often incompatible. In particular high stiffness (Young's modulus) is incompatible with high toughness. Desirable features for resisting fatigue damage are unclear, though probably toughness is important.

Bone property ⇒	Mineralisation bone	Remodelled integrity	Organic	Anisotropy	Homogeneity
Change with age ⇒	?	Up	Down	?	Down
Mechanical property ⇓	Change in mechanical property if the bone property increases ⇓				
Stiffness	Up	Down	?	Up	Down
Bending strength	Up (to a limit, then down)	Down	Down	Up	Down
Tensile strength	Varied (to a limit, then down)	Down	Down	Up	Down
Toughness	Down	Varied	Down	Up	Down
Fatigue	?	?	Down	?	Down?

Table 3. Relationship between properties, and how they change with age, and mechanical properties.

Architecture

Some important changes of architecture with age are understood. Perhaps best known is that the second moment of area seems to increase, and this has important implications for strength, because a higher second moment of area may increase bending strength (though not compressive strength) even if the amount of bone mineral is decreased[2].

Architecture, as well as material properties also suffers from conflicting requirements - in a bone loaded in bending the optimum shapes for stiffness and impact resistance are different. These differences are small, however. More important is the requirement that, in impact, the bone should be stressed as equally as possible. The loss of cancellous bone in the distal radius has a twofold effect - the end of the bone is weakened by the loss of the cancellous material and, in an impact tending to produce a Colles fracture, the energy of the fall will be disproportionately concentrated in the already weakened end of the bone, because the metaphysis becomes more compliant relative to the diaphysis[3]. This relative weakening of the metaphysis is also found in the femur[4]. Somewhat counterintuitively therefore, increasing the strength of one part of a bone, by orthopaedic or pharmacological methods, may actually reduce the ability of the bone to resist impact!

Particular problems

- Developing microcracks may turn into dangerous fatigue cracks, or may weaken the bone when it is loaded in impact[5]. On the other hand, there is some evidence that bone is not weakened in impact by a moderate amount of microcracking, and in certain circumstances a phenomenon called 'microcrack toughening' may occur[6]. We need to discover how dangerous microcracking is, and develop ways of measuring it *in vivo*.
- There is a considerable amount of clinical evidence about what kind of fractures are particularly characteristic of different bones at different ages. However, we have little understanding, in detail, of what kind of loadings, with their associated stress systems and strain rates, produce the characteristic kinds of failure seen in bones. Until this is known, in turn we have little idea of the characteristics of the bone that it is important to measure. For instance torsional strength requires a different cross-section from that required for resistance to bending in one direction.
- The interaction of cancellous bone and its surrounding cortex is complex. Roughly, the strength and stiffness of a short segment of a bone are proportional to the amount of cortical bone material, but to the square of the amount of cancellous bone material. The implication of this is that loss of bone is proportionally much more serious in cancellous than in cortical bone. Measurements of amount of bone material without distinction between compact and cancellous bone are therefore conflating two different materials with different mechanical properties. Useful measurements of bone should be able to distinguish cortical from cancellous bone.
- Adequately combining material properties (measured somehow) with architecture (measured somehow) is at the moment dauntingly histomorphometrically and computationally expensive and probably, for some time to come, we shall have to rely on rules of thumb.

Where next?

Prediction of failure of bone can be mainly 'epidemiological', based on relatively straightforward measures of the amount of bone present and fracture incidence, treating, in effect, the actual mechanical goings-on inside the bone as a black box. In most circumstances this is still very useful, and is often the only method available. Nevertheless, methods of non-invasive characterisation of bone, particularly its architecture, are becoming more sophisticated, and a mismatch may arise between the information that can be gleaned about bones, particularly their architecture, and the predictive use to which this information can be put.

The clinician is concerned with the individual patient, not a class of patients sharing some characteristics, and might expect that more sophisticated measuring methods will lead to a better prediction, for it will be necessary to have a much clearer understanding of the mechanics of the various kinds of fractures in the first place. Finally, in the consideration of individual patients, the ability to obtain a reliable measure of bone material quality will be increasingly needed.

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OR-2

Searching for Wolff's Law: The Anabolic Potential of Low Level Mechanical Signals

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The principal responsibility of the skeleton is to support the loads and moments which arise during activity, resulting in mechanical strain in the bone tissue. The skeleton's ability to adapt to these functional signals was recognized well over a century ago and is now referred to as Wolff's Law. The premise of this "law" is that bone strives toward an optimized structure which caters to an individual's level of activity. Thus, each individual would tune the mass and morphology of their skeleton such that it could safely withstand the extremes of functional loading. While mechanical signals are, in general, recognized as strongly anabolic, identifying those specific components which drive the osteogenic response has proven difficult.

There is increasing evidence that extremely low magnitude (<100 microstrain) mechanical signals can be strongly osteogenic if applied at a high frequency (15 to 60 Hz).[1] Such high frequency low magnitude strains comprise a dominant component of a bone's strain history[2], indicating that these mechanical events represent a significant determinant of bone morphology. With this in mind, we have been examining if small perturbations in high frequency loading, induced non-invasively into the lower appendicular skeleton, will stimulate an increase in bone mass without sacrificing bone quality. Short-term animal studies provide evidence that very low intensity (<10 microstrain) mechanical stimuli are strongly anabolic if applied above 20Hz (Fig. 1). Extremely low-level strains (80me), if induced at 20Hz, promote osseointegration[3]. Ten minutes per day of these

low level signals (0.25g), induced non-invasively using an oscillating platform, are able to retain bone mass despite 23 hours and 50 minutes of disuse, while ten minutes of normal weightbearing fails to do so[4]. Longer term animal studies (one year), have shown that low level mechanical loading, inducing cortical strains on the order of 5 microstrain, can increase cancellous bone volume fraction[5], thicken trabeculae, increase trabecular number[6] and enhance bone stiffness and strength (fig. 2)[7]. Considering these strain levels are far below (<1/1000th) those which may cause damage to the tissue, we believe these signals hold great potential as a mechanical prophylaxis for osteoporosis.

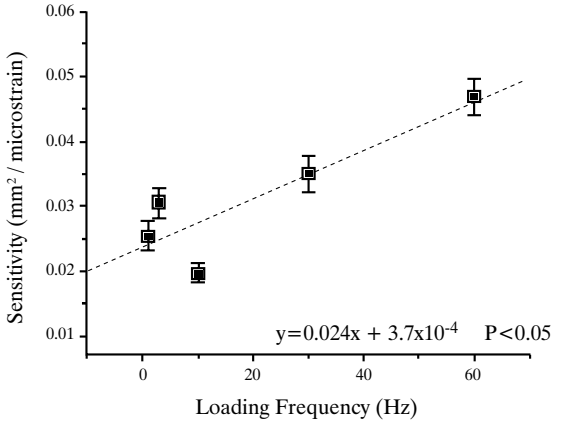

Fig. 1. The osteogenic potential of mechanical strain increases with loading frequency. Plot reflects the area increase (mm^2) in cortical bone measured for each additional one microstrain imposed on the turkey ulna, at each of five loading frequencies spanning the 1Hz to 60Hz range. Adapted from Qin et al., 1998[1].

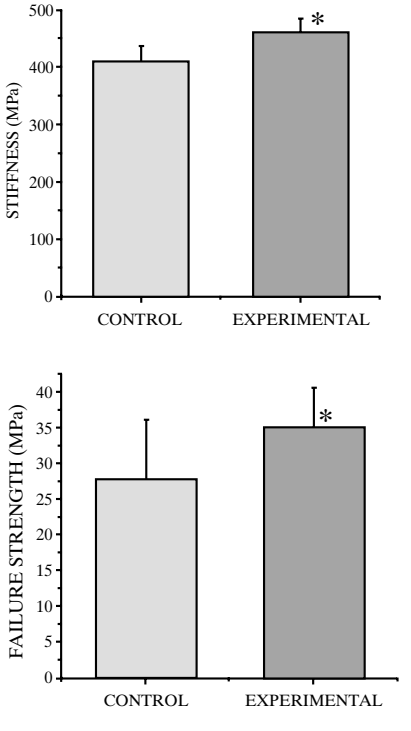

Fig. 2. Physical property measurements of cubes from the sheep femora demonstrate significant differences between the control and experimental animals, in both longitudinal stiffness (12.3% increase, left) and failure strength (26.7% increase, right); $p<0.05$ in both cases. These data indicate that the anabolic nature of the mechanical signal ultimately serve to enhance both the stiffness and strength of the bone, and thus improves both the quantity and quality of bone. Adapted from Rubin et al., 2002[7].

Importantly, this unique biomechanical intervention affords the ability to examine the molecular basis of an osteogenic signal, thus identifying novel targets for drug development. For example, osteoclast differentiation factor (ODF, or RANK-L) is a cytokine involved in the recruitment and activity of osteoclasts[8], and *in vitro* studies have linked its upregulation to the absence of mechanical strain[9]. In these experiments, we used rats as a model to examine the osteogenic efficacy of low-level high frequency mechanical stimuli and their ability to reverse the bone loss which arises under microgravity. We then hypothesized that the expression of RANK-L would be inversely related to altered tissue level bone formation rates.

Adult (6 month) female Sprague Dawley rats were assigned to controls (n=30), mechanically stimulated (n=21), tail suspension related disuse (n=11), disuse interrupted by 10min/d of normal weight bearing (n=7), and disuse interrupted by 10min/d of 90Hz stimulation at 0.25g (n=19). All experimental procedures were applied for 28d. Mechanical stimulation consisted of whole body vibration at 90Hz (0.25g). All rats were given injections with demeclocycline prior to the beginning of the study and calcein on day 18 of the protocol to determine histomorphometric indices of bone formation. RANK-L mRNA levels were quantified in three animals of each group (except disuse plus normal weight bearing group) via Northerns. RNA was extracted from whole left tibiae, including bone marrow and cartilage.

Body mass of the rats did not change significantly in any of the groups during the course of the 28d study. Mechanical stimulation at 90Hz for 10 min/d proved to be a strong osteogenic stimulus as indicated by increased trabecular bone formation rates (+97%, Fig. 3a). Hindlimb suspension significantly decreased trabecular bone formation rates by 92% as compared to controls. This suppression was not significantly different from the animals subject to disuse for most of the day (23h, 50min) and then allowed to freely bear weight for 10 min/d (D+WB). In contrast, when low-level mechanical stimulation was applied for 10min/d to combat disuse, the countermeasure served to normalize bone formation rates back to control values. Mechanical stimulation for 10 min/d decreased RANK-L mRNA levels by 78%. Disuse increased the expression of RANK-L by 72% with respect to control values while disuse interrupted by 10 min of daily mechanical stimulation decreased RANK-L levels by 49% (Fig. 3b). When linear correlation was used to relate bone formation rates to ODF expression levels across groups, the r2 value was 0.79 (inverse correlation).

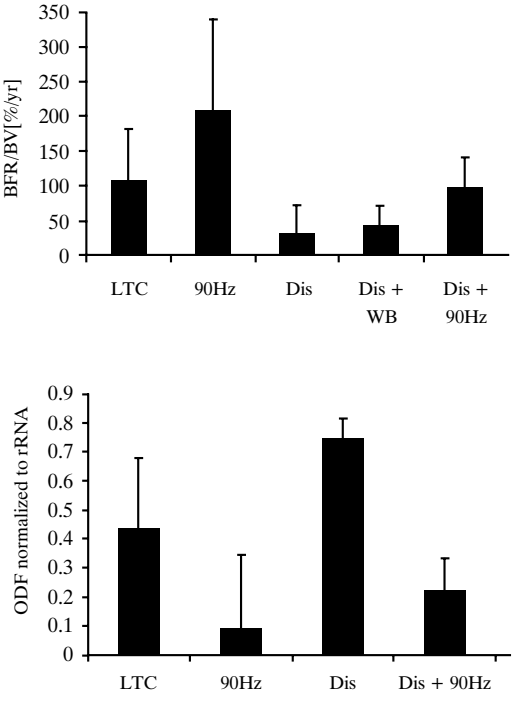

Fig. 3. Tibial trabecular bone formation rates (BFR/BV) of age matched controls rats (LTC) and after 28 days of mechanical stimulation for 10 min/d at 90Hz (90Hz), tail suspension (Dis), disuse interrupted by 10min of weightbearing (Dis + WB), and disuse interrupted by 10 min of mechanical stimulation (Dis + 90Hz). b. Relative expression of RANK-L (ODF) in

control, 90Hz stimulated, disuse, and disuse interrupted by 90Hz vibration rats (mean ± SD).

Using the mouse as a model, it is also apparent that the genetic make-up of the animal is a strong determinant of their sensitivity to mechanical stimuli[10]. Adult (16 week) female 16wk old C57BL/6J (low density), BALB/cByJ (medium density) and C_3H/He (high density) mice were assigned to control, mechanically stimulated, and disuse groups (n=13 each). Mice in the mechanically stimulated group were placed on a vibrating plate (45 Hz, 0.25g) for 10 min/d. Disuse animals were subjected to tail suspension. Four animals per group were culled 4d into the protocol for determining gene expression levels (semi-quantitative RT-PCR) while the remaining animals were sacrificed after 21d for the assessment of bone formation. Disuse failed to affect histomorphometric indices in C57BL/6J mice (Fig. 4). In BALB/cByJ, mechanical stimulation increased bone formation rates by 34% ($p<0.02$), but bone volume was unaffected. This increase in bone formation rate was primarily achieved by an increase in the ratio of double labeled surface to single labeled surface (+101%, $p<0.001$). Disuse in the BALB/cByJ mice suppressed bone formation rates by 48% ($p<0.01$), the ratio of double labeled surface to single labeled surface by 47% ($p<0.01$), and mineral apposition rates by 45% ($p<0.03$), resulting in trabecular bone volume that was 43% smaller ($p<0.01$) compared to control BALB/cByJ's.

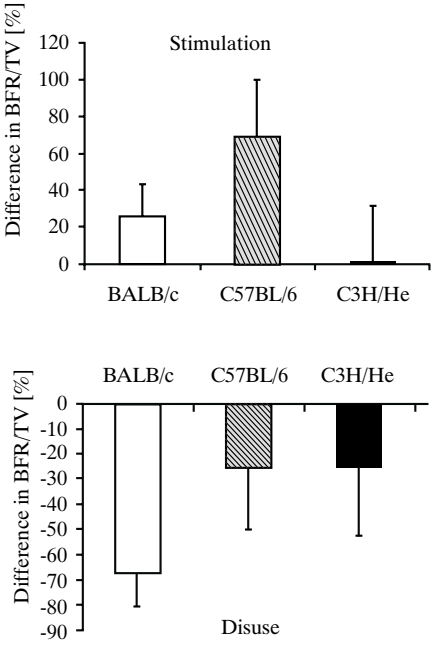

Fig. 4. Percent difference in bone formation rates (BFR/TV) between a. mechanically stimulated and age matched control mice and b. disuse and age matched control mice in the three genetically distinct strains of mice (mean±SD of the difference). Labels here refer to BALB/cByJ, C57BL/6J, and C3H/HeJ. It is clear that the genetic makeup of the animals helps define the extent to which they respond to anabolic and/or catabolic stimuli.

In contrast to the responsiveness of the skeleton of C57BL/6J and BALB/cByJ mice, no significant effects of mechanical stimulation or disuse were measured in tibial trabecular bone of C_3H/HeJ mice. These tissue level results were essentially mirrored at the molecular level. The transcriptional levels of collagen type I, the most abundant protein in bone, were significantly reduced in tibiae of hindlimb suspended BALB mice but not in those of any other group (Fig. 5a). This further emphasized the differential response of these mouse strains. The lack of upregulation of type I collagen mRNA after 4 days of mechanical stimulation may reflect its late occurrence in the cascade of events leading to new bone formation. Inducible nitric oxide synthase was significantly down-regulated by a similar percentage in mechanically stimulated mice of those strains that had responded to mechanical stimulation at the tissue level (Fig. 5b).

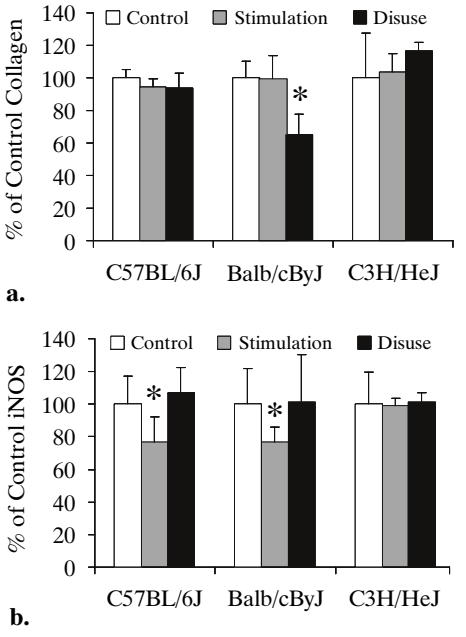

Fig. 5. a. Collagen type I was downregulated in BALB/cByJ mice subjected to disuse while the transcription of this gene was not affected in C57BL/6J or C3H/HeJ mice. b. Inducible nitric oxide synthase mRNA levels. Only the two strains that, at the tissue level, responded to mechanical stimulation, demonstrated a similar molecular (mean + SD, n=4).

Testing the anabolic potential of this biomechanical intervention in the human, sixty-two healthy women, 3-8 years past the menopause, enrolled in a double-blind, placebo controlled pilot study[11]. 32 women underwent mechanical loading of the lower appendicular and axial skeleton for two ten-minute periods per day, through floor mounted devices that produced a 0.2g mechanical stimulus at 30Hz (TX). 32 women received placebo devices (PL) and underwent daily treatment for the same period of time. Linear regression of the change in BMD shows a -3.3% (± 0.83) loss of BMD in the spine of the placebo group. Treatment reduces this loss to -0.8% (± 0.82), reflecting a net benefit of 2.5% ($p=0.03$). The trochanter of the femur shows a 2.9% (± 1.2) loss of BMD in the placebo group, while treatment stimulates a +0.4% (± 1.2) gain, reflecting a net benefit of 3.3% of treatment ($p=0.03$). Interestingly, the intervention was more beneficial in the group of women in the lower 50% of body mass, the same group that was most susceptible to bone loss. Subsequent studies in children with cerebral palsy[12] and girls, ages 10-13, in the lowest quartile of BMD[13] also demonstrate the anabolic nature of the signal.

In summary, evidence in both animals and humans, at the molecular, histomorphometric, densitometric and structural level shows that short exposure to extremely low-magnitude, high frequency loads are anabolic. Such a biomechanical intervention is self-targeting, endogenous to bone tissue, and auto-regulated. In essence, these studies lay the groundwork for a unique, non-pharmacogenic intervention for osteoporosis, based purely on the premise of "form follows function" in the skeleton, and that these low level signals can enhance both the quantity and quality of bone. These low level signals, perhaps providing a surrogate for the deteriorating musculature that occurs with age, implies that the persistent barrage of low-level signals provided by muscles during predominant functions such as standing may be as important as the high level signals, which occur far less often[14] in defining (and retaining) bone mass and morphology.

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OR-3

Changes of Muscle and Bone: Age vs. Immobilization

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Aging and deconditioning effects due to immobilisation lead to similar changes in the musculoskeletal system. With increasing age bone mass declines as muscle mass does, and the same is true in the course of immobilisation. Likewise, either condition leads to a decay of peak muscle force and power, and the control of balance is disrupted.

The modern lifestyle implies increasing sedentarism, particularly at higher ages. Hence both longitudinal and cross-sectional studies on the musculoskeletal system in aging populations may be biased. Moreover, the prevalence of most diseaeses is strongly age-dependant, which further complicates the identification of genuine age effects.

We have thus chosen two complementary approaches. Firstly, we studied young and veteran athletes at international competition levels. The veteran athletes' tendency of sedentarism can be regarded as negligible, and specifc diseases are of minor impact on their performance. Secondly, we immobilised young healthy subjects by strict bed-rest over 90 days (Long Term Bed Rest = LTBR study). In both groups, the muscle-bone relationship was investigated by peripheral computed tomography of the lower leg, and jumping force and power was measured on a ground reaction force plate.

As expected, immobilisation led to a reduction of muscle mass, muscle force and muscle power, and subsequently to losses of bone mineral content in the leg. Moreover, balance control was affected. The subjects recovered from these changes within different time ranges, depending on their physical efforts. Recovery appeared to be complete.

In the veteran athletes, muscle force and power turned out to be significantly and substantially greater than in age matched controls, but not if compared to younger subjects.

In conclusion, the effects of immobilsation are apparently completely reversible, whereas the physiological process of aging is irreversible and can at best only be slowed down, even by extensive exercise.

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OR-4

Aging of the Locomotor System: Frailty and Risk of Falling

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Pathogenetic and epidemiological background

Locomotor performance is a key determinant of frailty as well as of falling. Falling without loss of consciousness and without overwhelming external force or challenging activity can be regarded as manifestation of a decline of locomotor competence and as an indicator for frailty. The pathogenetic cascade leads from the decline in locomotor functions to falling, fall-related fractures and further to overall functional decline. Falls account for 87 % of all fractures in the age group of 65 and older, and resulted in 340.000 hip fractures in the USA 1996 (NCIPC). More than 90 % of hip fractures are caused by a fall. They result in a 15-25 % increase in mortality in the successive year and a 20 % increase in nursing home admissions. A third of all elderly people (65+) fall each year. 5 % of falls result in a fracture, a fifth are hip fractures.

Assessing fall risk and frailty risk

The EPESE test battery (Established Populations of Epidemiologic Studies of the Elderly) is a group of measures of lower extremity function and has been proven as predictive for (a) locomotor disability (b) functional decline in activities of daily living (c) premature death, (d) increased number of hospitalizations, and (e) of nursing home admissions. It comprises gait velocity, standing maneuvers (Romberg, semi-tandem and tandem standing) and timed chair rising. All three performances have also been found to be fall risk factors in several prospective studies.

Given a remarkable diversity of the investigated populations, diverse settings and diverse performance tests, the following characteristics are consistently found as independent fall risk factors:

1.) Muscle strength/ muscle power of the lower extremities, 2.) Lateral balance, 3.) Vision, 4.) Polypharmacy or certain CNS medications, 5.) cognitive impairment.

Age-related reference data

Assessing muscle function, fall risk, locomotion and balance requires age-, sex- and race-related reference values (cf. www.mobility-clinic.de). We are conducting a number of studies for establishing reference values. We have standardized the test procedures, and conducted reliability studies comparing the reliability of some performance tests (up and go test, freely chosen gait velocity, maximal gait velocity, chair rising test). We have compared these tests with a newly developed approach to assessing muscle function: the mechanography with the Leonardo system (Novotec, Pforzheim, Germany). Using force measurements with a force plate the Leonardo system can continuously measure force, velocity and power during unrestricted movements. We obtain objective data (watt, m/s, Newton) according to the conventions of physics, and can analyse human movements on a deeper level gaining new insights in the age-related capability to generate force and velocity and for storing energy in elastic elements of the neuromuscular system. We have found that power is the key measure for assessing neuromuscular function in a handicapped and aged population and has a very strong and functionally relevant correlation to the aging process.

OR-5

Physical Performance and Ageing

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Ageing is characterised by the progressive loss of functioning cells from

many organs. This results in progressive erosion of the safety margins between maximal function and critical threshold levels of function. These margins are generous in youth but may have vanished by old age.

The loss of muscle ('muscular cachexia', or 'sarcopenia') is central to the declining physical ability, increasing fatiguability and progressive frailty of old age. It begins in middle age, proceeds at approximately 1% per year, and impairs all aspects of muscle function. Much of the loss is due to the loss of muscle fibres, probably resulting from a slowly progressive and incompletely compensated denervation and unrelated to habitual inactivity. The loss of muscle fibres may also be due to impaired regeneration of muscle after damage. A variable degree of shrinkage of some of the surviving muscle fibres also contributes to the loss of active muscle tissue and may reflect individual variation in habitual activity. Although most of the weakness is directly attributable to the reduced muscle mass, older muscle may also be weak for its size.

The age-related loss of muscle performance is greater than the loss of body weight. This has important implications for gait and mobility, especially for women, as they have a lower percentage of their body weight as muscle than men of the same age. In the English National Fitness Survey, nearly half of all women (but 15% of men) aged 70 to 74 had a power/weight ratio below the value at which they could still be confident of managing a 30 cm step (without using a hand rail). The loss of muscle, together with changes in cardiovascular function, also limits the ability to perform endurance (aerobic) exercise. In the English National Fitness Survey, 80% of women (but 35% of men) aged 70 to 74 had an aerobic power/weight ratio such that they would be unable to walk comfortably at 3 miles per hour. By age 80, it seems likely that even just 2mph (in comfort) is impossible for most 80-year-old women. Indeed, even just sitting still is probably as fatiguing for a detrained 80-year-old female patient as the average intensity of work over an 8 hour shift is for a young man in heavy industry.

The loss of muscle, and its disabling impact, may be further exacerbated in disease states common in old age. This may be secondary to enforced immobility (e.g. after stroke) or may be a result of increased catabolism (e.g. after surgery). The loss of muscle may be so severe that the patient becomes unable to perform ordinary activities of everyday life. With elderly patients, effective acute treatment of an illness, whilst essential, is no longer sufficient to ensure a return to the patient's usual level of functional ability and independence. For example, 12 months after surgery to repair a hip fracture, fewer than half of the survivors previously able to do so are able to return to walking alone outdoors.

Fortunately, octogenarians undergoing physical training experience gains in strength and in aerobic power equivalent to some 10-20 years 'rejuvenation'. Adequately controlled studies have confirmed a beneficial response resistance training for healthy octogenarians, for residents in institutional care, and for those who have sustained a hip fracture. Improvements in aerobic power produced by endurance training have been confirmed for healthy octogenarian women.

Strength training may also provoke valuable enlargement of remaining muscle fibres although the underlying, progressive, age-associated reduction in the number of muscle fibres appears to continue.

There are urgent humanitarian and financial reasons for further research into the sarcopenia of old age - into its pathophysiology, its response to potential interventions and, the ultimate test, the effect of putative interventions on the elderly person's ability to perform everyday tasks independently, safely and without distressing fatigue.

OR-6

Impact of Sarcopenia on Aging

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Age-related loss of muscle (sarcopenia) in the elderly has a significant impact on morbidity, mortality and healthcare costs. This reduction of muscle mass in the elderly results in reduced strength and performance and compromises the quality of life. One serious consequence of sarcopenia in the elderly is an enhanced propensity to fall and suffer injuries from the fall. It has been reported that approximately 30% of individuals > than 65 years of age and 50% of individuals > than 80 years fall each year. Also, 10% of these falls result in serious injury and about 4 - 6% result in skeletal fractures. In the U.S., the average cost of hospitalization due to falls among the elderly is about $12,000. Approximately one-third of all admissions to long-term institutional care of the elderly is directly due to loss of physical performance resulting from poor muscle strength. With the 65 year old plus segment of the population growing at the highest rate, the impact this changing demographic profile will have on healthcare systems throughout the world will be substantial. In 1900, the percentage of the world's population that was 65 + yrs of age was less than 1%. In 1992 this increased to about 6.2%. In 2050, the percentage of individuals in the population is expected to increase to greater than 20%. Thus, it will become an important healthcare imperative to develop strategies that will improve physical strength and performance in the elderly to prevent falls leading to loss of independence, quality of life and high healthcare costs.

OR-7

Muscle and Bone Changes with Age: A Male Rat Model for Frailty Research

Age Related Changes in Muscle Mass and Function in Rats

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Aims: Declines in skeletal muscle mass and strength (sarcopenia) and in bone mass (osteopenia) occur with aging and contribute to increased falls and fractures and to a decline in ability to perform activities of daily living in the elderly. There is presently no established animal model to study the etiology of or to evaluate the utility of targeted interventions in the treatment of age-related sarcopenia. To establish a rodent model of age-related frailty, we have monitored changes in skeletal muscle mass and strength as well as changes in bone parameters in a colony of naturally aging male Sprague Dawley rats.

Methods: Male Sprague Dawley rats were studied at 6, 9, 12, 15, 18, 21, 24, 27 and 30 months of age. Body composition was measured by dual energy X-ray absorptiometry, peak indirectly evoked tetanic force and fatiguability of the tibialis muscle by electrophysiology, and organ and muscle weights following dissection. PQCT analysis of the distal femoral metaphysis, and trabecular bone histomorphometric analysis of the proximal tibial metaphysis were also performed.

Results: Body weight peaked at ~600 g by 12-18 months followed by a gradual 12% decline to 520 g by 30 months. Tibialis muscle mass and peak evoked tetanic tension peaked at ~1050 mg and ~950 g force, respectively, by 12-15 months and declined progressively thereafter to 770 mg and 700 g force at 24 months and 470 mg and 300 g force by 30 months. These muscle changes were paralleled by declines in bone mass. Trabecular content and density of the distal femur declined progressively after 18 months while the cortical bone content of the femoral shaft and trabecular bone volume of the proximal tibia declined progressively after 21 months of age.

Conclusions: Age-related sarcopenia occurs in parallel with osteopenia in Sprague Dawley rats, beginning at ~18 months and progressing slowly until 24 months when progression is accelerated. Thus, this rat model would be suitable for studies of naturally occurring frailty (sarcopenia and osteopenia).

OR-8

Strength and Fatigue in Ageing Human Muscle

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Muscle strength, defined as the amount of force a muscle may produce during a maximum voluntary isometric contraction, declines with increasing age. Cross-sectional data suggest that in the knee extensor muscles this decline may occur at a rate of ~1-2% per year (relative to a 77-year-old's value) after 65 years of age. The loss of muscle strength is most closely associated with a reduction in muscle cross-sectional area. However, this

does not explain all of the strength loss, i.e. there is a loss of force per unit area of muscle. When measured *in vivo*, this may result from an underestimate of the increased proportions of the aged muscle occupied by fat and connective tissue and potentially from poorer levels of neural drive. However, at the intracellular level, there are a number of reported changes which may contribute to this phenomenon, such as changes in the kinetics of Ca^{2+} release from the sarcoplasmic reticulum. Recent studies using human single muscle fibres which have been chemically skinned, suggest that fibres from older people may be intrinsically weaker then those from a young muscle. The mechanisms are unclear, but a reduction in the number of cross-bridges, a lowering in the force potential of individual cross-bridge or a change in the density of myofilaments have all been postulated as contributory factors. Fundamental changes in the contractile component of the older muscle are supported by a slowing in the speed of movement of actin filaments studied using the *in vitro* motility assay and an increased stretch force to isometric force ratio. There is also some evidence to link the role of certain hormones such as oestrogen and progesterone to the loss of specific force. Muscle power, the product of force and velocity of contraction appears to be affected by the ageing process to an even greater extent than isometric strength. This may result, in part, from a selective atrophy of the type II fibres leading to a reduction in the overall proportion of the muscles occupied by fast MHC-IIA and MHC-IIX isoforms. This will result in a slower velocity of shortening adding to an already weaker muscle in reducing power output. This phenomenon may be further compounded by a reduction in the maximum velocity of shortening of fibres expressing the same myosin isoforms.

Whole body exercise capacity, as indicated by maximal oxygen consumption ($VO_{2\,max}$) is reduced in older people, resulting in an increase in the percentage of $VO_{2\,max}$ needed to perform exercise at a given workload. The muscles of weaker older people are thus performing at higher relative intensities than the young people in order to perform similar absolute levels of power output. Such an unfair comparison suggests that old muscles are thus more fatiguable. However, if muscle fatigue resistance is studied under controlled conditions such as those in which subject volition is removed by electrically evoking contractions and fatigue resistance is normalised to allow for differences in muscle mass, then an aged muscle appears to be no more fatiguable than a young muscle. However, increasing evidence suggests that ageing is associated with increased mutations in mitochondrial DNA. The functional implications of such changes remain unclear, because in contrast, analysis of key mitochondrial enzymes involved in the oxidation of glucose and fats suggest that their activities, are similar to young subjects, particularly if the level of physical activities of the subjects from which the muscle samples are taken is controlled. Furthermore, the results of nuclear magnetic resonance studies have similarly failed to show differences in skeletal muscle energetics between young and elderly subjects.

OR-9

Catabolic Effects Of Growth Hormone On Bone Under Low Protein Intake

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Isocaloric protein undernutrition is associated with bone loss and decreased bone strength, which seem to be related to a decrease in Growth Hormone (GH) and/or IGF-I secretion and/or action. Whether GH administration can reverse the protein undernutrition-induced alterations in bone turnover, bone mineral mass and bone strength under a low protein diet, which is a situation frequent in the elderly, is not known. Six-month old female rats were fed isocaloric diets containing 2.5% (low Protein/LP) or 15% (normal Protein/NP) casein for 2 weeks. At this time, plasma IGF-I is markedly decreased in LP rats. Then, GH (0.5 or 2.5mg/kg BW) or its solvent were given subcutaneously to rats on either diet twice daily for 4 weeks. Proximal tibia (PT) and spine bone mineral density (BMD, mg/cm^2) and ultimate strength (N), together with urinary deoxypyridinolin excretion (Dpyr, nmol/day), osteocalcin (ng/ml) and IGF-I were measured. GH caused a dose–dependent increase in IGF-I under both NP and LP diets (585±17, 709±20 for 0.5 and 2.5 mg GH vs 476±22 ng/ml in controls, $p<0.05$, and 450±30, 556±29 vs 304±19, $p<0.05$), respectively.

	NP Control	NP GH 0.5	NP GH 2.5	LP Control	LP GH 0.5	LP GH 2.5
PT BMD	253.1±4.9	254.4±5.0	263.0±4.5	262.7±3.5	248.8±5.4	234.0±4.7*
PT Strength	208.0±11.9	209.9±5.4	191.1±14.9	225.7±9.4	199.7±12.2	139.9±13.9*
Osteocalcin	16.2±1.6	17.5±1.1	25.7±2.5*	10.92±0.9	13.5±0.7	18.7±1.6*
Dpyr	2275±164	3295±326*	4223±628*	1832±221	1893±217	2591±113*

Values are means±SEM, * $p<0.05$ as compared to controls by ANOVA

GH dose-dependently decreased bone strength in rats fed the LP diet. Similar trends were observed for midshaft tibia and spine. This was associated with a decrease in BMD. GH also increased bone turnover in rats on both NP and LP diets as indicated by change in plasma osteocalcin, urinary Dpyr. Thus, a stimulation of bone turnover results in a negative bone balance, when the protein intake is low. These results emphasize the major importance of dietary protein intake in the bone response to GH administration. This question is of clinical relevance since protein malnutrition is frequently observed in the elderly.

OR-10

Effects of Age, Estrogen Depletion, and Parathyroid Hormone or Prostaglandin E2 Treatments on the Calcaneus in Female Rats

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The main goals of this study were to determine age-related changes and estrogen-depletion effects on the calcaneus of rats and the envelope-specific responses of the calcaneous to parathyroid hormone (PTH) or prostaglandin E2 (PGE2) treatments. Female Sprague Dawley rats were subjected to sham surgery or bilateral ovariectomy at 6 months of age. Two months after surgery, ovariectomized (ovx) rats were injected subcutaneously (s.c.) for 60 days with vehicle or 80 mg/kg hPTH (1-34) or with 6mg/kg PGE2. The calcaneus was collected from each rat and the mid- transverse sections were processed undecalcified for bone histomorphometry measurements. Results: In the calcaneus, periosteal expansion ceased at 6 months of age. Despite the fact that periosteal bone formation and endosteal bone turnover were increased after ovx, either cortical or cancellous bone loss was observed. Both PTH and PGE2 treatments of ovx rats did not increase periosteal bone formation significantly. However, PGE2 increased the cancellous bone volume by 20% by thickening the trabeculae and increasing bone formation by nearly 300%, while PTH treatment did not increase cancellous bone volume despite the bone formation rate being increased by about 100% compared with vehicle treatment of ovx rats. These results indicate that the magnitude of anabolic responses to PTH and PGE2 treatments in calcaneus vary from those commonly seen in other cancellous bone sites such as the proximal tibial metaphysis and the lumbar vertebral body or cortical bone sites such as the tibial shaft. At these skeletal sites, augmented cancellous bone mass and markedly stimulated bone formation is generally observed with PTH treatment and stimulated periosteal bone formation is usually seen with PGE2 administration.

Groups	Ct. Ar %	BV/TV %	Ps-BFR mm/d	Es-BFR/BS $mm^3/mm^2/d$	Es-BFR/BV %/yr
Baseline	68±2	35±4	1.7±3.6	0.7±1.0	4.0±6.2
8mo-Sham	71±3	35±10	0±0	1.5±1.9	10.9±12.9
10mo-Sham	70±4	34±5	1.3±3.0	1.8±1.0	14.1±7.8*
8mo-OVX	74±2	30±4	17.8±8.7*	4.2±2.6*	31.8±20.0*
10mo-OVX	74±5	35±4	0.5±1.3	6.3±9.7*	31.6±38.5*
PTH	72±3	34±12	5.8±6.1	13.4±4.4*	80.0±30.3*
PGE2	79±2*	43±5*	0.4±0.8	24.4±4.8*	117.2±20.18

* = $p < 0.05$ from the baseline group. Ct. Ar, cortical bone area; Ps, periosteal surface; Es, endosteal surface; BFR, bone formation rate.

OR-11

Non-Invasive Monitoring of Changes in Structural Cancellous Bone Parameters Over 20 Weeks in Rats With a Novel Prototype Micro-CT

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At present, obtaining information on changes in structural parameters of cancellous bone has only been possible after necropsy of animals followed by histomorpometric analysis or time intensive measurements on dedicated microCT systems *ex vivo*.

In our study we tested a novel prototype micoCT scanner from SCANCO which allows for the first time to monitor such changes repeatedly in anaesthetised animals at 26µm resolution with an acquisition time of less than ten minutes for mapping of the entire proximal tibia metaphysis.

Skeletally mature, 8-month-old virgin Wistar rats (n:10 / group) were treated the following way to induce specific changes in cancellous bone architecture: 1. Sham OP + placebo s.c., 2. hPTH(1-34) (25µg/kg/day s.c.), 3. OVX + placebo p.o., 4. OVX + zoledronic acid (5µg/kg s.c. 2 inj/week) or 5. OVX + 17-alpha ethinylestradiol (aEE, 0.3mg/kg/day p.o.). Cancellous bone structure was assessed at baseline as well as 1, 2, 4, 8, 12, and 16 weeks after surgery.

Sham operated animals did not show any significant change in trabecular number (TbN), thickness (TbTh), bone volume/tissue volume (BV/TV), connectivity density (ConnD) or the Structure Model Index (SMI). Administration of hPTH(1-34) increased BV/TV, TbTh significantly at 2 weeks with the effect starting to plateau at 4 weeks. TbN did not change significantly but tended to be lower than in Sham OP rats. The SMI dropped dramatically and even assumed negative values in the PTH-group indicative of the development of a plate-like structure. In OVX-rats BV/TV, TbTh and TbN dropped to reach statistically significant differences from Sham OP animals at two weeks. The increase in the SMI indicated development of rod-like characteristics. ConnD also decreased significantly at 8 weeks and thereafter.

Rats treated daily with aEE or zolderonic acid were not different from Sham OP rats at any time-point or parameter. A tendency towards an increase in TbTh and BV/TV was observed in rats treated with the bisphosphonate.

Our results demonstrate for the first time that this protoype micro CT is capable to assess 3D-trabecular microarchitecture accurately, repeatedly, reliably and quickly in anaesthetised rats. Furthermore it is capable to pick up the structural changes induced by OVX, anabolic and antiresorptive treatment and to distinguish between them.

OR-12

The Effect of Post-Exercise Nutrition on Skeletal Muscle Training Adaptations in the Elderly

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Resistance exercise is known to be able to counteract the age-related muscle atrophy by increasing the net protein synthesis of the exercised skeletal muscles in the elderly. Further, muscle protein metabolism is also affected by nutritional intake during rest. Thus, if protein is ingested in the post-absorptive state it will create a positive protein balance whereas a carbohydrate-intake only reduces the negative protein balance. Further, an additive response in net protein synthesis has been observed in young subjects with administration of protein after a bout of resistance exercise. Yet, controversy exists whether a daily nutritional supplementation has an additional effect on hypertrophy of the trained muscles in the elderly when evaluated over 10-12 weeks of resistance exercise. However, this may be explained by the timing of the nutritional intake. Accordingly, it was found in a group of the elderly that an immediate post-exercise intake of a protein supplementation resulted in muscle hypertrophy evaluated over a 12-week training period, whereas a matched group receiving the same supplementation but two hours post-exercise had no change in muscle mass. Evidently, amino acid availability plays an essential role in the regulation of the muscle protein metabolism following resistance exercise and apparently is the anabolic state affected by ingestion of small amounts of protein. In order to provide valuable recommendations of post-exercise protein intake in the elderly knowledge on the first-pass splanchnic extraction of amino acids and the potential decrease in amino acid sensitivity of muscle with aging must be taken into account as well as the information of the fate of circulating amino acids.

OR-13

Tendon Collagen Turnover in Exercising Humans

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Connective tissue of the tendon and within skeletal muscle plays a major role in force transmission during muscular activity, but studies of its collagen turnover have been limited. Recent development of methods have allowed for a demonstration of exercise induced increases in tendon tissue blood and metabolism *in vivo*. In association with this, determination of procollagen peptides and degradation peptides for collagen type I in peritendinous tissue has shown local increases in both collagen synthesis and degradation in response to both acute exercise and prolonged training. Increased degradation of collagen after loading is also supported by findings of increased tissue activity of metallo proteases (e.g. MMP-9) and their regulating inhibitors (TIMPs). After weeks to months of training, a marked net synthesis of tendon related connective tissue is shown, and results in an improved amount of tendon content and thus in an increased tendon cross-section area. This adaptation is associated with the larger load that a trained tendon is subjected to, but results in a lowered stress on the tendon in the trained vs. the untrained at maximal voluntary loading. Regulatory hormones and cytokines of the collagen turnover are shown to be released from tendon-related tissue in a time fashion that make them likely candidates for regulating post-exercise collagen synthesis. These include TGF-beta, IL-6 and IGF-1, for which tissue changes in the content of IGF-binding proteins in response to exercise suggest a shift favouring an increase in the fraction of free IGF-1 in response to loading. The fact that training results in a tissue IGF-BP3 proteolysis is compatible with a central role for metallo-proteases in both collagen degradation and synthesis signalling in human tendon connective tissue in relation to mechanical loading.

OR-14

Adenovirus Vector Mediated alk Gene Transduction to Synovial Cells Induces Chondrogenic Differentiation

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Background: We previously reported that the expression of constitutively active forms of bone morphogenetic protein (BMP) type I receptors (BMPR-IA and BMPR-IB; BMPR-I group) and those of activin receptor–like kinase (ALK)-1 and ALK-2 (ALK-1 group) induced alkaline phosphatase activity in C2C12 cells. Chondrogenic differentiation of ATDC5 cells was induced by the receptors of the BMPR-I group but not by those of the ALK-1 group. Synovium is a thin tissue, which lines the nonarticular surfaces of diarthrodial joints. There is evidence that synovium contains cells with chondrogenic potential, and it was previously reported that synovial cells demonstrated chondrogenesis when treated with TGFb1. In the present study, we demonstrated that adenovirus vector-mediated ALK-3 and ALK-6 expression induced chondrogenic differentiation of synovial fibroblasts.

Methods: Synovial tissues were obtained from synovial tissues of the knee joints of adult rabbits or rheumatoid arthritis patients at the time of total knee arthroplasty, and synovial cells were obtained from the tissues by enzymatic digestion. After 3-5 passages, subcultured synovial cells are mainly composed of synovial fibroblasts with fibroblastic morphology and free from T cell or macrophage markers. The cells were then infected with adenovirus vectors carrying LacZ (control), constitutively active forms of ALK-3, 5, or 6 genes for 2 hours. Chondrogenic phenotypes of the cells were examined by Northern blotting or RT-PCR of type II collagen, aggrecan, and type X collagen genes as well as Alcian blue staining.

Results: After 3 days of infection, dramatic induction of type II and aggrecan genes were observed in rabbit and human synovial cells infected with ALK-3 or ALK-5 virus, while no chondrogenic phenotypes were

observed in LacZ or ALK-5-infected cells. Type X collagen expression or calcification of the cells was not observed.

Conclusions: These results suggest that adenovirus vector mediated ALK-3 or 6 gene expression can induce chondrogenic differentiation of synovial fibroblasts, and that they are promising candidates for developing novel cell-based therapeutic approaches for postnatal articular cartilage repair.

OR-15

Intervertebral Disc Disorganisation and its Relationship to Vertebral Body Morphometry and Vertebral Bone Architecture

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Introduction

The aetiology of back pain due to osteoporotic vertebral crush fracture, osteoarthritis and ageing is poorly understood. Vertebral deformity, intervertebral disc disorganisation, and change to vertebral bone architecture are morphological features that are associated with degeneration of the spine and with back pain[1]. Degenerative disc disease is one of the major causes of back symptoms and is believed to be associated with segmental instability of the spine. It is postulated that these changes result in increased or abnormal segmental spinal motion, modified load distribution across the spinal joint and altered cancellous bone architecture with consequences that influence well being.

It has been found that with age, there is increased disorganisation of the intervertebral disc and decreased quality of vertebral cancellous bone[2,3]. Age changes in vertebral trabecular architecture are seen as an alteration of trabeculae from plate-like, densely connected trabeculae to the rod-like structures seen in patients susceptible to vertebral crush fractures. Amling et al.[4] found that the osteoporotic sample of their study had overall values of bone density within the range of normal subjects, but the selective loss of structural elements reduced the load-bearing capacities of these vertebrae. An important concept here is that even for a given bone mass, fracture risk increases with age[5], supporting the notion that there is a component of bone fragility that is independent of mass[6].

The incidence of vertebral fracture or deformity or both, the hallmark of osteoporosis, increases with age. Severe structural failure (multiple vertebral fractures) is associated with significant morbidity, especially in elderly people. Disc disorganisation usually is observed earlier than vertebral deformity[7]. Intervertebral disc disorganisation first appears in the second decade, and by age 50 years, 97% of lumbar discs show signs of degeneration[8]. In osteoporotic patients, disc disorganisation and vertebral deformity commonly are observed together and these degenerative processes are considered to contribute to chronic back pain. Cancellous bone of the same mass can have very different mechanical properties depending upon its structural integrity (cancellous bone architecture) or material properties (accumulation of tissue microdamage).

Changes in vertebral body shape are not necessarily the result of osteoporotic collapse[9]. The shape of the vertebral body may be influenced by many factors. Mild or even moderate vertebral deformity may arise from lifelong age related changes in vertebral bone architecture[3]. It has been reported that vertebral wedging resulting from remodelling in osteoarthritis should not be confused with wedging due to osteoporotic fracture[10].

Mechanical loading through the intervertebral disc alters as disc integrity deteriorates, resulting in non-uniform load distribution across the vertebral endplate[11,2,12]. The altered load distribution results in adaptive bone remodelling and osteophyte growth, which in turn alters vertebral body and endplate dimensions[13] Furthermore, in degenerate discs, the effective stresses are in the peripheral area of the endplates, in the cortical wall and in the vertebral rim[2]. In addition, load transmission may be directly influenced and lead to various failure modes of the vertebral body[3].

The normal disc acts as a fluid-filled cushion that distributes stress or load evenly across the vertebral endplate. Compression under normal circumstances creates pressures in the nucleus, leading to compressive stresses at the centre of the endplate, and tension at the periphery where the annulus fibres attach, suggesting a relationship between the intervertebral disc and vertebral body bone[11]. Changes in the loading of the disc may cause responses in the adjacent bone, for example the development of osteophytes at areas of annular attachment[14]. A Finite Element Analysis of changes in bone and disc properties found that the distribution of load on a vertebral body moved from beneath the nucleus to beneath the annulus with increasing disorganisation of the disc. It was suggested that the unloaded trabeculae of central regions might undergo resorption[2].

In many cases where estimations of bone volume are presented, these figures represent average values of the whole vertebrae studied. Research performed on vertebral bone trabeculae has found regional variations within vertebrae, such as the differences between endplate and central regions[15,11] and between anterior and posterior regions[15]. In addition, findings that overall bone volume may remain the same in the presence of differential responses of trabecular architecture to mechanical loading suggest that average values of bone volume for whole vertebral bodies may obscure regional variations in trabecular bone morphometry[4,16].

Finally, while the relationship between disc disorganisation and changes in vertebral bone is commonly invoked, the mechanisms of this relationship have largely been overlooked, with age changes given more attention. However, it may be that intervertebral disc disorganisation modulates age-related bone changes within the spine. Disc disorganisation may influence trabecular bone responses before changes with age put the patient at risk of vertebral crush fracture. It has been proposed that the mature disc cannot effectively regenerate after damage, and thus responses to disc damage will be more readily observed in vertebral bone architecture than in the disc[15].

Aim of the study

The purpose of this study was to investigate the association between the morphological disorganisation of the intervertebral disc, vertebral body shape and vertebral cancellous bone architecture, independent of age-related change.

Materials and methods

Lumbar spines (T12 – L5) of 19 males and 8 females were removed at autopsy from a sample with no clinical history of bone-related disease. Spines were stored at -20°C until used for study. Each spine was divided into three functional units consisting of a superior vertebra, an inferior vertebra, and the IVD (T12–L1, L2–L3, L4–L5). This resulted in 80 functional units being available for study, consisting of 26 vertebrae of the T12–L1 group, and 27 vertebrae from both the L2–L3 and L4–L5 groups (the T12 – L1 unit was unavailable for one case). The average age of the whole sample was 59.3 ± 22.1 years (range = 20 – 94 years). The average age of males was 56.7 ± 23.0 years (range = 20 – 90 years) and the average age of females was 65.6 ± 19.7 years (range = 35 – 94 years).

Intervertebral disc evaluation

In order to examine the morphological changes in the intervertebral disc transaxial disc slices were examined. An initial transaxial slice was made through the disc centre, followed by cranial and caudal parallel transaxial slices midway between the disc centre and the vertebral endplate. Owing to central cavitation of the disc and complex annulus tears, it was apparent that the abnormalities of the two cut surfaces of the initial central transaxial slice often did not present a mirror image, therefore features of these two surfaces were recorded separately. At the other two surfaces, features were recorded from the inferior surface of the upper slice and the superior surface of the lower slice. Thus, information was recorded from the examination of four transaxial surfaces at three levels in the disc[17].

An intervertebral disc grade signifying the severity of disc disorganisation was assigned to each disc using the macroscopic disc grading method of Hansson and Roos[3] briefly outlined below.

- Grade 1 included discs without changes visible to the naked eye.
- Grade 2 consisted of discs with macroscopic changes only in the nucleus pulposus.
- Grade 3 discs had macroscopic changes in both the nucleus pulposus and the annulus fibrosus.
- Grade 4 discs had pronounced macroscopic changes with fissure formation and cavities in both the nucleus and the annulus.

Vertebral body shape indices

Vertebral shape indices (VSIs) and vertebral body bone histomorphometric analyses were performed on 160 (80 x 2) vertebral bodies. Direct measurements of vertebral body dimensions in the axial, sagittal and coronal planes were made using a digital calliper. The VSIs, wedging,

concavity and specific compression were determined from these direct measurements. The VSIs were calculated using the method described by Evans et al.[18].

- Wedging is defined as the percent reduction in the anterior vertebral body height (AH) relative to the posterior vertebral body height (PH) [Wedging (%) = ((PH - AH)/PH) x 100].
- Concavity is defined as the percent reduction in the mid-body height (MH) relative to PH [Concavity (%) = ((PH - MH)/PH) x 100].
- Specific compression is defined as a percent reduction in the PH relative to the inferior antero-posterior width (IAP) [Specific compression (%) = ((IAP - PH)/IAP) x 100].

Histomorphometric analysis

After classification, the disc of each functional unit was removed, along with the cartilaginous end plates of the adjacent vertebral bodies. Two para-sagittal slices of 5mm thickness were cut close to the midline of each vertebral body. Each slice was fixed using 10% neutral buffered formalin and decalcified with 9% nitric acid in 1% EDTA. Following dehydration with graded ethanol and clearing with xylene, slices were embedded in paraffin wax on their medial surface. Sections of 7μm thickness were then cut from each block using a Leica base sled microtome. Sections were mounted on slides and stained with van Gieson stain. This produces a red stain where bone matrix is present, yellow for marrow, and leaves no stain in fat spaces.

Each slide was divided into 9 sectors, with sectors 1, 4 and 7 at the anterior surface of the vertebral body, and sectors 3, 6 and 9 at the posterior surface. Sectors 1, 2 and 3 were closest to the disc of each functional unit. The sectors of the inferior vertebrae of the functional units were labelled so as to be mirror images of the sectors of the superior centrum (Figure 1). Division of vertebrae into sectors was based on the individual dimensions of each vertebral body (each side divided into three equal parts). Trabecular bone within each sector was measured using a Quantimet 500 MC (Leica, Cambridge, UK), which was linked to a spreadsheet (Microsoft Excel 97). A Quips program was prepared which allowed tracing the area of trabecular bone in each sector, with the exclusion of cortical bone from the measurement field. Using the 'plate model' of trabecular bone, the histomorphometric variables bone volume/total volume (BV/TV), bone surface/total volume (BS/TV) and bone surface/bone volume (BS/BV) were measured and trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and the number of trabeculae per millimetre (Tb.N) were calculated. Measurements and calculations were performed separately for each of the nine sectors on the slides. During measurement, any osteophytes, Schmorl's nodes or other abnormal bony features were excluded from the morphometric analysis. The type and location of these features in the section was noted. Histological assessment identified one abnormal case that was consequently excluded from analysis.

Once data collection was complete, differences between left and right para-sagittal sections for all of the morphometric variables were assessed. No statistically significant differences were identified between the left and right sides of each vertebra. The data for left and right sections of each vertebra were then pooled. No differences were found between males and females in this sample that could be attributed to sexual dimorphism.

Statistics

A modified analysis of covariance (the Z-score method) was used to adjust BV/TV for age. This removes the effects of age from the data and allows investigation of variation in BV/TV, trabecular architecture and vertebral body shape associated with disc disorganisation. Using the Z-score method, subjects are grouped into age ranges by decades, and means and standard deviations of these groups are calculated. The age range containing the mean age of the entire sample is used as the reference category. Analyses were performed, on both the raw data and the age-adjusted data, using one-way analysis of variance (ANOVA), Student-Newman-Kuels comparison of means, and the Student's t-test. Statistical significance was set at $p<0.05$.

Results

Our investigation has examined vertebral body shape, regional bone architecture of the sagittal plane of vertebrae and condition of the related intervertebral discs. Twenty-five discs were classified as slightly disorganised (grade 2 and aged 43 ± 22 years), 30 as moderately disorganised (grade 3 and aged 62 ± 13 years) and 25 as severely disorganised (grade 4 and aged 75 ± 12 years). Each grade was significantly older than the lower grade. No discs of the sample possessed characteristics typical of the grade 1 disc condition, limiting the range to exclude macroscopically normal discs and their associated vertebrae.

Differences in BV/TV were found between vertebral body sectors (Table 1). Following adjustment for age, it was found that there is significantly less BV/TV ($p < 0.04$) in vertebrae associated with grade 2 (10.4 ± 5.1 [%]) than in grade 3 (11.4 ± 6.9 [%]) or grade 4 (11.8 ± 6.5 [%]). No significant differences were found between different vertebrae, however, the vertebrae at lower regions of the lumbar spine did have slightly higher BV/TV (L5 = 12.0 ± 6.0 [%]) than those more cranial (T12 = 11.0 ± 5.0 [%]), which have a higher incidence of crush fracture. These results identify a potentially significant role for the degree of disc disorganisation in the incidence of vertebral crush fracture.

Sector	BV/TV (%)	BV/TV (%) age-adjusted
1	12.4±5.5	13.2±7.5
2	11.0±4.0	11.08±6.2
3	13.6±4.2	15.9±6.6
4	9.2±3.7	7.5±5.3
5	8.1±2.5	5.8±3.7
6	10.9±3.4	11.0±5.4
7	11.4±3.7	11.8±5.5
8	10.5±3.5	10.2±5.2
9	12.8±3.6	14.4±5.4

Table 1. Summary statistics (mean ± sd) for BV/TV (%) in each sector before and after age adjustment.

We have shown that BV/TV is minimal at the anterior mid vertebral body level, sector 5, and that the highest bone volume occurs in the posterior region, sectors 3, 6 and 9 (Figure 1) (Table 1). Anterior and posterior sectors of vertebrae were investigated by examining sectors 4 and 6. For disc grades 2, 3 and 4, BV/TV and Tb.N are significantly greater and Tb.Sp is significantly smaller, for the posterior region (sector 6). Tb.Th is not significantly different between anterior and posterior regions (Table 2).

		Grade 2	Grade 3	Grade 4
BV/TV (%)	Sector 4	6.4±4.4	7.0±5.4	8.4±5.9
	Sector 6	10.3±5.2*	11.4±6.2*	11.2±4.9
Tb.Th (mm)	Sector 4	89.2±28.2	94.1±30.9	93.6±39.6
	Sector 6	90.4±34.7	94.5±32.3	93.1±33.3
Tb.Sp (mm)	Sector 4	965.1±205.7	925.5±307.5	869.7±236.3
	Sector 6	770.6±169.5*	743.0±170.4*	35.3±154.9*
Tb.N (mm-1)	Sector 4	1.0±0.2	1.0±0.3	1.1±0.2
	Sector 6	1.2±0.3*	1.2±0.3*	1.2±0.2*

* Significantly different to sector 4, $p<0.05$.

Table 2. Summary statistics (mean ± sd) of age-adjusted morphometric variables for sector 4 (anterior) and sector 6 (posterior) at each disc grade.

Due to the lack of significant differences between sectors 1 and 7, 2 and 8 and 3 and 9, the data were pooled to form horizontal regions within vertebrae. The superior layer represented sectors 7, 8 and 9 of the superior vertebrae of T12, L2 and L4, and sectors 1, 2 and 3 of the inferior vertebrae of L1, L3 and L5. The inferior layer consisted of sectors 1, 2, and 3 of T12, L2 and L4, and sectors 7, 8 and 9 of L1, L3 and L5 (Figure 1).

Significant differences between these regions were seen at all states of disc disorganisation (Table 3). The central region has the lowest BV/TV for all disc grades, and the inferior region has the highest BV/TV. For the other morphometric variables, Tb.N has a similar pattern to BV/TV, with the central regions having the lowest Tb.N and the inferior region the highest Tb.N. Tb.Sp for disc grades 2, 3 and 4 is similar, with the central region having the greatest Tb.Sp and the inferior regions the lowest Tb.Sp. However, for grade 2 the central region Tb.Sp was the greatest. In grade 2

discs, Tb.Th in the superior region of the vertebrae is significantly lower than Tb.Th in the central region. For grade 3 discs, Tb.Th in the superior region of the vertebrae is significantly lower than the central and inferior regions. In grade 4 discs, no significant differences were observed between the three regions for Tb.Th (Table 3). In summary, for each disc grade the central region has the lowest BV/TV and Tb.N and the highest Tb.Sp.

		Grade 2	Grade 3	Grade 4
BV/TV (%)	Inferior	13.3±5.5	14.3±6.9	14.3±6.7
	Central	7.3±5.0*	8.6±5.6*	8.4±5.3*
	Superior	10.8±5.3**	11.6±7.1**	12.6±6.0**
Tb.Th (mm)	Inferior	84.9±27.5	89.4±28.7	89.6±33.0
	Central	87.4±30.8	92.8±31.6	87.1±34.3
	Superior	79.6±24.4***	82.6±30.9**	86.7±32.5
Tb.Sp (mm)	Inferior	642.7±126.3	640.5±161.7	630.6±121.3
	Central	901.2±198.2*	872.8±247.6*	845.4±216.8*
	Superior	712.0±159.8**	714.8±183.6**	669.7±131.7**
Tb.N (mm-1)	Inferior	1.4±0.3	1.5±0.4	1.5±0.3
	Central	1.0±0.2*	1.1±0.3*	1.1±0.3*
	Superior	1.3±0.3**	1.3±0.4**	1.4±0.3**

* Significantly different to inferior region, $p<0.05$.
** Significantly different to inferior and central regions, $p<0.05$.
*** Significantly different to central region, $p<0.05$.

Table 3. Summary statistics (mean ± sd) of age-adjusted morphometric variables for vertebral regions at each disc grade.

Anterior vertebral body wedging and concavity decreased as disc disorganisation increased. In contrast, vertebral body specific compression increased as disc disorganisation increased (Table 4).

	Grade 2 (52)	Grade 3 (60)	Grade 4 (48)
Wedging (%)	min med max	min med max	min med max
	-27.3 8.6 35.1a	-42.7 5.1 32.5a	-33.7 -0.3 19.6b
Concavity (%)	min med max	min med max	min med max
	-9.8 6.8 27.6a	-12.2 9.9 27.5a	-33.8 0.4 19.5b
Specific compression (%)	25.1 ± 5a	26.6 ± 6a	31.3 ± 5b

Table 4. Vertebral body morphometry according to disc grade, age adjusted data (number of vertebrae). The data is designated by mean ± standard deviation. For wedging and concavity minimum (min), median (med) and maximum (max) values are listed.

a, b or c - Statistically significant differences occur when the letters are different.

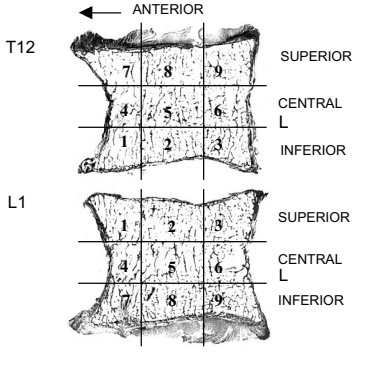

Figure 1. A functional unit showing sagittal vertebral body bone slices divided into nine sectors for bone morphometric analysis. Superior, central and inferior regions represent horizontal rows of three sectors.

Conclusions

The decrease in bone volume has been identified as a major factor contributing to vertebral crush fracture. However, there are problems associated with this mass based paradigm for identification of individuals that will experience vertebral crush fracture. We have shown that one of the factors influencing this outcome may be trabecular bone architecture in specific locations within the vertebral body. In particular, disc disorganisation, altering mechanical loading of the spine, may modulate vertebral body trabecular bone architecture. This is based on the premise that responses to spine impairment are more likely to occur in the bone than in the disc, as the mature disc cannot effectively regenerate after damage[15,19]. Many authors have postulated a co-dependency between vertebral bone volume and the state of the adjacent IVD[2,3,15,11,19]. Keller et al.[15] found that as trabecular bone in mid-sagittal endplates became weaker, the adjacent disc was more degenerated. They related this to an adaptive response in bone with changes in disc pressure. Some researchers have suggested that as disc disorganisation increases the transmission of forces through the vertebral body changes[2,20].

When adjusting BV/TV to account for age differences, grade 2 discs had the lowest BV/TV, while grades 3 and 4 had similar BV/TV values, both being significantly greater than BV/TV of vertebrae with grade 2 discs. This shows an increase of BV/TV with more severe disc disorganisation.

Anterior sector 4 and posterior sector 6 have significantly different trabecular bone architecture. Posterior sector 6 has greater BV/TV and Tb.N but lower Tb.Sp than anterior sector 4. This observation is consistent with clinical data, which reports anterior vertebral body wedge fractures[21]. We have shown that trabecular thickness is the same in both regions, for all disc grades. Trabecular bone architectural changes based on Tb.Sp and Tb.N can significantly influence cancellous bone strength and the risk of fracture. Non-uniform distribution of trabecular bone in the vertebrae can create stress concentrations that could increase fracture risk and accelerate failure. The anterior margin of the vertebral body is subject to especially high compressive loads, as predicted from finite element models, in forward flexion[22]. Preferential bone loss in the anterior half of the vertebra would reduce the mechanical load bearing properties of the vertebra much more greatly than predicted by a bone mass measurement averaged over the entire vertebra. In histological sections, we have observed that anterior vertical trabeculae are more susceptible to buckling and trabecular microfracture than trabeculae oriented in a more horizontal direction.

The danger of reliance on the mean values of trabecular bone architecture, with the mass based paradigm of bone strength, is clearly illustrated in the comparison of inferior, central and superior regions of the vertebral body sagittal slices. The central region is clearly the region at greatest risk of failure with the minimum BV/TV greatest Tb.Sp and lowest Tb.N. Kothari and colleagues[16] reach a similar conclusion, stating that mean values of trabecular bone architecture do not adequately describe the true trabecular bone morphology, due to significant variation or heterogeneity within each vertebral body. In addition, increased intra-specimen variation in trabecular architecture increases the risk of vertebral fracture[23]. In a study to determine the sector of vertebral body sagittal slices in which bone damage is most harmful to its structural integrity, Kopperdahl at al.[24] reported that damage to the central region of the centrum led to the largest reduction in structural stiffness. In particular, bone damage was most critical to sector 5 in this central region.

Vertebral body shape and consideration of disc condition and trabecular architecture in 9 sectors of vertebral body sagittal slices has not previously been reported. The decreased vertebral body wedging and concavity associated with increased disc disorganisation is consistent with the change in the cancellous bone architecture of the vertebral body. In contrast, vertebral body specific compression increased as disc grade increased. The increased specific compression may be a result of improved bone quality in the anterior region of the vertebral body and the more homogeneous architecture favouring vertebral body compression.

Change in the cancellous bone architecture of the vertebral body was associated with increased disc disorganisation, consistent with decreased vertebral deformity. Vertebral bodies adjacent to severely disorganised discs showed structurally enhanced cancellous bone architecture compared to vertebral bodies adjacent to mildly disorganised discs. It appears that vertebral bone morphology is related to the condition of the intervertebral disc. This shows that disc disorganisation modulates vertebral cancellous bone architecture such that it may protect against age related bone changes and the occurrence of osteoporotic vertebral body crush fracture.

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OR-16

Narrowing of Lumbar Disc Space and Osteophyte Formation are two Independent Risk Factors for Spinal Deformity in Elderly Japanese Women

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Aim of the study: The study was designed to assess the contributions of physical and constitutional factors to osteophyte formation, disc degeneration and bone mineral density (BMD) in lumbar vertebrae of elderly postmenopausal women.

Materials and methods: A total of 80 women with back pain, aged 61-86 years (average, 3.2), were invited to participate in the study. Lumbar radiographs, MRI and DXA measurements were performed. VDR genotypes were also assessed.

Results: Prevalence rates of osteophytes on radiographs and disc degeneration on MRI were 61% and 68%, respectively. Body weight and BMI correlated significantly with anteroposterior (AP) and lateral (LAT) BMDs ($r=0.354$ for body weight, $r=0.347$ for BMI), mean osteophyte area ($r=0.557$ for weight, $r=0.486$ for BMI) and number of discs with osteophytes. However, these did not correlate with the disc area or the number of degenerated discs. Stepwise regression analysis revealed that body weight and LAT-BMD values significantly influenced the osteophyte area. Disc area ($r=0.386$ for AP view) and osteophyte area ($r=0.384$ for AP view) significantly correlated with BMDs. However, disc area and osteophyte area did not correlate with each other ($r=0.045$). The proportion of degenerated discs was higher in the lower lumbar discs, but not in discs with osteophytes. Frequencies of T and t alleles of VDR did not correlate with disc degeneration, osteophyte formation or osteoporosis.

Conclusions: Our data showed that increases in osteophyte formation and BMDs in the lumbar vertebrae are influenced by body weight and BMI, but do not correlate with disc area narrowing, which correlated inversely with BMD. Disc degeneration and osteophyte formation seem to represent two different factors that affect the lumbar spine in elderly women.

OR-17

Alendronate as a Countermeasure to Disuse Induced Bone Loss

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Microgravity, similar to disuse immobilization on earth, causes rapid bone loss. This loss is believed to be an adaptive response to the reduced musculoskeletal forces in space and occurs gradually enough that changes occurring during short duration space flight are not a concern. Bone loss, however, will be a major impediment for long duration missions if effective countermeasures are not developed and implemented. Bed rest is used to simulate the reduced mechanical forces in humans and was used to test the hypothesis that oral alendronate would reduce the effects of long duration (17 weeks) inactivity on bone. Eight male subjects were given daily oral doses of alendronate during 17 weeks of horizontal bed rest and compared with 13 male control subjects not given the drug. Efficacy was evaluated based on measurements of bone markers, calcium balance and bone density performed before, during and after bed rest. During bed rest, the BMD of the control group decreased in all regions except the arms and radius while the treated group showed no loss in BMD except the calcaneus which decreased about 5% compared to 10% in the controls. The Wilk's Lambda statistic, used to compare groups of variables, showed a significant treatment effect on BMD ($p<0.02$), resorption markers ($p<0.001$), and urine and fecal Ca excretion ($p<0.02$). These results show that oral alendronate attenuates most of the characteristic changes associated with long duration bed rest and presumably space flight.

OR-18

GH Effects on DXA-Assessed Whole-Body and Lower-Limb Mineral and Lean Masses in Men and Women With GH Deficiency

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The GH effects on muscle-bone relationships have been scarcely investigated. This study compares the whole-body and lower-limb mineral and lean masses (BMC, LM) in 15 men and 16 women (9 of which also received HRT) aged 23-60 yr with GH deficiency (AGHD), both before and after treatment with replacement GH during 12-18 months, with those of 600 age-matched, normal men and postmenopausal women.

Both the slopes and intercepts of the correlations between BMC (y) and LM (x) were similar to those shown by controls, either before or after treatment. Treatment enhanced both BMC and LM correlatively in men and women, keeping the already shown, natural proportionality [Ferretti, Bone 1998;22:683]. However, the intercept of that relationship was significantly higher for the HRT-treated than -untreated women. The parallel evolution of LM and BMC as affected by GH treatment was generally more evident in women than men, and in the lower limbs than the whole body.

Assuming a direct proportionality between LM and muscle masses, results suggest that the AGHD affected bones and muscles following the natural proportionality, while the GH replacement tended to neutralize that impairment also keeping the normal relationships. The parallel evolution of the bone-muscle proportionality was more evident in the lower limbs than the whole body perhaps because the fat-mass interaction with the DXA-BMC measurement was significantly lesser in the former. The more evident parallelism between the evolution of bone and muscle indicators in women, on the one hand, and the potentiation of GH effects by HRT, on the other, suggest that the positive GH effects on bones and muscles are sex-hormone dependent. Therefore, GH treatment would have enhanced both bone and muscle masses following the physiological relationships, but would have been either unable or much less effective than that to improve the bone-muscle proportionality. Results do not allow proposing that GH effects on muscles and bones are or not interdependent. Although they suggest at the least that GH treatment would not interfere negatively with the control of the skeletal status according to customary mechanical usage by the bone mechanostat.

OR-19

Two-Way Interactions Between Intervertebral Discs and Vertebral Bodies.

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Aim of the study

To investigate how degenerative changes in either structure threaten the integrity of the other.

Materials and methods

Cadaveric thoraco-lumbar spines were removed post-mortem and stored at –20°C. Subsequently, they were dissected into "motion segments" consisting of two complete vertebrae and the intervening disc and ligaments. Specimens were subjected to 2kN of compressive loading on a computer-controlled materials testing machine. The distribution of compressive "stress" was measured within each loaded disc by pulling an instrumented needle, 1.3mm in diameter, along its mid-sagittal diameter. "Stress profiles" were integrated to calculate the compressive force acting on different regions of the disc, and these forces were subtracted from the applied 2kN to quantify load-bearing by the neural arch. The first experiment examined how disc degeneration affected intradiscal stress distributions, and how these in turn influenced the compressive strength of the adjacent vertebrae. The second experiment investigated how damage to a vertebra in turn affects the adjacent disc.

Results

In specimens with non-degenerated discs, the 2kN compressive force was distributed evenly on the vertebral body. Severe disc degeneration, however, caused up to 80% of the 2kN to be resisted by the neural arch in simulated erect standing posture, and much of the 2kN was transferred to the anterior vertebral body during simulated spinal flexion. As a result, vertebral compressive strength (tested in flexed posture) was inversely proportional to neural arch load-bearing in erect posture ($R2=0.55$, $P<0.01$). Vertebral endplate damage resulted in high stress concentrations acting on the annulus fibrosus of adjacent discs, and subsequent cyclic loading caused the annulus to collapse inwards.

Conclusions

Degenerated discs become narrowed, leading to stress-shielding of the anterior vertebral body by the neural arch in habitual erect postures. This leaves the vertebra vulnerable to "osteoporotic" anterior wedge fracture during forwards bending movements. Conversely, compressive damage to a vertebral body leads to progressive adverse changes in the adjacent discs. We suggest that these interactions explain observed associations between disc degeneration and vertebral fractures.

OR-20

Collagen Quality in Osteoporosis: Under The Microscope

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In the clinical setting, bone density, reported as bone mineral density (BMD), has been used as a predictor of fracture risk. But BMD does not explain all of the risk because it does not account for all of the mechanical properties of bone. For example, it is known that collagen intermolecular cross-linking in fibrillar matrices affects tensile strength and viscoelasticity, and such properties certainly play a role in the mechanical competence of bone.

In this study, Fourier transform infrared imaging techniques were used to determine the ratio between the pyridinoline (pyr) and dehydro-dihydroxylysinonorleucine (deH-DHLNL) cross-links in the collagen of not-previously demineralized stained thin sections of bone from human iliac crest biopsies (acquired under an appropriate IRB protocol) that showed the histological / histomorphometric features of normal bone, low-turnover osteoporosis, or high-turnover osteoporosis N = 9 / group). This technique allows the analysis of thin tissue sections (400x400 um areas) at a spatial resolution of 6.3 um. In the present study, only trabecular bone was examined, and measurements were taken at various depths in the bone relative to the trabecular surface, which was either formative or resorptive. Distinctive, but complex differences were found in the collagen cross-link ratios at the different depths in the different conditions at both formative and resorptive surfaces, with osteoporotic bone invariably exhibiting a statistically significant ($p<0.01$) higher pyr / deH-DHLNL ratio when compared to normal at anatomically equivalent microscopic loci, suggesting that osteoporotic matrix is of different "quality" than normal even at actively bone forming trabecular surfaces.

A better understanding of the quality of bone at this level may help explain the pathogenesis of failure in osteoporotic bone and thus provide the basis for the development of new therapeutic protocols.

OR-21

The Serotonin Transporter (5-HTT) Regulates Osteoclast Differentiation: Evidence For a Novel Neural-Skeletal Regulatory Mechanism

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Interactions between the neural and skeletal systems are suggested by various clinical observations, but remain poorly understood. Reductions in bone mineral density have been reported in patients suffering from depression syndromes that appear to be related to derangements in

serotonergic function. Fluoxetine ('Prozac'), a selective serotonin reuptake inhibitor (SSRI) that blocks 5-HT uptake by its specific transporter (5-HTT), reduced bone resorption in mice with adjuvant-induced arthritis. These observations suggest that there may be a significant relationship between the 5-HT system and bone remodeling. Bone resorption is regulated by a complex system of hormones and locally-produced cytokines that stimulate osteoblasts and stromal cells to express Receptor Activator of NFkB Ligand (RANKL), and results in the differentiation and activation of osteoclasts, the bone resorbing cells. The aim of this study was to characterize the role/s of the components of the serotonergic system in bone remodeling. Using gene micro arrays, we found the serotonin transporter (5-HTT) to be strongly expressed in Receptor Activator of NFkB Ligand (RANKL)-induced osteoclasts. The osteoclast 5-HTT exhibited typical serotonin (5-HT) uptake activity that was blocked by fluoxetine. Fluoxetine reduced osteoclast differentiation in part through effects on NF-kB activation, whereas 5-HT itself enhanced differentiation. Fluoxetine had no significant effect on the proliferation of unstimulated or RANKL-stimulated cells. Reserpine inhibition of intracellular transport of 5-HT into cytoplasmic vesicles potentiated RANKL-induced osteoclast formation, and also enhanced the expression of the osteoclast marker TRAP in the absence of RANKL. Taken together, these data indicate that the 5-HT system plays an important role in bone homeostasis, and suggests that commonly used anti-depressive agents may affect bone mass.

OR-22

Inducible Knockout of a Phospholipase C Isoenzyme

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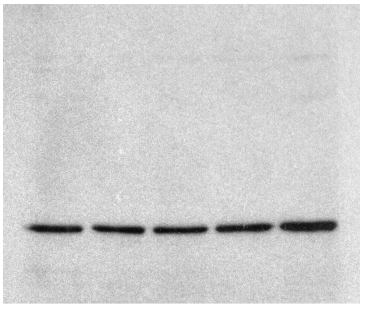
It is well known that Phosphoinositol Specific Phospholipase C (PLC) plays a crucial role in mechanotransducion in osteoblasts. Yet the PLC isoenzyme taking part in the mechanotransduction process is unknown. In order to identify the isoform which is mainly involved in mechanotransduction we established a genetically modified, stably transfected MG-63 cell line with an inducible knockout of two isoforms we consider to play an important role in this process. Whereas other studies use a pharmacological or an oligonucleotide inhibition which only allow single experiments we use this system to induce PLC knockout to minimize physiological damages to the cell.

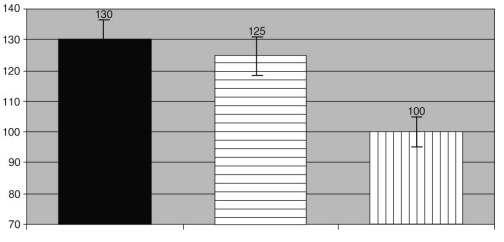
As osteosarcoma cells lacking the PLC β2 isoform did not show any increase in metabolic activity after applying mechanical forces within physiological ranges we concentrated on investigating the PLC β2 and as a control group the PLC β1. In previous experiments we found that certain osteosarcoma cell lines such as MG-63 show a signicant increase in metabolic activity. Mechanosensitive MG-63 osteosarcoma cell line was furnished with the inducible Ecdysone Expression system, consisting of two vectors with different tasks. One vector contains the two Ponasterone (an insect molting hormone) receptor units, EcR and PvG, the second vector (pIND) containing a 190bp PLC beta2 cDNA sequence, inhabiting the start of translation, cloned into the plasmid in antisense direction. Transcription of the antisense mRNA could be induced by adding 10μM Ponasterone. The transcribed mRNA is complementary to the physiological mRNA and allows binding between those two oligonucleotides. Thus, translation of the physiological product is made impossible.

Cells were cultured on 3cm plastic-petri-dishes and antisense expression was induced with 10μM Ponasterone. Western-Blotting of these cells after 6, 24 and 48 hours showed a 8% decrease of PLC beta 2 after 6h, and a 30% knockout maximum after 24h. PLC beta2 levels increased after 48h while physiological MG63 kept up the same PLC beta 2 level at all times. *Biological effects* Applying mechanical forces of 3,000 μstr on two consecutive days with 1Hz by a 4-point-bending-machine to the knockout cells did not show any significant changes in metabolic activity whereas MG63 and primary osteonlasts (POB) cells show a significant increase in metabolic activity. Metabolic activity was tested with MTT cell proliferation assay modified by Jones. Further investigations showed that the antisense cells lose attachment to the surface when loaded with a shear stress of 30 dyn/cm^2. Furthermore the physiological calcium signal as a response to induced forces by shear-flow of osteoblasts could not be found in the knockout cells.



not	6h	24h	48	MG63
Induced				Control

Response to mechanical forces



OR-23

Muscle Contraction And Bone Modeling/Remodeling: a Pilot Project

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Exercise has been prescribed as a means of maintaining bone mass. However, the mechanism by which this occurs is not clear. Until the late 1970s the prevailing dogma followed the 1892 edict of Wolff that bone strain controlled remodeling. In 1952, Yasuda used the studies of Fukada to propose that piezoelectricity was the mechanism by which bone bending transmitted information to osteocytes. When it was discovered that piezo-electricity is significant for generating the necessary electrical force to stimu-late osteocytes only when bone is dry, a new paradigm had to be found. In 1977 Piekarski and Munro provided bone fluid flow as the new model. Since then two mechanisms for its operation have been proposed to function in canaliculi and osteocyte lacunae: 1) Streaming potentials and 2) Fluid shear stress. Both of these mechanisms require that fluid percolate through the bone matrix at convective rates. It has been difficult to demonstrate that bone bending alone generates sufficient flows. Neither does blood pressure in a resting subject. We investigated the possibility that bloodflow accelerated by skeletal muscle contractions and heartrate increase during exercise provides sufficient pressure to drive convective filtration through osteonal capillary walls. To explore this idea we subjected rabbits, which had been implanted in their tibias with optical bone chambers to electromyographic stimulation of their adjacent gastrocnemius muscle. Fluorescein isothiocyanate-dextran 70 (FITC-D70), a 70 kDa molecule was injected IV and its capillary filtration recorded. In the absence of stimulation FITC-D70 did not filtrate. After 5 seconds of 2 Hz stimulation at 85 volts, however, extravasation of the molecule was evident. These results suggest that blood pressure from muscle contractions can stretch capillaries to open pores normally too small for an albumin sized molecule. Previous work, using a single vessel model has suggested that when such pores are opened filtration through them is convective. These results provide a basis for further investigation, testing the hypothesis that muscle contraction is the major source of pressure driving bone fluid flow.

OR-24

Percentilized BMC/LM Curves for Diagnosing "Physiological" and "True" Osteopenias in Males and Females of all Ages

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Bone mass is indirectly determined by the biomechanical control of bone stiffness by the bone mechanostat, a system that modulates the spatial distribution of the mineralized matrix according to the osteocytic sensing of strains provoked by mechanical influences on the skeleton. As an evidence, 1. the bone / muscle masses are linearly related, showing the same slope for any gender, age or body habitus, and are mutually affected by physical activity; and 2. the intercepts of those relationships vary with the gender and endocrine status. In addition to an intrinsic disturbance of the mechanostat cells (specific diseases), a low bone mass may only correspond either to 1. a constitutional feature (small persons, "physiologic" osteopenia); 2. a prolonged inactivity, muscle weakness or weightlessness ("disuse" osteopenia), or 3. a systemic disorder ("true" osteopenia). Only when a "disuse" or a "true" osteopenia induces a demonstrable bone fragility can the condition be regarded as a "disuse" or a "true" osteoporosis, respectively. The DXA-BMC is the best resource for measuring bone mass and diagnosing an osteopenia; not so for diagnosing an osteoporosis, because it does not assess bone tissue quality or distribution. However, as DXA is able to assess all bone (BMC), fat, and lean (proportional to muscle) masses, it allows approaching a differential diagnosis between "physiologic" and "true" osteopenias (appropriate or inappropriate bone/muscle mass proportionality) as pre-conditions for diagnosing "disuse" or "true" osteoporoses, respectively, by other means.

We have developed reference BMC vs. lean-mass charts for whole-body DXA data (Norland XR-26) in normal Argentine boys and girls (n=545), men (n=228), and pre- and post-MP women (n=330, 347) showing the CI for 1, 2 and 3 SD's and the 1-99 percentiles in each case. The BMC was also plotted after statiastical adjustment to a common fat mass value in order to compensate for fat interference with BMC determination. The BMC (crude or fat-adjusted) / LM ratio vs. age relationships were also percentilized for males and females. The developed percentiles offer a suitable reference scale from which the limits for distinction between "disuse" and "true" osteopenias could be determined regarding significant, functional muscle-bone relationships.

OR-25

Mechanism of Iliac Bone Development

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The ilium is of interest to bone researchers because it is the site of the skeleton where bone tissue can be easily obtained. When the patient receives two courses of tetracycline prior to biopsy, histomorphometric analysis offers the unique possibility to study human bone cell function *in vivo*. The pelvis is often thought to increase its diameter by periosteal apposition on the outer cortex and periosteal resorption on the inner cortex. To investigate normal iliac bone growth, histomorphometry was performed in biopsy samples from 9 children (age 9.1 to 12.9 years) without metabolic bone disease. On endocortical surfaces, all bone surface-based measurements (osteoid surface, osteoblast surface, mineralizing surface and bone formation rate) were two to four times higher on the inner as compared to the outer cortex ($p<0.05$ each). In contrast, eroded surface per bone surface was significantly higher on the external cortex ($p=0.0001$). As to periosteal surfaces, osteoid surface, osteoblast surface and mineralizing surface relative to bone surface, as well as adjusted apposition rate were between three and six times higher on the external cortex ($p<0.05$ each). Bone formation rate per bone surface was 212 ± 174 $\mu m^3/\mu m^2/y$ on the external, but 27 ± 44 $\mu m^3/\mu m^2/y$ on the internal periosteum, whereas relative eroded surface was much lower on the external periosteal surface ($1.7\pm3.4\%$ vs $55.7\pm36.8\%$; $p=0.003$). These results indeed suggest that an outward modeling drift occurs on both cortices during growth. However, the rate of external drift is in the order of one mm per five years, and as such contributes little to pelvic growth. The asymmetric behavior of the two endocortical surfaces provides indirect evidence that these events are not determined by 'marrow environment' (which should be similar for both surfaces) but rather are under the control of signals that are generated in mineralized bone.

OR-26

Associations Between Muscle Strength, Lean Mass and Bone Density are Genetically Determined

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Cross-sectional studies report positive associations between muscle strength, muscle mass and bone mineral density (BMD), athletes generally have greater muscle strength, muscle mass and BMD than sedentary controls, and immobilization is associated with bone loss. The inference made is that resistance exercise, or a lack of it, is responsible for these associations. An alternate view is that difference in BMD between sedentary and active individuals could be due to differences in genetic composition, not differences in exercise patterns. Based on within pair differences in 56 monozygotic (MZ) and 56 dizygotic (DZ) female twin pairs, mean age 45 years (range 24-67), BMD was associated with lean mass, independent of fat mass and height ($p < 0.05$). A 10% increment femoral neck (FN) BMD was associated with a 15% increment in lean mass ($\sim$6 kg). BMD was associated with muscle strength before, not after, adjusting for lean mass. Same-trait correlations ($\pm$ se) in MZ pairs were double those in DZ pairs; FN-BMD (0.62 ± 0.08, 0.33 ± 0.12) and lean mass (0.87 ± 0.03, 0.30 ± 0.11) (all $p < 0.001$). The cross-trait correlation between lean mass and FN-BMD in an individual was 0.43 ± 0.06. The cross-trait cross-twin correlation between lean mass in one twin and FN-BMD in the other was 0.31 ± 0.07 in MZ pairs, about 75% of the cross-trait correlation in the same individual, and greater than 0.19 ± 0.09 in DZ pairs ($p < 0.001$). After adjusting for height and fat mass, the MZ and DZ cross-trait cross-twin correlations were no different (0.16 ± 0.08 and 0.13 ± 0.09, respectively). Genetic factors account for 60-80% of the individual variances of both for FN-BMD and lean mass, and over 50% of their covariance. The association between greater muscle mass and greater BMD is likely to be determined by genes regulating size.

OR-27

Muscle-Bone-Relationships Analysed by DEXA in Health and Disease

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The correct interpretation of bone mineral density data is critical to the diagnosis and management of osteoporosis in children. We have examined the important influences on bone mineral density by initially investigating 1074 healthy schoolchildren aged 5 –18 years. Bone mineral content (BMC) of the lumbar spine and whole body was measured using a Lunar DPX-L DXA scanner using the appropriate software. Stepwise linear regression identified lean body mass (LBM) as the strongest single predictor of BMC in the lumbar spine and whole body. A significant gender difference in the relationship between BMC and LBM was observed with girls having significantly more bone per unit of lean body mass beyond 30 kg.

As lean body mass is predominantly muscle mass we have subsequently examined the relationship between muscle mass and whole body BMC in different diseases. A two stage algorithm was used to examine LBM for height and BMC for LBM by calculation of Z-scores using mean and SD values from the normative data.

12 children with muscular dystrophy had a mean LBM for height Z-score of –1.96 but a mean BMC for LBM Z-score of + 2.06 indicating their primary abnormality was reduced muscle mass (sarcopenia) with no

evidence of osteopenia. In contrast 27 children with Osteogenesis Imperfecta had a mean LBM for height Z-score of + 0.15 but a mean BMC for LBM Z-score of – 2.38 indicating normal lean body mass for size (i.e. no sarcopenia) but significantly reduced BMC for LBM (ie osteopenia) confirming a primary bone abnormality. A group of 26 children with low trauma fractures (vertebral and non vertebral) who were subsequently treated with the bisphosphonate Pamidronate had little evidence of sarcopenia (Mean LBM for height Z-score – 0.25) but significant osteopenia (Mean BMC for LBM Z-score – 2.78).

The muscle bone relationship we have examined using DXA may be a satisfactory means of diagnosing osteoporosis in children and identifying if the primary abnormality is in muscle or bone.

OR-28

Tomographic (pQCT) Analysis of Bone Structure and Strength and Muscle-Bone Interactions in 15 Children With Osteogenesis Imperfecta (O.I.)

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This study analyzes the skeletal status and muscle-bone relationships in 5 boys and 10 girls aged 2-16 years with untreated type I (5), III (5) and IV O.I. (5) and 31 3-21-year-old controls. The cross-sectional bone area (CSA) and vBMD (attenuation threshold = .710 cm^{-1}), and the moments of resistance and Stress-Strength Indices (SSIs) for bending and torsion of the tibia; and the whole- and fat-free CSA's of the calf muscles were measured by pQCT (XCT-3000, Stratec) at the standard, 66% site of the leg.

The vBMD, increasing with age in controls, remained around 900 g/cm3 showing great dispersion in the O.I. children, the lowest values corresponding to type IV. Bone CSA and SSIs increased with age in controls and more slowly in type I O.I., remaining constantly low in types III-IV. The "mass / accumulation" curves between bone CSA (y) and vBMD (x; usually negative hyperboles) plotted higher for controls than O.I. children, these showing no significant slope. Bone CSA and SSIs increased with mobility score, a variable affected by the O.I. type. Both bone CSA and SSIs increased in all groups with the fat-free muscle CSA, more in control than in O.I. children. The whole O.I. group showed single slopes with increasing values of both variables for types IV < III < I. The vBMD of all the O.I. children increased logarithmically with the muscle CSAs, reaching normal values at approximately 600 mm^3 of fat-free muscle CSA regardless of O.I. type. The fat-free / whole muscle CSA ratio decreased in the order: controls > type I O.I. > types III-IV O.I.. Fracture incidence rate decreased exponentially with SSIs and mobility score in O.I. children of all types together.

This shows that untreated O.I. children (especially types III and IV) are unable to improve compact vBMD, mass and strength with age, presumably because of both, mass / architecture and mineralization defects (low bone formation, enhanced intracortical porosity by disuse-mode remodeling) in proportion with muscle weakness. These effects could be associated with shifts in the mechanostat setpoints for triggering bone modeling and disuse-mode remodeling. Despite that pQCT determinations neglect the mineralization-unrelated, microstructural factors which are affected in O.I., the SSIs predicted adequately the fracture incidence in O.I. children.

OR-29

Low or Normal Bone Mineral Density in Female Elite Triathletes: Relation to Muscle Strength and Biological Bone Markers

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Aim of the study: To test the hypothesis that bone mineral density (BMD) in female elite triathletes is high and correlated to maximal muscle strength (MMS) and sex hormone concentrations, irrespective of endogenous or exogenous origin.

Materials and methods: Eight female elite triathletes (training 8-24 hrs/wk) and seven sedentary controls, age 21-37 years, participated in the study. Three triathletes and three controls were taking oral contraceptives (OCs). Regional and total BMD (g/cm^2) were measured by DXA. MMS was measured as isokinetic peak torque (Nm) in hip extension, hip flexion and knee extension. Serum concentrations of progesterone (P), estrogen (E), LH, FSH, osteocalcin (O) and carboxyterminal telopeptide of type I collagen (ICTP) were measured in fasting state in follicular and luteal phase.

Results: Neither BMD nor MMS were higher in triathletes than in controls. In contrast, BMD tended to be lower (significant only in left radius and left and right tibia, $p<0.05$). In triathletes peak torque in hip flexion correlated to BMD in femur and wards, L2-L4 and total BMD ($0.62<r<0.86$, $p<0.05$). Furthermore BMD L2-L4 was negatively correlated to ICTP in luteal phase ($r = -0.69$, $p<0.05$). BMD did not correlate to sex hormones. OC-users had lower P, E and LH in luteal phase, lower LH in follicular phase and higher ICTP/O ratio ($p<0.05$) than non-users. In controls OC-users had lower BMD in whole body ($p<0.005$), right radius ($p<0.005$), left femur ($p<0.05$) and right tibia ($p<0.05$), lower P in follicular and luteal phase ($p<0.05$), and lower LH in follicular phase ($p<0.05$) than non-users.

Conclusions: Despite a high training volume, BMD in triathletes seemed to be compromised compared to sedentary controls. BMD correlated to maximal hip flexion but there was no correlation to sex hormones. In both triathletes and controls OC-users had low concentrations of sex hormones and in triathletes a high ICTP/O ratio. This could indicate a sex hormone mediated change in remodeling in favour of resorption, however low BMD was only found in controls. We could speculate that this difference between OC-users in triathletes and controls could be ascribed to the osteogenic impact from triathlon, opposing the low concentrations of sex hormones.

OR-30

Body Image and External Appearance Influence the Daily Activities of the elderly

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Objectives: To investigate the self estimation of elderly people of the changes of their body image and the interference of these changes with several parameters of their life as well as its correlation with gender, body type, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was given to 318 aged subjects. The completion of the questionnaire was done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ± 5,9 years. In the questionnaire the opinion of the subjects about their mobility problems, their body image, their activities of daily living (ADL), their emotional condition, and other parameters was reported. An SPSS spread sheet was used and correlation has been done with chi square.

Results: Some decrease of their height is reported by 30.8%, whereas 65.4% did not report any decrease. Protrusion of the belly is reported by 37.4%, thoracic kyphosis is reported by 23.6%. Some differences on the size of the clothes is reported by 36.8% whereas 29.6% on the size of the shoes. Modification of locomotion is reported by 53.4% of the subjects as follows: 34% shorter step, 16% drawing step, 12.3% instability, 28.6% difficulties in ascending stairs and 48.7% weakness. Change of the body image annoyed 23% in subjects and 17.6% of them feel that are unable for simple activities. Emotional disorders caused by body image disorders are observed in 21.1% of the elderly. External appearance did not bother ADL according to 75.2% of the subjects. Change of the body image is correlated with the gender ($p=0.002$), especially with changes in body height in women (66%), the area of residence ($p=0.001$) and the body type ($p=0.001$) especially with belly protrusion ($p=0.001$) and with differences on the size of the clothes ($p=0.0008$). Gender is highly correlated with disorders in locomotion ($p=0.015$, women 59%) the ascent of the stairs ($p=0.016$, women 66%) and instability during locomotion ($p=0.049$, women 71%). Body type is correlated with the emotional disorder due to changes in body image ($p=0.020$) and the concomitant inability ($p=0.018$). Weakness due to the external appearance is correlated with the gender (0.011, women 58%) and body type ($p=0.003$).

Conclusion: Two out of 5 elderly people report change of the body image and this leads to emotional disorders in 1 out of 5. Half of the subjects have changes in locomotion and weakness. External appearance did not affect ADL in 3 out of 4. Gender and body formation are correlated with disorders in locomotion, weakness and emotional disorders due to the change of body image.

OR-31

Interpretation of Data on Muscle Mass and Function During Childhood Relationship to Skeletal Development

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Bone densitometric data often are difficult to interpret in children and adolescents because of large inter- and intraindividual variations in bone size. Here, we propose a functional approach to bone densitometry that addresses two questions: Is bone strength normally adapted to the largest physiological loads, that is, muscle force? Is muscle force adequate for body size? To implement this approach, forearm muscle cross-sectional area (CSA) and bone mineral content (BMC) of the radial diaphysis were measured in 349 healthy subjects from 6 to 19 years of age (183 girls), using peripheral quantitative computed tomography (pQCT). Reference data were established for height-dependent muscle CSA and for the variation with age in the BMC/muscle CSA ratio. These reference data were used to evaluate results from three pediatric patient groups: children who had sustained multiple fractures without adequate trauma (n = 11), children with preterminal chronic renal failure (n = 11), and renal transplant recipients (n = 15). In all three groups mean height, muscle CSA, and BMC were low for age, but muscle CSA was normal for height. In the multiple fracture group and in renal transplant recipients the BMC/muscle CSA ratio was decreased ($p < 0,05$), suggesting that bone strength was not adapted adequately to muscle force. In contrast, chronic renal failure patients had a normal BMC/muscle CSA ratio, suggesting that their musculoskeletal system was adapted normally to their (decreased) body size. This functional approach to pediatric bone densitometric data should be adaptable to a variety of densitometric techniques.

OR-32

Foetal Origins of Bone Formation - *Ex Vivo* Cell and Bone Analysis

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An understanding of the normal development and regulation of skeletal growth during fetal life and in the young individual has tremendous implications in later life and an individual's predisposition to fracture. Skeletal ageing is associated with a progressive decrease in bone mass, which can result in osteoporosis, a disease characterised by low bone mass and deterioration of trabecular architecture with devastating socio-economic consequences. However, there is considerable variation in the degree of bone loss as a consequence of genetic, environmental and nutritional factors. With an increasing ageing population, elucidating the mechanisms involved in normal skeletal maintenance with age has tremendous healthcare implications for all.

The bone mass of an individual in later life depends on the growth and mineralisation of the skeleton during the first two decades of life as well as the subsequent loss of bone, which commences during the fourth decade. Epidemiological studies have indicated that poor growth during fetal life and infancy is associated with decreased bone mass in adulthood. We have shown, in a longitudinal study of 21-year old women born in Bath, a positive association between weight at one-year age and adult bone mineral content. This relationship has also been observed in a population of Hertfordshire men and women aged between 60 and 75 years of age (Cooper et al. 1997, Fall et al. 1998)[1-2]. The mechanisms underlying this association are believed to include the programming of skeletal growth by adverse environmental influences during early fetal development. During intrauterine life, the growth of the fetus is related to maternal nutrient supply and it is thought that programmed adaptations by the fetus in response to maternal under-nutrition may result in an increased risk of chronic diseases in later life (Barker et al. 1990, Barker 1998)[3,4]. However, to date, no experimental data exists about the cellular and molecular consequences of altered fetal development on skeletal maintenance in normal ageing or on the mechanisms by which nutritional deficiencies *in utero* influence bone quality. Animal studies provide a reproducible laboratory model with which to investigate the mechanisms of programming, and have replicated the epidemiological associations found in humans. In this study, a rat model of maternal protein insufficiency was used to investigate the cellular mechanisms involved in the programming of bone development. In this model, the feeding of a low protein diet to pregnant rats produces offspring that subsequently develop functional changes in adulthood such as hypertension and impaired renal function (Langley-Evans et al. 1999)[5]. We have undertaken two studies to examine skeletal growth with age in the offspring of protein-deficient mothers.

In the first study we examined the bones in aged rats (78-88 months) to establish whether bone structure and quality in the offspring from protein-deficient rats differed from normal rats. Ten female rats mated and fed diets containing 180g casein/kg (control), or 90g casein/kg (low protein) throughout pregnancy. Offspring of the low protein (23) and control (31) group were maintained on a normal chow diet throughout life and were allowed to expire normally. Bone mineral measurements were obtained by dual energy X-ray absorptiometry, and tibiae were processed for histology. Maternal protein restriction resulted in a significant reduction in whole body bone area and whole bone mineral content and altered growth plate morphology in later life of the offspring (Table 1).

Table 1. Body composition of adult offspring of dams fed low protein or control diets during gestation.

Variable	Maternal diet (Control, n=31)	Maternal diet (Low protein, n=23)	P-value Unadjusted	Adjusted*
Bone area (cm2)	58.1 ± 2.2	53.6 ± 2.5	0.04	0.01
BMC (g)	9.01 ± 0.40	8.29 ± 0.43	0.02	0.06
BMD (g/cm2)	0.155 ± 0.003	0.154 ± 0.003	0.98	0.88

* Adjusted between groups for gender, body weight, animal and mother interrelationships (random effects model)

There was no difference in whole body bone mineral content. The growth plates of the offspring from protein-deficient mothers were found to be wider, more irregular and contained more acellular areas (Fig. 1a and 1b). In addition, the altered skeletal development could not be compensated for by adequate nutrition during the lifetime of the animals consistent with the possible programming of bone and cartilage growth by maternal undernutrition in early life.

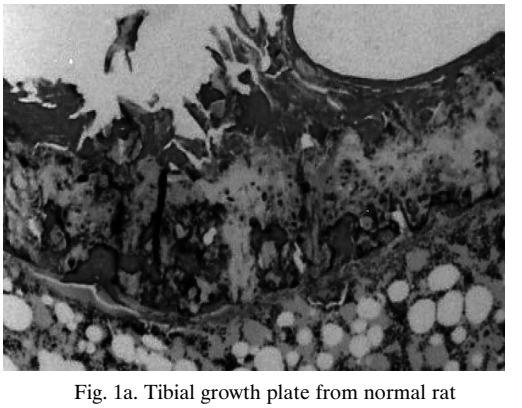

Fig. 1a. Tibial growth plate from normal rat

Fig. 2: Variation in a) total colony number and b) & c) alkaline phosphatase positive colony number in control and low protein groups at 4 weeks

a) Control 18% low Protein 9% b) Control 18% low Protein 9% c) Control 18% low Protein 9%

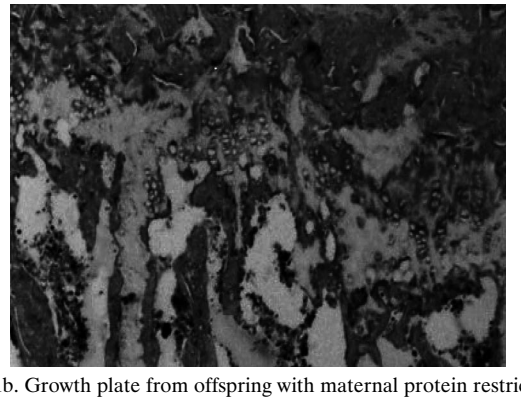

Fig. 1b. Growth plate from offspring with maternal protein restriction

In a second study, we examined whether colony formation (colony forming unit-fibroblastic, CFU-F), proliferation and differentiation of bone marrow stromal cells from offspring of female rats maintained on normal (18% casein) or low (9% casein) protein diets was altered and / or their response to the key endocrine factors, Growth Hormone (GH), $1,25(OH)_2D_3$ and IGF-1. Dams, as in study one, were fed an 18% casein control diet or 9% casein low protein diet from conception until the end of pregnancy. Offspring (n=40) were fed a normal protein diet and CFU-F formation, proliferation and differentiation examined in young (4 weeks), pubertal (8 weeks) or skeletally mature rats (12 -16 weeks).

As shown in Figure 2 and Table 2, results suggest that normal proliferation and differentiation of bone marrow stromal cells was suppressed in offspring from mothers on low protein diets as assessed by colony forming unit-fibroblastic (CFU-F) formation at four and eight weeks. CFU-F are derived from stem cells within bone marrow and can differentiate into cells of the osteogenic, adipogenic, fibroblastic and reticular lineages. A proportion of these cells have high proliferation and differentiation capacity, as shown by the formation of bone in diffusion chambers in a variety of *in vivo* model systems.

The reduced proliferation and differentiation capacity observed is suggestive of a reduced capacity for bone formation differentiation of mesenchymal stem cells was delayed by restricted maternal nutrition during early life.

Table 2. Difference in bone activity - Low protein compared to control group

Time (weeks)	Total CFU-F AP+ve	CFU-F	Alk.Phos specific activity
4	-21%**	-41%**	-60%***
8	-38%**	-90%**	-92%***
12	+22%	-9%	+ 220%*
16	+211%***	+349%***	+7,278%***

*P<0.05, **P<0.01, ***P<0.001

At 12 weeks, no significant differences were observed in colony formation. Modulation of osteoblast proliferation and differentiation was observed in cells from the 18% group by $1,25(OH)_2D_3$, IGF-1 and GH at 8 weeks and the low protein group at 12 weeks.

This observed reduction in osteoblast activity in the low protein group may represent altered programming of skeletal growth by maternal dietary manipulation. In skeletally mature rats (12-16 weeks), the reverse was found with "catch-up" or a physiological shift in bone cell activity observed in the low protein group. These results suggest that bone growth may be programmed in early life; an effect that can be demonstrated at the cellular level. Furthermore, these results indicate this animal model provides a useful model system to investigate the cellular biochemical mechanisms involved in bone development with programming *in utero*. These alterations in mesenchymal stem cell function by the early environment represent an important candidate mechanism for the programming of osteoporosis and associated consequences in later life.

In summary, these results demonstrate that maternal protein restriction has significant effects on bone cell activity of related offspring. The reduced CFU-F numbers and specific activity in osteoblasts derived from the low protein group may have profound effects on bone formation, with changes persisting to adulthood. Understanding the factors regulating lineage potential and activity and control of bone formation will prove central in our understanding of bone growth and development to skeletal maturity and beyond as well as the role of foetal programming.

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OR-33

Are Statins Scientifically Still an Option for Osteoporosis Treatment?

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The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors widely used for treatment of hyperlipidemia have been shown to have not only cholesterol-lowering effect but to have also other pleiotropic effects such as stimulation of nitric oxide production, anti-apoptosis, anti-proliferation, and immunomodulation. One of the possible pleiotropic

effects of statins is modulation of bone metabolism. The first evidence that the statins may have an anabolic effect on bone came from a 1999 study by Mundy et al., in which statins induce the production of bone morphogenetic protein-2 (BMP2) and addition of lovastatin or simvastatin into a mouse-calvaria organ culture increased osteoblastic bone formation. Furthermore they have shown oral statin therapy prevented bone loss in female rats after ovariectomy. Triggerd by this finding an increasing number of studies of the effect of statins on bone metabolism, osteoporosis and osteoporotic fracture risk *in vitro* as well as *in vivo* has been published recently.

Mechanism of action

Several *in vitro* studies on the effect of statins, including simvastatin, mevastatin and fluvastatin, but not the hepatospecific pravastatin, on cultured osteoblastic cell lines showed that the statins induced expression and production of bone morphogenetic protein-2 (BMP-2), a protein of the transforming growth factor-b superfamiliy, which plays an important role in osteoblast differentiation and bone formation. This effect appears to be due to the inhibition of HMG-CoA reductase. Furthermore it seems that only a brief exposure of 24 hours to a statin is enough to induce a "bone-building" activity for 2 weeks.Thus BMP-2 plays a central role in mediating the effects of statins on bone. Statins may also have effects on osteoclasts, in a similar way as do bisphosphonates. The nitrogen-containing bisphosphonates inhibit bone resorption by inibiting one or more enzymes down-stream of HMG-CoA reductase in the mevalonate pathway in osteoclasts and prevent the synthesis of isoprenoids required for the prenylation of small GTPases such as Rho and Rac, necessary for osteoclast function. Inhibition of bone resorption, involving osteoclast apoptosis and the loss of ruffled border, was shown to be induced by statins. In some of the *in vitro* experiments the statins were even more potent inhibitors of resorption and osteoclast numbers than bisphosphonates. However, their effects *in vivo* would not be as dramatic as the bisphosphonates specifically target bone while the pharmacokinetics of the statins favor the liver. Thus products of mevalonat metabolism in the cholesterol synthesis pathway have important effects on both bone formation and resorption.

Effect on bone metabolism, bone mineral density and fracture risk

In animal studies systemic administered statins increased new bone formation by approximately 2- to 3-fold. Histomorphometric analysis showed a 50% increase in new bone formations in mice and significantly increased cancellous bone volume in rats. Studies in humans assessing bone turnover by using specific markers of bone formations and resorption in control and statin-treated subjects showed variable results. In an observational study with the use of pravastatin in postmenopausal women an increase in procollagen I amino-terminal propeptide, a marker of bone-formation, was observed. In another study there was an increase in osteocalcin serum concentration on simvastatin. However, in both studies using pravastatin or simvastatin another more widely used assay for bone formation, the bone specific alkaline phosphatase levels, no effect was detectable. No effect on bone formation or resorption was found with the use of fluvastatin in elderly women. Others found a weak inhibition of bone resorption with fluvastatin in postmenopausal women with osteoporosis or osteopenia. The effect on bone mineral density has been addressed in a few cross-sectional studies suggesting that the use of statins is associated with a higher bone mineral density in diabetics, postmenopausal, as well as osteoporotic elderly women compared with aged matched controls.

Recently, an increasing number of epidemiological case controlled studies appeared in the literature, suggesting a possible association of reduction in fracture risks with statins, however there were also studies which did not show a reduction in fracture risk with the use of statins. The most recent study (Geelong osteoporosis study) examining the effect of statin use on fracture risk showed that the odds ratio for fracture associated with statin use was 0.4 (95% CI 0.23 – 0.71), after adjusting for BMD at the femoral neck, spine and whole body the odds ratio increased to 0.45 (95% CI 0.25 – 0.80). Adjusting for age, weight, concurrent medications and lifestyle factors had no substantial effects on the odds ratio for fracture. An exception to this finding reports no effect of pravastatin use on fracture frequency. In contrast to other statins, the hepatospecific pravastatin has very little effect on bone cells, which explains the negative finding. Intriguing is also the finding that 2 studies using the same database (UK General Practice Research Database) arrive at different results, one group reporting a significant reduction in fracture risk on statin use, while the others found no association. However one has to bear in mind, that all of these case controlled studies differed in recruitment criteria, definition of statin exposure and outcome assessment. Furthermore there might be other uncontrolled confounding factors, such as differences in physical activity or in nutritional factors and others. Despite the encouraging consistencies of the first reports on fracture risk and the use of statin, the more recently presented data remind us to be cautious in interpreting observational data. Diseases and treatment modalities that are associated with other factors as nutrition, weight and lifestyle changes can be very difficult to separate. To date, no prospective study has been conducted to investigate the relationship between the use of statins and bone metabolism and fracture risk.

Conclusion

Available data appear consistent that statins have a profound effect on bone metabolism. There is growing evidence from both *in vivo* and *in vitro* animal studies as well as experimental models on cell lines, supporting that statins are a group of stimulator of new bone formation and may also affect bone resorption. There is indication that the use of statins in humans may be associated with increased BMD and reduced fracture risk, however this has not been confirmed by all studies. Clearly, the last word has not yet been spoken regarding the effect of statins on bone health. Data from randomized prospective trials are needed to determine the impact of statins on bone mineral density and fracture risk.

OR-34

Clinical use of Raloxifene (Evista) in Patients With Postmenopausal Osteoporosis

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One third of women aged 60 to 70 years and up to 2/3 of women over the age of 80 are considered suffering of osteoporosis worldwide. Approximately 20 to 25 % of women over the age of 50 will have one or more vertebral fractures. While hip fracture is a dramatic consequence of osteoporosis, the impact of vertebral fracture on survival rates and well-being of patients should not be underestimated. The age-adjusted relative risk of death following a spinal clinical fracture is 8.6. Suffering of one or more vertebral fractures systematically induces a reduction in the ability of performing daily living activities and the highest the number of fractures, the poorest the quality of life of the patient. One of the major and less known risk factors for experiencing a new vertebral fracture is the prevalence of prior vertebral fractures. Having suffered from fracture increases by 60 % the risk of developing a new vertebral fracture independently of the level of bone mineral density and, in case of the presence of two prevalent fractures, the risk is increased by 30 %. Raloxifene is a new non-hormonal treatment for postmenopausal osteoporosis. As other selective estrogen receptor modulators, raloxifene has an estrogen-like effect on the skeleton while it exhibits anti-estrogen activities on other body systems, mainly on the uterus and the breast. The effects of raloxifene in the treatment of osteoporosis were derived from the Multiple Outcomes of Raloxifene Evaluation (MORE study). This multicentre, double-blind, placebo-controlled trial investigated the effect of 60 mg or 120 mg of raloxifene in a population of 7 705 postmenopausal women with osteoporosis. The population was characterized either by the prevalence of one or more prevalent vertebral fracture (n = 3 203) or the prevalence of a low bone mineral density without vertebral fracture (n = 1 638). Over the whole duration of the trial (4 years), raloxifene significantly reduced the rate of incident vertebral fractures by 49 % in women without prevalent vertebral fractures and 34 % in women with prevalent vertebral fractures. Interestingly, the effect of raloxifene was present as soon as the first year of the trial with a 68 % reduction of new clinical vertebral fractures after one year in the raloxifene 60 mg/day group compared to the placebo-treated population. In order to be able to claim for a sustained efficacy, it is of prime importance to demonstrate that the investigated compound is still able to significantly reduce the incidence of vertebral fracture during the last year of the treatment. This observation is the basis for being able to recommend a long-term treatment with this particular compound. During the 4th year of the trial, raloxifene significantly reduced the incidence of new vertebral fractures by 50 % in the population who had no prevalent vertebral fractures at baseline and by 38 % in the population of women who had prevalent fractures at baseline. When considering the risk of

multiple new vertebral fractures in women without pre-existing fractures, the reduction observed under raloxifene at the dose of 60 mg/day was 93 %, over a period of 3 years. While no specific effect of raloxifene on the incidence of hip fracture was observed during this trial, the overall assessment of the anti-fracture efficacy of raloxifene, taking into account the occurrence of any type of fractures, showed a reduction of the risk of 20 % compared to the placebo-treated population for the dose of 60 mg/day, a reduction which was not only clinically relevant but also statistically significant. No impairment in bone quality was observed during treatment with raloxifene and, more specifically, no woven bone, marrow fibrosis, mineralisation defect, cellular toxicity or histologically abnormal appearance were recorded on bone biopsies analysed by quantitative histomorphometry. The effect of raloxifene on bone was linked to a reduction in biochemical markers of bone remodeling and a significant increase in vertebral and femoral bone mineral density. In conclusion, the new SERM, raloxifene, at the dose of 60 mg/day, was shown to significantly reduce new vertebral fractures incidence in a population of post-menopausal women with or without prevalent vertebral fractures. This effect was statistically significant as soon as the 1st year of treatment and a sustained efficacy was demonstrated by the reduction in new vertebral fractures observed also during the last year (4th) of treatment both in women with fractures or with densitometric osteoporosis. Due to its outstanding safety profile and collateral benefits, raloxifene at the dose of 60 mg/day, can now be considered as a first-line treatment of osteoporosis able to provide a rapid and sustained benefit for the patients. It should be directed to women presenting either with prevalent vertebral fractures or with low bone mineral density of the spine or the femoral neck.

OR-35

Age, Genes and Environment Explain the Important Association Between Lean Body Mass and Bone Density: a Twin Study

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There are strong cross-sectional associations between lean mass (LM) and measures of bone mineral density (BMD). Moreover, change in LM is a strong predictor of change in BMD during growth. These associations have important implications, but their genetic-environmental basis is controversial. We studied the extent to which genetic and environmental factors explained LM-BMD associations in healthy female twins (760 monozygotic pairs, MZ; 770 dizygotic pairs, DZ) aged 8 to 89 years, divided into young (8-16), mid-range (17-44) and older (45-89 years) groups. Age- and age-height-adjusted lumbar spine, total hip and total body bone mineral measures were moderately-to-highly heritable in adult twins (Falconer estimate (h) = 0.50 -0.83). Heritability (h) of age-height-adjusted LM was 0.60-0.61 in adult twins. There was evidence for additional, environmental effects both on BMD and LM shared within pairs of young twins. Analysis of age- and age-height-adjusted cross-trait correlations (LM vs. BMD) within individuals and within MZ and DZ pairs revealed that:

(i) In young twins, within-pair shared environment plus additive genetic factors (partly related to height) largely explained a strong correlation between LM and BMD (within-individual r 0.60-0.87 for age-height-adjusted LM and BMD);

(ii) In mid-range twins, additive genetic factors (probably not related to height) largely explained a moderate correlation between LM and BMD (within-individual r 0.37-0.68 for age-height-adjusted LM and BMD);

(iii) In older twins, additive genetic factors (probably not related to height) plus a marginal effect of the individual twins' environment explained a moderate correlation between LM and BMD (within-individual r 0.36-0.62 for age-height-adjusted LM and BMD).

In conclusion, by studying female twins across a wide age range, it was found that:

(i) LM and BMD were highly correlated in young twins, the relationship diminishing with age;

(ii) Genetic factors were important determinants of the LM-BMD association, with evidence for a role of height-related genes in young twins;

(iii) Environmental factors (shared within young twin pairs; marginally evident in older twins) contributed to the LM-BMD association, in keeping with experimental evidence that interventions can increase both LM and BMD, especially during growth.

Keywords: Lean Body Mass, Bone Mineral Density, Heritability

OR-36

Molecular Effects of Testosterone on Muscle in Males

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As humans age there is a progressive loss of muscle mass and strength or as it is commonly named, sarcopenia. We study the effects of androgen administration in older men on muscle function, physiology, and molecular mechanisms. We assess function using measurements of muscle strength (1-RM assessment) and muscle volume (MRI assessment). We use stable isotope methods to assess muscle physiology by determining muscle protein synthesis and breakdown. Molecular mechanisms are determined by Western analysis of key regulatory proteins (androgen receptor and IGF-I) and cDNA array analysis.

From studies in older men, we found that testosterone will increase net protein balance and increase muscle strength and volume after 6 months of testosterone administration. The mechanisms of testosterone effects on muscle change over time during continuous administration. Initially, androgen receptor and IGF-I expression are increased in muscle as well as key nuclear-derived mitochondrial oxidative phosphorylation enzymes making the major physiologic response one of increased protein synthesis. However, after 6 months, all of these markers except IGF-I have returned to baseline with now the major determinant of an increased net protein balance deriving from a decrease in protein breakdown. This is shown by a decrease in the expression of calpain p94, an important breakdown protease and an increase in protein concentrations of the gigantic structural protein, titin. These findings indicate that studies are needed to assess the effects of testosterone cycling paradigms on muscle metabolism and strength in men.

Objectives

1. Discuss androgens and their administration in humans
2. Understand physiological effects of androgens on muscle in older men.
3. Understand physiological effects of androgens on muscle in older women.

OR-37

Physical and Cognitive Performance in Elderly Males; The Influences of Testosterone, Estradiol and DHEAS

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There is considerable variation in the effect of aging in healthy individuals. Genetic factors, lifestyle, and social investments in a safe and healthful environment are important aspects of successful aging. Loss of muscle mass and strength are important predictors of a decrease in physical performance, which in turn results in a loss of independence. In a study we performed amongst 403 independently living elderly men in The Netherlands, muscle strength was strongly positively related to quality of life. Physical performance, muscle strength and quality of life were all predictors of mortality.

Since changes in activity of different hormonal axes during aging occur in parallel with the decline in physiological functions, it has been suggested that part of the aging process might be related to the endocrine system. There is an abrupt cessation of estradiol production in women accompanied by a decrease in bone mineral density. While in men the decrease in testosterone production occurs more gradually and is accompanied by a decrease in bone mineral density as well as muscle strength. During aging, serum estrogen levels also decrease in men. Amongst the 403 elderly men, we demonstrated that serum estradiol was positively related to bone density and, interestingly more strongly compared

to testosterone levels, as well as to quality of life. Finally, high estradiol levels predicted a poorer cognition 4 years later. In both men and women there is a progressive gradual decline in serum dehydroepiandrosterone (DHEA) concentration. It is so far not elucidated whether DHEA plays a functional role in the aging process. The same accounts for the decrease in serum growth hormone and insulin-like growth factor 1 concentrations which occurs during aging.

Muscle strength and functional ability are considered to be the key physical characteristics of successful aging. Circulating levels of testosterone and estradiol in men seem to play an important role in physical and cognitive aspects of successful aging. The role of DHEA remains to be established.

OR-38

Selective Androgen Receptor Modulators (SARMs): Therapeutic Potential in the Aging Male

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A novel approach for the treatment of certain disorders associated with aging in men, and possibly women, is the development of selective androgen receptor modulators (SARMs) that can stimulate formation of new bone, increase muscle mass and strength and enhance libido and sexual function with substantially diminished proliferative activity in the prostate, as well as reduced virilizing activity in women. Over the last several years, we have developed a program to discover and develop novel, non-steroidal, orally-active selective androgen receptor modulators (SARMs) that provide improved therapeutic benefits and reduce risk and side effects. In recent studies, we have used a skeletally mature orchiectomized (ORDX) male rat as an animal model of male hypogonadism for assessing the efficacy of novel, non-steroidal, non-aromatizable, and non-5alpha-reducible SARMs. We assessed the activity of these molecules on bone turnover, bone mass and bone strength, and also evaluated the effects exerted on classic androgen-dependent targets, such as prostate, seminal vesicles and muscle. A substantial loss of bone density was observed in ORDX animals, and this loss was prevented by SARMs, as well as androgens. Biochemical markers of bone turnover revealed an early increase of bone resorption in androgen-deficient rats that was repressed in ORDX animals treated orally with a SARM during a 4-month treatment period. Differences in architectural properties and bone strength were detected by histomorphometric and mechanical analyses, demonstrating beneficial effects of these molecules on bone quality in androgen-deficient rats. Histomorphometric analysis of cortical bone revealed clear and distinct anabolic activity of SARMs in periosteal bone. The compound was able to prevent bone loss and maintain bone quality in ORDX rats by stimulating bone formation, while also inhibiting bone turnover. Similarly, SARMs exert anabolic activity in muscle, restoring muscle mass to eugonadal levels or even above those. Taken together, these results suggest that orally-active, non-steroidal SARMs are useful therapeutics to enhance both muscle and bone mass in elderly hypogonadal men through their anabolic activities. Since SARMs both prevent bone loss, and also stimulate formation of new bone, they may have significant advantages relative to currently used anti-resorptive therapies. Coupled with their activity in muscle and their ability to maintain or restore libido, they offer new therapeutic approaches for male and female hormone replacement in aging subjects.

OR-39

Regenerative Biology and Medicine

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Regenerative biology is the science of understanding the mechanisms of regeneration by cellular and molecular comparisons of regenerating and non-regenerating tissues. Regenerative medicine applies this understanding to restore tissue structure and function after damage due to injury or disease.

Vertebrates regenerate via three mechanisms, each of which involves regeneration-competent cells in different states of differentiation. The first is compensatory hyperplasia, which involves the proliferation of cells while maintaining all or most of their differentiated functions. Compensatory hyperplasia is characteristic of hepatocytes of regenerating liver and capillary regeneration from the walls of venules.

The second mechanism is the activation of adult stem cells. These are undifferentiated cells set aside in various tissues during embryonic and fetal development and used for juvenile growth and for regeneration throughout life. Mammalian tissues known to regenerate by stem cell activation are blood, bone, skeletal muscle, epithelia, liver, and olfactory neurons. Some stem cells, such as those residing in bone marrow (hematopoietic and mesenchymal stem cells), appear to have wide developmental plasticity when placed in microniches other than the one they normally occupy. Thus mesenchymal stem cells of the bone marrow normally have the potential to differentiate into bone, cartilage, and adipocytes; hematopoietic stem cells of the bone marrow give rise to blood and lymphoid cells. MSCs and HSCs can, however, circulate and give rise to skeletal muscle (Ferrari et al., 1998), cardiac muscle (Bittra et al., 2000), liver (Petersen et al., 1999; Theise et al., 2000), astrocytes and neurons (Kopen et al., 1999; Brazelton et al., 2000), and epithelia of the respiratory and digestive systems, kidney tubules, and skin (Krause et al., 2001) when injected into lethally X-irradiated or severe combined immunodeficiency (scid) mice, injured mouse tissues, or after marrow transplants in humans. The developmental plasticity of HSCs and MSCs (or alternatively, the presence of a pluripotent stem cell in bone marrow), as well as other adult stem cells, has generated great interest in their potential use to replace damaged tissues. Marrow cells could be cultured to expand their numbers, then transplanted into the injured region, either as a suspension or aggregate of cells, or after seeding into a shaped biomimetic scaffold to make a bioartificial tissue construct.

The third mechanism is the production of stem cells from mature cells by dedifferentiation. The urodele amphibians (salamanders and newts) are unique among the vertebrates in their ability to regenerate by this mechanism (Brockes, 1997). For example, adult newts can regenerate the neural retina and lens of the eye, cardiac muscle, intestine, jaws, tail, and limbs by the dedifferentiation of cells local to the injury. Dedifferentiation involves the loss of tissue organization by proteolytic degradation (acid hyrdolases, matrix metalloproteinases) of extracellular matrix (ECM) and the liberation of cells which are then triggered to dedifferentiate. Like conventional stem cells, dedifferentiated cells exhibit plasticity, being able to transdifferentiate into cell types other than the one of origin (Stocum, 2002).

Limb skeletal muscle has been used to study dedifferentiation and re-entry into the cell cycle, because it is a multinucleate cell that must be cleaved into mononucleate cells and has a high degree of internal structure that is disassembled. What is known so far is that thrombin in the serum of the wound activates an as yet unidentified protein that induces myofiber nuclei to enter G1 and synthesize DNA (Tanaka et al., 1999). The mechanism of myofiber cellularization is independent of re-entry into the cell cycle, but mitosis and cytokinesis does not occur in the absence of cellularization (Velloso et al., 2000). Mouse myofibers are not known to cellularize and dedifferentiate in response to serum factors, but will do so if transfected with the msx1 gene *in vitro* (Odelberg et al., 2001), or cultured in the presence of extract derived from amputated newt limbs undergoing dedifferentiation (McGann et al., 2001). These observations have significant implications for regenerative medicine, because they indicate that a large part of the dedifferentiation potential of amphibian myofibers is conserved in mammalian myotubes and perhaps other mammalian cells.

Regenerative medicine is using three major approaches based on regenerative biology: isolation and expansion *in vitro* of adult stem cells for (1) transplantation as aggregates, or (2) construction of a bioartificial tissue made by seeding the cells into a biomimetic scaffold, and (3) stimulation of regeneration *in vivo* by activating resident stem cells or producing them by dedifferentiation. Derivatives of embryonic stem cells (ESCs), in addition to adult stem cells, have been used in protocols designed to restore the structure and function of injured or genetically deficient tissues. Cardiomyocytes, as well as multipotent glial precursors derived from ESCs have been successfully transplanted to regenerate new tissue in dystrophic mice and mice suffering from genetic or injury-induced myelin deficiency (Klug et al., 1996; Brustle et al., 1999). ESCs have the advantage of unlimited growth potential and pluripotency for transplantation. Among their disadvantages is immunorejection of the derivatives, but therapeutic

cloning techniques have been developed to make autogeneic ESCs from blastocysts that would be unable to form a more developed embryo.

Transplants of ASCs have also been successful in regenerating new tissue. Grafts of fetal midbrain cells, some of which are neural stem cells, into the striatum of rodents, monkeys, and humans suffering from Parkinson's or Huntington's disease, reduced motor and cognitive deficits (Bjorkland and Lindvall, 2000). Satellite cells of skeletal muscle (Taylor et al., 1998), and bone marrow HSCs and MSCs (Orlic et al., 2001), are able to differentiate into cardiomyocytes in injured cardiac muscle. MSCs have also shown promise in the regeneration of dystrophic muscle, cartilage, tendon, and segmental bone defects in rats, rabbits, and dogs (Caplan and Bruder, 2001). Recently, transfusions of normal bone marrow cells from HLA-identical normal siblings were sown to improve the fracture-withstanding capacity of bones in infants with osteogenesis imperfecta (Horwitz et al., 1999). Umbilical cord blood has been found to have high concentrations of HSCs that are as efficient as bone marrow HSCs in reconstituting the hematopoietic system of compromised patients. Cord blood HSCs also elicit graft vs. host disease at lower frequency (Smith et al., 2000).

Stem cell transplants will most likely constitute the first wave of regenerative medicine in the next decade or two. The most compelling vision for the future, however, is one in which we are able to induce or enhance regeneration directly from stem cells in injured tissues using a cocktail of regenerating-stimulating proteins, in combination with neutralizers of proteins that inhibit regeneration. Even if a tissue contains no stem cells itself, it may be possible to intensify the ability of stem cells like those in bone marrow to circulate, home to injury sites throughout the body, and exhibit developmental plasticity in order to regenerate new tissue. Alternatively, we may be able to induce the dedifferentiation of mature cells in the injury region by transfecting a combination of regeneration-stimulating genes, or delivering a combination of proteins that triggers the dedifferentiation mechanism. Induction of regeneration *in vivo* is the most biologically desirable way to regenerate tissues; furthermore, it bypasses bioethical and legal issues associated with the use of exogenous stem cells.

Making this vision a reality requires a great deal more research on regenerative biology. How are stem cells activated and how do they home to sites of injury? What signals do they require to differentiate into new tissue and are those signals present at injury sites? What inhibitors of regeneration are present at injury sites? What are the molecular mechanisms by which stem cells are created by dedifferentiation? In essence, what are the differences in gene activity that define repair by scar tissue formation vs. regeneration of the original tissue architecture?

We have adopted a comparative molecular approach to answer the last three questions. The frog, *Xenopus laevis*, regenerates structures such as limbs, neural retina, lens, and spinal cord as a tadpole, but loses regenerative capacity as it approaches metamorphosis. We are comparing regeneration-competent stages of these tissues with regeneration-incompetent stages in order to identify as many genes as possible that promote or inhibit regeneration. This process has revealed that many more genes are involved in regeneration and in wound repair than are currently known. The task now is to use bioinformatic analysis to categorize these genes and get an idea of the function of their proteins. Then these genes must be tested in functional assays for their ability to extend regenerative capacity into post-metamorphic stages of frog development, or inhibit regeneration in tadpole stages. Finally, human homologues of effective frog genes will be cloned and tested for their regeneration-promoting or inhibiting capacity in mammalian systems.

This is a daunting task that will require the development of some new tools, but one that will have a high pay-off in terms of reduction in health-care costs and increase in quality of life.

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OR-40

Frequency Dependent Bone Formation in an *Ex Vivo* Trabecular Bone Core Percolation and Loading System

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The purpose of these studies was to observe *ex-vivo* trabecular bone cylinders exposed to different loading frequencies in a newly developed Percolation-Loading System (PLS), The PLS can be programmed to apply compressions of a predetermined amplitude and waveform to the bone core in a loading chamber at a given waveform, frequency and cycle number. The stiffness and morphological responses of the loaded bone cores were evaluated in an initial experiment lasting 33 days. We used a waveform derived from force plate measurements of a person walking (Figure 1) which was analysed by FFT to derive the frequency Spectrum (Figure 2)

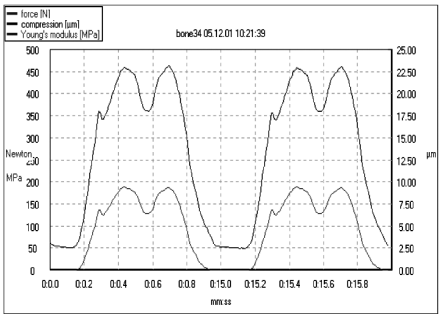


Figure 1 walking waveform

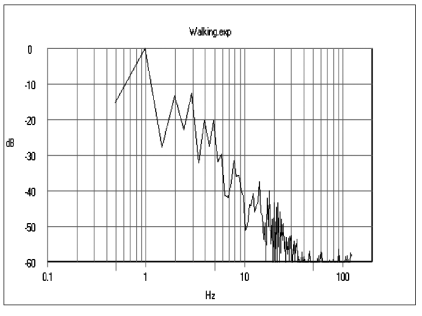
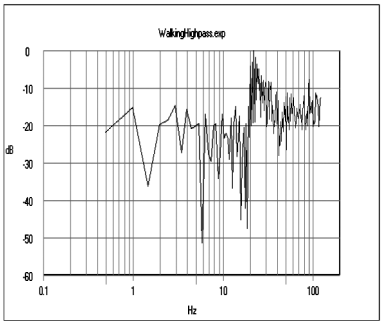
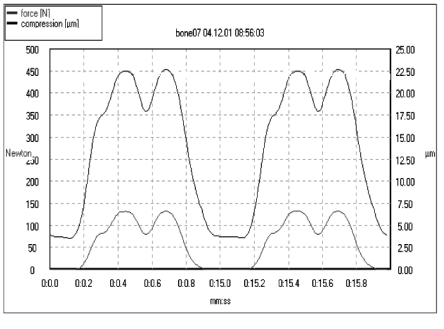


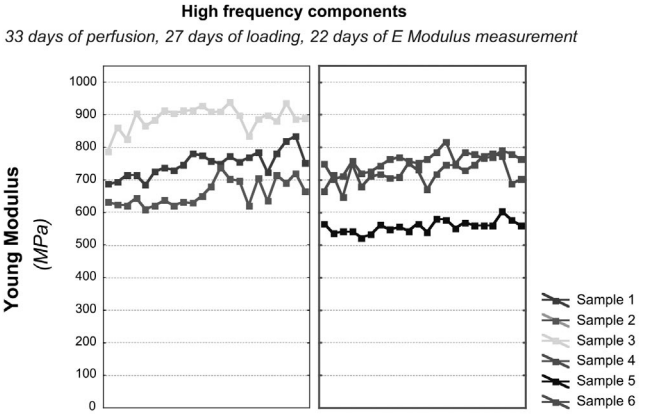
Figure 2 Fourier frequency spectrum

The frequency components were then separated by a cut off filter into frequencies above and below 30 Hz as shown in Figure 3 and the resultant waveform (Figure 4). The same was done for the frequencies above 30 Hz.





The samples were percolated for 33 days (from the 2nd of November until the 4th of December) with a flow rate of 3,3ml/h. After the first 6 days of percolation, the bone cores were submitted to the daily loading procedure until the end of the experiment. Each bone sample was loaded every day (during 27 days), 5 minutes at 1 Hz. Simultaneously to the loading procedure, the apparent bone stiffness of each sample was determined by a "Static Young's Modulus" measurement using a new calibration method and which was carried out at 8°C (cold conditions).



The results indicate that loading during cooling does not change the mechanical properties (ie cold bone does not receive mechanical stimulation). We find in these experiments no significant difference between high frequency components of the walking pattern over low frequency components or the full pattern in increase in stiffness, all waveforms increased over control. Figure 4 above show the results for the high frequency wpart of the waveform

OR-41

Long Term Cell Viability, Modeling and Remodeling in an *Ex Vivo* Trabecular Bone Core Perfusion and Loading System

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The purpose of these studies was to observe trabecular bone core response in a newly developed Perfusion-Loading System (PLS), designed to maintain cylindrical trabecular bone cores (10mm in diameter X 5mm in height) viable under controlled conditions. The PLS is programmable to apply compression loads to create a predetermined deformation of the bone core in a loading chamber at a given waveform, frequency and cycle number. Both the physiological and morphological responses of the loaded bone cores were evaluated in seven experiments lasting 7 hours to 49 days. These studies were designed to establish methods and technologies for bone core preparation and to determine trabecular bone core cell viability and response to mechanical loading and chemical stimuli in the PLS. Each bone core provides approximately 2 million viable cells per sample.

MTT (30 mg/ml), (Sigma, 3-[4,5- Dimethylthiazol-2-y 1]-2,5-diphenyltetrazolium bromide) was used as an indicator of cell viability. MTT showed that 95 to 98% of the osteocytes from 120 sections representing 20 bovine bone cores were viable at 7 hours to 31 days.

Prostaglandin E_2 (PGE_2) was measured as a marker of cell response to mechanical stimulation at approximately 3000 με for 300 cycles of loading at 1 Hz. In 18 bovine samples loaded for 28 days, PGE2 was elevated 2 and 5 hours after loading compared to the non-loaded controls.

Mechanical loading combined with PTH 1-38 in the culture medium was conducted for 20 days. The bovine bone cores were perfused for two hours, Monday, Wednesday and Friday for 20 days at four concentrations (0, 10^{-10}, 10^{-8} or 10^{-6} M) of PTH 1-38. All bone cores were loaded in the perfusion chambers at 3000 me (15mm) for 300 cycles/day at 1 Hz. The apparent stiffness of each core was determined daily. The percent change in apparent stiffness was calculated. Day 1 was defined as after 48 hours rest in the chambers. The average stiffness for all groups on Day 1 was 4.5± 2 N/µm.

In a 49-day study with human bone cores, bones were perfused on days 19, and 34 with Calcein (30 mg/ml) in the medium to label mineralizing surfaces and determine mineral apposition rates (MAR) and on day 44 with Alizarin Complexone.

In the bovine bone cores treated with mechanical loading and PTH, PTH at 10^{-10} M concentration showed the greatest increase in apparent stiffness while PTH at 10^{-6} showed a loss in apparent stiffness over the 28 day

experiment. In the human cores, double and triple labels were observed and MAR for the cores averaged 0.6 ± μ/day. Toluidine blue stained sections from these human cores showed numerous cell types including osteoblasts, osteoclasts, and lining cells at 49 days.

These studies demonstrate that bone cores remained viable for 31 to 49 days. Bone cores loaded in the PLS produced prostaglandins and the MAR was similar to that of human *in vivo* studies. The bone cores' response to PTH concentrations, catabolic and anabolic, was similar to that seen in other *in vivo* and *in vitro* models. Active osteoblasts, osteocytes and osteoclasts were observed at 49 days indicating the potential for bone modelling and remodelling in the system.

P-1

Emotional and Cognitive Condition of Elderly People

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Objectives: To investigate the emotional and cognitive conditions of elderly people and their attitude towards their overall status and the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of day living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 75% did not report feelings of frustration, 66,7% feelings of anger, 62,6% weakness and 74,2% hopelessness. Moreover 76,4% did not report feelings of isolation and 66,4% did not report inability. 57,9% and 64,8% respectively did not report disorders of memory and attention, while 56,9% and 70,8% respectively did not report any change in their ability for calculation and problem solving ability. 50% reported improvement of their condition when they were involved in community activities. The family status is correlated with the feelings of frustration (p=0,0006), anger (p=0,0002), feelings of weakness (p=0,003), feelings of isolation (p=0,0001), inability (p=0,004). The educational level is correlated with the ability of calculation (p=0,005) and disorders in temporo-spatial orientation (p=0,00001).

Conclusion: One out of four subjects reports psychological disorders (frustration, anger, weakness, isolation and inability). Three out of four have no cognitive disorders. Family status and body formation are correlated with the psychological status of the elderly people and educational level with the cognitive status.

P-2

Epidemiological Data for Elderly People – Protocol Presentation

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Objectives: To present the epidemiological data concerning problems of the elderly people as well as the presentation of the protocol used.

Materials and methods: A special constructed questionnaire was administrated to 318 elderly people, in Community Centers for Elderly People. Completion of the questionnaire was done by themselves with the assistance of the social worker when requested. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of day living, his emotional condition, and other parameters.

Results: 52,5% of the sample (n=167) were female and 47,5% (n=151) men. Mean age was 69,7 ±5,9 years. The sample was divided into tree groups according to the residential area, as rural (20,4%), urban (26,7%) and rurban (30,5%). Marital status was: 62,9% married, 27,4% widowed, 1,6% divorced, 1,9% singles. Financial status classified as average (91,2%), rich (4,1%) and poor (2,8%). Educational level was: unliterary 13,2%, lower 65,7%, medium 17,3% and high 2,5%. Body formation classified as: obese (24,5%), normal (58,2%) and slim (15,7%). Nutrition was adequate in 90,9% and poor in 3,5%. The outfit was careful in 88,4% and dishabille in 2,2%. Information concerning problems of the elderliness derived from: books 27,7%, TV 89%, pamphlets 44,7% and chatting with friends 46,9%. The majority of the subjects (63,5%) lived with their families, while 18,9% lived alone. Commonest diseases were: heart diseases 20,1%, lung diseases 8,8%, high blood pressure 45,9%, diabetes mellitus 20,8%, vasculopathy 8,2%, arthritis 20,4%, spondylopathy 25,1%, gastrointenstinal diseases 14,8%, reumatological diseases 6,9%, psychiatric diseases 1,6%, obstetrics 12,3% and prostate related diseases 14,8%.

Conclusion: There is an homogenous distribution of the sample concerning gender and age. The majority of the sample derives from rurban areas, they are married, with average financial status, lower educational level, normal body formation, adequate nutrition and with careful outfit.

P-3

Disorders in Mobility of Elderly People

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Objectives: To investigate the musculoskeletal and mobility problems of elderly people, as well as their qualitative and quantitative characteristics and the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of day living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: No pain reported the 39,3% and 29,6% had pain 1 – 2 days per week. Pain at the upright position had the 54,7% and during weight lifting the 53,5%. Limitation of the ROM reported the 52,5%, difficulty during flexion reported the 64,3% and during transportation and motivation the 53,4%. 59,1% reported locomotion disorders. High statistical correlation was found for the pain existence and the body formation (p=0,008), the family status (p=0,009), the gender (p=0,001) and the area of residence (p=0,0001). Similar correlation appeared for the intensity of the pain, the presence of the pain at the upright position or the presence of fatigue. Gender contributed in weight lifting (p=0,015) and difficulty in motivation (p=0,022), the body formation contributed in the limitation of the ROM (p=0,011), in difficulty during flexion (p=0,003) and locomotion disorders (p=0,003)

Conclusions: More than 50% of the subjects reported pain during their activities (lifting and carrying weights) and limitation in rate of motion. Body formation had positive correlation especially with the rate of motion, bending of the trunk, disorders in locomotion and early coming weakness.

P-4

Ergonomically Modification of Daily Activities for Elderly People With Musculoskeletal Pain

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Objectives: To investigate the modification in the environment of the elderly people with pain in order to improve their Activities of Daily Living (ADL) and the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of day living, his emotional condition, and

other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 52,5% avoid household activities and 77% do not use helping devices. No elimination reported: 57,9% in social events, 58,8% in friend's visits the and 64,4% in telecommunication. 52,8% decreased weight lifting and 61,9% walked with more alertness. 72% did not mention about any modification of their place and their clothes. 41,9% asked medical advice and 54,7% followed the doctor's instructions. 58,2% reported a good support from their environment. Avoidance of the household activities is correlated with the gender (p=0,0004) and elimination in social activities with the area of residence (p=0,00031). Elimination of daily activities is correlated with the area of residence (p=0,018) and the family status (p=0,008). Desire for more special information is correlated with the area of residence (p=0,017) and the educational level(p=0,052), while the inquiry of medical help is correlated with the area of residence (p=0,001), the educational level (p=0,009) and the body formation (p=0,0008).

Conclusion: Pain did not restrain ADL, or social activities in 3 out of 5. More than 50% reported sufficient support from their environment. Elimination of daily activities, is correlated with the gender, the family status, the educational level and the residential area.

P-5

Social Support of Elderly People from their Family and Friends

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Objectives: To investigate the opinion of elderly people about their socialization and the support they have from their family and their friends as well as the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of daily living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 66,7% had daily personal contact with their children and 70,8% by phone. 31,1% visited friends on a daily base and 25,5% had communication by phone. 39,9% had 1 to 3 friends while 14,8% did not trust anybody. In 11,3% the support of the relatives increased after the appearance of osteoporosis. 17,6% were fully satisfied from this support. 24,8% needed help for their ADL and 40,6% needed psychological help for their problems. Educational level correlated with the contact of the elderly people with their children (p=0,0001), their friends (0,0004) and the phone contact with friends (p=0,006). Assistance in the ADL correlated with the gender (p=0,00006). Assistance in solving daily problems correlated with the gender (p=0,0005), the family status (p=0,007) and the body formation (p=0,049).

Conclusion: Three out of 4 have contact with their children and friends, while 2 out of 5 visit them daily. Approximately 50% need psychological support, while 1 out of 4 need more help with ADL. Educational level and gender are correlated with all the above.

P-6

Sexual Behavior of Elderly People

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Objectives: To investigate the attitude of elderly people to their sexual desire and behavior and the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of daily living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 53,5% reported changes of sexual desire while 37,4% maintained some sexual life and 35,2% no sexual life at all. 26,1% reported sexual intercourse once a month, 15,4% once a week and 31,4% none. 70,1% disliked sexual movies and 14,8% reported desire of masturbation. 71,45% were not interested in meeting a new companion. The disturbance of sexual desire is correlated with gender (p=0,001), family status (p=0,0006), educational level (p=0,0002) and the area of residence (p=0,0004). Moreover the body formation is correlated marginally with the desire of masturbation (p=0,049) and significantly with the interest in meeting a new companion (p=0,023). The educational level is correlated with the active sexual life (p=0,030), the watching of sexual movies (p=0,012), the desire of masturbation (p=0,053) and the perspective of a new acquaintance (p=0,041).

Conclusion: Almost 50% of the subjects did not reported any libido disorder, while 2 out of 5 had some sexual life. One out of six watched sexual movies or had desire of masturbation. Libido disorders is correlated with the gender, the family status and the educational level.

P-7

Activities of Daily Living (ADL), and Social Activities of Elderly People

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Objectives: To investigate the participation of elderly people, in ADL and social activities and the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of daily living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 86,5% reported no difficulties with hygiene, in household activities 49,4% and 85,8% dressed by themselves without difficulties. 33% reported a long trip distance once a month and 27,7% leisure activities. Dance was the preferable activity for the 57,9%, while 48,7% used to go to the church and 70,8% visited frequently friends and relatives. Gender is highly correlated with: house cleaning ability (p=0,0001), shopping (p=0,050) and hobbies (p=0,039). Body formation is correlated with personal hygiene (p=0,039), leisure activities (p=0,0008), dance (p=0,028) and visits to friends and relatives (p=0,023). Educational level is correlated with trips (p=0,003), leisure activities (p=0,016), dance (p=0,002) and church related activities (p=0,0019).

Conclusion: More than 80% of the subjects were self-served (personal hygiene, house cleaning, shopping) and 1/3 participated in travels and other leisure activities. Body formation, educational level and family status are correlated with the personal care and leisure activities (dancing, visiting friends and relatives and church related activities).

P-8

Osteoporosis Diagnosis and its Implication to Elderly People

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Objectives: To investigate the opinion of elderly people concerning the diagnosis of osteoporosis and its implication in several parameters of their

life, as well as the correlation with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of daily living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: Only 11,3% of the subjects were aware that suffered from osteoporosis, while 23,6% underwent laboratory investigation. 17,9% were under the supervision of a special doctor and 16,7% were under therapy. The diagnosis of the osteoporosis did not affect 15,4% while 23,6% did not restrict ADL and social activities. 19,2% had fear for fall, 21,4% had psychological disorders, while 15,1% had high anxiety. 4,7% had weakness. In 10% of the subjects the changes of the body image were obvious and only in 24,2% did not annoyed them. The area of residence and the gender were correlated with the ability to complete the laboratory investigation of osteoporosis (p=0,0001) and (p=0,009) concomitant, the supervision from a special doctor (p=0,024) and (p=0,024) and the use of medication (p=00004) and (0,023). Change of the emotion and augmentation of the anxiety are correlated with the educational level (p=0,022) and (p=0,039) concomitant.

Conclusion: Only 1 out of 10 knew he suffered from osteoporosis, 1 out of 5 underwent special laboratory tests and was under medical supervision. Osteoporosis diagnosis did not affect 1 out of 6, while did not restrict ADL in 1 out of 4. One out of five of the subjects reported psychological disorders (fear for fall, anxiety). The diagnostic procedure had correlation with the residential area and the educational level.

P-9

Information About Osteoporosis and Allied Problems

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Objectives: To investigate the qualitative and quantitative information of elderly people about osteoporosis and allied problems. Correlation was done with gender, body formation, educational level, family status and area of residence.

Materials and methods: A specially constructed questionnaire was used in 318 aged subjects. The completion of the questionnaire has been done in Community Centers for Elderly People for 247 subjects and in their places for 71 subjects. 167 were women and 151 men. The mean age was 69,7 ±5,9 years. In the questionnaire was reported the opinion of the subject about his mobility problems, his activities of daily living, his emotional condition, and other parameters. An SPSS spread sheet was used and correlation has been done with chi square.

Results: 67,3% had average to good information about the problems of the elderly and 62,9% about osteoporosis. Information was derived from special pamphlets in 9,4%, from TV in 19,5%, from the radio in 7,2%, from doctors in 32,1%, from friends in 17,3%, from chats in 14,2% and from lectures in the Community Centers for Elderly People in 41,5%. 67,9% of the subjects asked for more information, 51,9% looked for it. 45,3% believed that more information could improve their lives and 12,9% had no interest at all. Information about the problems of the elderly had high statistical correlation with the family status (p=0,002), the body formation (p=0,00001) and the area of residence (p=0,0001). Information about osteoporosis was farther more positively correlated with the educational level (p=0,004). The residential area was correlated with the information about osteoporosis as derived from: doctors (p=0,006) and lectures in the Community Centers (p=0,0001) as well as the need for more information (p=0,00001) and the improvement of the quality of life (p=0,026).

Conclusion: Three out of four subjects had adequate awareness about the problems of the elderly and osteoporosis. Television programs, doctors and lectures in the Community Centers for Elderly People, are the main information sources. Awareness about osteoporosis is correlated with the family status, educational level and area of residence.

P-10

The Operative Treatment of Osteoporotic Post-Traumatic Vertebral Collapse

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Study Design and Objectives: The study examines the reliability of spinal implants to re-establish the stability of the osteoporotic post-traumatic vertebral collapse.

Summary of Background Data: Osteoporosis is the most frequent cause of vertebral compression fractures. In spite of severe kyphosis due to osteoporotic compression fractures in the thoracolumbar spine, neural compression, in the spinal canal is rare. Recently there have been several reports of small series of patients involving neural compromise after osteoporotic compression fractures. The operative treatment of such fractures however, faces the problem of the hardware failure due to osteoporosis.

Materials and methods: Between January 1997 and May 2001, 13 patients (4 male and 9 female) with osteoporotic post-traumatic vertebral collapse of the thoracic and lumbar spine were treated surgically. The average age was 70 years (range 59 – 82 years). Indications for surgery were neurological compromise or increasing unstable kyphosis at the fracture site. Eight of the 13 patients demonstrated late neurological deficits and all patients had severe pain accompanied with increasing thoracolumbar kyphosis. Initial treatment was a TLSO orthosis with or without bed rest and analgesics for all patients. Time intervals between fracture and onset of neurological compromise were between 3 months and over 12 months. Three patients were submitted in anterior spinal canal decompression by resection of the collapsed vertebrae, correction of kyphosis, anterior column reconstruction with a titanium spacer filled with bone grafts and anterior instrumentation and posterior stabilization. Ten patients were submitted in posterior decompression and stabilization with transpedicular screws and rods. Reinforcement of the vertebral bodies and the pedicles with methylmethacrylate or carbonated hydroxyapatite (Norian) was applied in all patients. All patients were mobilizied within 3 – 5 days after surgery, patients wearing a TLSO orthosis.

Results: The follow up of the patients was on average 18 months (range 10 months – 4 years). Neurological improvement was evident in all patients, as was the level of pain and the quality of life. No hardware failure was noticed in any patient.

Conclusions: The stabilization of osteoporotic, post-traumatic vertebral collapse of the spine showed that in spite of the technical difficulties involved, it can substantially improve the neurological status, the pain and the quality of life of these patients.

P-11

PC - Simulation of Scoliosis Treatment by Bracing

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Aim of the study: In childhood scoliosis of the spine is usually treated by brace (when the degree of scoliosis according to Cobb is above 20). The shape of brace, and the time of its use is determined according to orthopaedist and/or orthopaedic prosthetics experiences. The laminate brace made according to plaster form pushes to child trunk as a small shoe with aim to correct the scoliosis curves. There is no science theory how a brace influences the stress state of spine and curve remodelling in time. The aim is to show the computer algorithms for determine a stress states at vertebras, intervertebral discs and ligaments under brace force effects. The stress state has effect on correction of pathologic spine curves. The spine curve corrections have two components. The first is elastic effect and it disappears after the orthosis is put off. If the orthosis has been putting on trunk for a long time than the second part of correction can be observed - it is permanent effect.

Materials and methods: The paper is based on many years of ongoing experience with bracing. The authors use the Cheneau-brace (CH-B) from 1991 and a new developed dynamic corrective spinal brace (DCSB) according to Cerny (Patent No. 281 800 CZ) from 1996. The evaluation of effectiveness

of the CH-B (a group of about 400 patients) and DKTO (a group of approximately 100 children) is a subject of our other communications. The presentation shows a manner of determination the spinal curve correction under brace force effect for concrete child patient and stress state at vertebrates and fibrous tissues. The first part of simulation program calculates the coordinates of spinal curve according to parameters measured with help X-ray. The second part calculated for all intervertebral discs moments of inertia. The third part solves spinal deformation and stress state. It is used follow presumption. The vertebral body is stiff and has no deformation. The intervertebral disc and ligaments are elastic and the nucleus pulposus is liquid. The spine is solved as an elastic and stiff beam on an elastic grunt (soft tissue) with help finite element method.

Results: The computer simulation program calculates at each time period the stress state at spine and it judges on permanent spine curve correction. There is searched the dependence of activation and velocity of the spinal curve remodeling on stress state for many patients.

Conclusion: Simulation program makes possible to design an optimal shape of orthosis, and predict the treatment course and a final form of spinal curve.

Acknowledgments

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P-12

Computer Control of Bone Deformities Treatment by Limb Orthoses

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Introduction: In childhood the valgus and varus deformities of the knee are known. Genu valgum in infancy (about 3 years) is often joint with overweight, no epiphyseal abnormalities are proved and the knock knee usually corrects spontaneously by the age of 6 or 7 years. Genu valgum in adolescence does not correct spontaneously and stapling of the inner side of the epiphyses of the tibia or femur or both is indicated. Also an osteomalatia should be excluded and treated. On the other hand, developmental genu varum is observed at 18 months of age and spontaneous correction is likely to occur by the time the child is 4 or 5 years old. Mild forms of rickets (tibia vara and some causes of disturbance of epiphyseal growth) should be excluded and treated.

There is a possibility to treat a significant (severe) valgus and/or varus deformities of legs (in various levels of shank and distal part of femur) by the new limb orthoses with high bending pre-stressing that were developed by authors. Step by step correction of bone deformities by orthoses is based on viscoelastic properties of bones.

The aim is searching of dependence between the bone remodelling and bone stress state under orthose force effect.

Materials and methods: The orthotic treatment was introduced in a group of more than 30 children suffering from valgus or varus deformities of legs. Limb orthoses are made according to plaster patient leg forms (negative and positive). The orthosis has two parts connected at definite level (position) by joint and screw. The screw operates with known force and the orthosis pushes on leg for a sufficient time (during night). Efficacy of orthotic treatment is evaluated according to correction of tibia-femoral angle measured at X-rays of lower limbs in standing patient. There is possibility to calculate the tibia-femoral angle according to measurement of intermalleolar or intercondylar distance in standing child, anthropological estimation and comparison with X-rays. These results are presented in charts, too. Children's femurs and tibias were measured with help of a computer tomograph, and/or X – ray. Space models of the bones have been composed. The model is transformed to special model of concrete patient according to patient dimensions (bone length and diameter), osteoporosis state (bone curvature, tibia/femur angle).

Results: It is supposed that orthosis and bone deformation is small with regard to the soft parts of leg and the press on unit length of leg and it is directly proportional to compressive deformation and indirectly proportional to a thick of soft leg tissue. The prismatic beam theory with respect Navier – Bernoulli's hypotheses or the finite element method is used for the calculation of femur and tibia load and stress state. The algorithm was implemented on a computer. The stress state will be calculated at each patient visit at orthopaedist for the actual leg deformities and a bone remodelling will be predicted.

Conclusion: The computer model of bone stress state and bone remodelling is verified with patient's treatment course. A part of this research is focused on determination of algorithms for a calculation of stress state at femur and tibia under force effects of orthosis. The algorithms make possible to calculate a stress state at bone tissue and growth epiphyses for concrete child patients. Mathematical relations of bone remodelling are composed according to stress state and time of bending pre-stressing activity. An algorithm of bone stress state calculation, a computer program of stress calculation and a simulation program of system bone stress state and bone remodelling is a top of the presentation. Contemporary results are very encouraging. That is why the new developed limb orthoses with high bending pre-stressing appear as a perspective therapeutic method for some congenital and acquired bone deformities especially in preschool age.

Acknowledgments

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P-13

Relationships Between the Densitometric Mineral, Fat and Lean Masses in the Whole Body and Limbs of 3,000 Normal Columbian Men and Pre- and Post-Menopausal Women

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In whole-body studies with DXA [Ferretti; Bone 22:683,1998, n=1,450] we had shown that the densitometric mineral mass, either crude (BMC) or statistically adjusted to fat mass (FA-BMC) in order to avoid any fat interference with its determination, correlated linearly with the lean mass (LM) showing similar slopes but decreasing intercepts in the order: pre-MP women >men>post-MP women> children. This evidenced 1. the homogeneous control of bone status by muscle strength in the species through the bone mechanostat, and 2. the interaction of sex hormones with that regulation. Now we aim to expand that evidence by studying a large sample of 3,000 normal, Caucasic and Hispanic Columbian men and pre- and post-MP women, including also determinations of the same variables in the upper and lower limbs.

In all the studied regions the slopes of the BMC or FA-BMC vs LM relationships were always parallel. However, interesting region-related differences were found between the intercepts of the curves. In the whole body the crude-BMC/ LM relationships showed the same intercept differences as previously observed (pre-MP women > men > post-MP women). In the lower limbs these differences were less significant and showed the order: pre-MP women > men = post-MP women. In the upper limbs they showed very clearly the decreasing order: men > pre-MP women > post-MP women. After fat-adjustment of the BMC, the order of the intercept magnitude in the limbs was always men > pre-MP women > post-MP women, the differences being much more significant for the upper than the lower limbs. In the upper limbs the slopes of the correlation curves between the FA-BMC and the LM were very low and statistically nonsignificant.

Assuming that LM is proportional to muscle mass, and that the fat adjustment of the BMC renders this variable a more reliable indicator of the bone status than its crude values, results suggest that the sex-hormone-induced differences in the DXA-assessed muscle-bone proportionality in humans would vary noticeably according to the region studied, perhaps in connection with the weight-bearing nature of the musculoskeletal structures in question.

P-14

Effects of Load on Bone and Cartilage Following Amputation in Guinea Pig Osteoarthrosis

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Aim of the study: The subchondral bone structure in osteoarthrosis was

studied by stereology after amputation in guinea pigs.

Materials and methods: We performed trans-tibial amputation in guinea pigs with mild OA changes. The volumes and density of bone and cartilage were studied by stereology on the light microscopic level. In addition proteoglycan content was measured biochemically.

Volume, densities and fibrillation were measured by point-and intersection counting on a projection light microscope ANOVA at a rejection of 5% was used.

Results: Three months after amputation, the osteoarthrosis changes in the knee in the amputated knees, were decreased. This was confirmed by volume density of cartilage, cysts and osteophytes, in addition to measurements of bone thickness, cartilage fibrillation, and also cartilage horizontal splitting. However, we also found a decrease in subchondral bone volume and density. Osteoarthrosis changes were, however, increased in the control group and were most pronounced in the contralateral knee (table 1).

Conclusions: We conclude that in osteoarthrosis, cartilage changes are associated with subchondral bone changes. Moreover, subchondral bone is sensitive to longitudinal load, and responds by increasing its volume. In situations with an increased load subchondral changes may play an important role in the development of osteoarthrosis.

Table 1. Means (SD) for volumes (mm^3) for subchondral bone and articular cartilage in the medial and lateral condyles respectively in the control, the loaded (non-amputated side) and the non-loaded (amputated side) groups (n=8).

	Medial condyle	Lateral condyle		
	bone	cartilage	bone	cartilage
control	11.2 (1.1)	9.6 (1.5)	6.1 (0.5)	7.4 (0.6)
loaded	11.3 (0.9)	8.2 (1.3)	7.1 (1.0)[a]	7.6 (1.2)
nonloaded	5.1 (1.1)[a,b]	7.1 (0.7)[a]	4.6 (1.0)[a,b]	7.1 (0.7)

a: $p < 0,05$ compared with control group, b: $p < 0,05$ compared with loaded group

P-15

Protective Phytoestrogen Effect on Bone Density of Ovariectomized Rats

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The aim of the present study was to investigate the possible protective effect of the aqueous solution of the methanolic extract of the plant *Onobrychis ebenoides*, with proven *in vitro* phytoestrogenic action, on bone mass loss of the ovariectomized (OVX) rat model of osteoporosis.

Materials and methods: Forty intact mature (10-month-old) Wistar rats were separated into 3 groups according to the experimental procedure: OVX (n=13), OVX plus phytoestrogen (PH) (n=20) and sham-operated (Control)(n=7). PH administration in the drinking water at a dose of 250mg/kg body weight/day commenced immediately after OVX. Dual energy X-ray absorptiometry scans of the whole and proximal tibia were carried out pre-OVX, 1, 3 and 6 months (mo) post-OVX. The bone mineral density (BMD) values and the percentage change from the baseline measurement were examined and compared between groups using independent samples t-test and one-way analysis of variance (Tukey test for multiple comparisons).

Results: BMD of the total tibia of the OVX animals exhibited a progressive decrease, which, compared to baseline, was –2.17, –6.34 and –12.14 % at the 1, 3 and 6 mo post-OVX measurements, respectively. OVX+PH animals had –0.48, 0.88 and –2.04 % changes compared to baseline, respectively. Control animals had 1.25, 1.47 and 1.44 % changes, respectively.

Comparison of the % changes at 1 mo post-OVX between groups showed no significant differences. At 3 mo, the difference between OVX and OVX+PH animals was highly significant ($p < 0.0005$), also between OVX and Controls ($p<0.0005$), whereas between OVX+PH and Controls there was no significant difference. At 6 mo, as previously, the difference between OVX and OVX+PH, as well as between OVX and Controls was highly significant ($p < 0.0005$), whereas between OVX+PH and Controls there was no significant difference.

BMD of the proximal tibia of the OVX animals exhibited a severer progressive decrease, which, compared to baseline, was –6.31, –14.11 and –27.65 % at the 1, 3 and 6 mo post-OVX measurements, respectively. OVX+PH animals had –3.89, –6.88 and –12.75 % changes, and Controls had –0.49, 4.79 and 9.26 % changes compared to baseline, respectively.

Comparison of the % changes at 1 mo post-OVX between groups showed no significant differences. At 3 mo, the difference between OVX and OVX+PH animals was non-significant, whereas between OVX and Controls the level of significance was $p=0.002$, and between OVX+PH and Controls it was borderline ($p=0.052$). At 6 mo, comparisons between all groups were statistically significant; i.e. OVX vs OVX+PH ($p=0.012$), OVX vs Controls ($p<0.0005$), and OVX+PH vs Controls ($p=0.002$).

Conclusions: The PH studied showed a highly significant protective effect on BMD of the total tibia of OVX rats after 3 and 6 mo of treatment, compared to the non-treated animals. The PH effect on BMD of the proximal tibia was less pronounced, but also statistically significant compared to the non-treated group, after 6 mo of treatment.

P-16

pQCT Study of Bone Development in Thoroughbred Horses up to 410 Days

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Aim of the Study: To describe the changes in bone content, area, mineral density and strength (SSI) parameters of the distal limb bones in relation to age and body weight in Thoroughbred foals up to 410 days of age.

Materials and methods: Seventeen Thoroughbred foals (8 colts & 9 fillies), born and raised on pasture, were scanned with a Stratec XCT2000 pQCT scanner approximately every 6-8 weeks from 4 days to 410 days of age. The radius, third metacarpal bone (Mc3) and proximal phalanx were scanned at respectively 25%, 45% and 50% of bone length from their distal extent. For any site or parameter, the coefficient of variation did not exceed 1.2%. The relationship of bone parameters to age and body weight were described by a piecewise polynomial equation. The effect of sex was examined using a General Linear Model, with foal age blocked into 40 day cohorts.

Results: For all parameters in all three sites the data best fitted a linear-linear regression model. The rate of parameter change in relation to age was greater before the inflection point than after. The ratio of slope coefficients ranged between 0.03-3.67 for periosteal circumference, to 17.80-50.38 for cortical density. The age at inflection varied with parameter and bone. Cortical bone density increased rapidly and plateaued at 927-1115 mg/cm3 before 110 days old, and subsequent increases in SSI were from increases in bone size.

The relationship of body weight and bone parameters was relatively homogeneous across sites, the inflection point occurring between 225.5 and 289 kg body weight.

Females had a significantly greater radius periosteal circumference and Mc3 cortical content, density, and area compared to males, indicating that the two bones had higher SSI in females than in males, but for different reasons.

Conclusion: pQCT has advanced the study of bone development in the young horse. Bones increase in size, strength and density very rapidly in the first 3-4 months, after which density increases little. The mechanism by which bone strength is affected by sex is mostly through greater circumference in the radius, and greater content, area and density in Mc3.

P-17

Accuracy and Precision Error of Muscle Cross-sectional Area Measured Using Peripheral Quantitative Computed Tomography in Adults

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Purpose: Using muscle area from standard spiral Computed Tomography (CT) as the gold standard this study assessed the accuracy of muscle cross-sectional area derived from pQCT images acquired at 0.4 mm

and 0.8 mm voxels. The precision error for muscle area derived at these two voxels was also assessed.

Materials and methods: Twenty subjects participated in this study. Two pQCT images of the leg were obtained at 66% of the distance from the medial maleolus to the medial condyle of the tibia using a STRATEC XCT2000. Image #1 was acquired with standard scan parameters (voxel=0.8 mm, scan speed=30 mm/sec). For image #2 the voxel and speed were lowered to 0.4 mm and 20 mm/sec. The spiral CT images were acquired on a General Electric scanner with 0.4 mm voxels and 2 mm thick slices. pQCT muscle cross-sectional area was derived using standard analysis parameters. To reduce noise, median filtering was applied to the 0.4 mm voxel image before analysis. Custom software was used to extract muscle area from the spiral CT images. To assess precision, 10 subjects had the 0.8 mm and 0.4 mm voxel pQCT images acquired on three different days within three weeks. Subjects were not repositioned between the scans.

Least squares analysis was used to compare pQCT and spiral CT muscle area. Precision error was assessed as percent coefficient of variation.

Results: There was good agreement between pQCT and spiral CT muscle cross-sectional area ($R2 > 0.9$, $p < 0.001$). As well, good agreement was found between muscle area derived from the 0.8 mm voxel pQCT image and the filtered 0.4 mm voxel image. The precision error for muscle cross-sectional area derived at either voxel setting was small (1-2%).

Conclusion: Muscle cross-sectional area derived from pQCT images is accurate when compared to muscle area from spiral CT. Second, filtering 0.4 mm voxel pQCT images to remove noise does not reduce the accuracy or precision of muscle measurements.

P-18

Metabolic Effects of Daily Supplementation of 1 G Elemental Calcium and 880 IU Vitamin D in Ambulatory Postmenopausal Women With Vitamin D Insufficiency.

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Aim: Calcium and vitamin D deficiencies are relatively common. Systematic vitamin D and calcium supplementations are therefore recommended.

Materials and methods: To prove their utility and innocuity, we, therefore, administered elemental calcium (1 g) and vitamin D (880 IU) daily for 2 months to 30 ambulatory postmenopausal women with lumbar spine osteopenia [T-score = - 1.5 (1.2)], aged 67.7 (6.4 SD), whose 25OHD level was lower than 75 nmol/L.

Results: Following therapy, 25OHD increased from 36.8 (17.5) to 73.0 (17.5) nmol/L, 50 % of patients reaching levels higher than 75 nmol/L. Serum calcium and creatinine clearance did not change significantly. iPTH decreased significantly from 34.1 (14.8) to 25.5 (8.7) pg/ml ($p < 0.01$). 24-H urinary calcium excretion increased from 173 (83) to 223 (93) mg and from 2.7 (1.2) to 3.5 (1.6) mg/kg, respectively ($p < 0.01$). No significant response of iPTH was observed for initial values of 25OHD higher than 65 nmol/L. 24-H urinary N-telopeptide (NTx)/creatinine and NTx/whole body BMC decreased non significantly. 13.3 % of patients increased their total serum calcium and 24-H urinary calcium/body weight over 10.4 mg/dl (up to 10.9 mg/dl) and 5 mg/kg (up to 6.6 mg/kg), respectively, and 20 % developed 24-H urinary calcium excretion superior to 300 mg (up to 444 mg). If we divided the patients in tertiles, according to the initial serum levels of 25OHD (9.3-25.0, 25.3-43.8, 44.0-75.0 nmol/L), significant decreases in iPTH were confined to the lower tertiles, and significant increases in 24-H urinary calcium restricted to the higher tertiles.

Conclusions: Calcium and vitamin D supplementations in elderly patients are able to induce a significant decrease in serum iPTH and a non-significant decrease in urinary NTX excretion, potentially favorable for bone strength. However, 24-H urinary calcium excretion should ideally be checked after 2 months in order to detect frank hypercalciuria which can occur in up to 20 % of patients. This could be of clinical significance, however, only in a minority of patients, particularly in those in which the positive metabolic effect on bone expected from vitamin D supplementation will be poor.

P-19

Proteoglycan Profiles in Chicken Gastrocnemius Tendons Change With Age and Exercise

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Tendon function depends on the proper organization of type I collagen fibrils in the tendon. Collagen fibrillogenesis is an essential process during embryonal development as well as during remodeling. The biochemical structure of the tendon adjusts to facilitate the required function. Fibrillogenesis, including the rate of formation and final sizes of the fibrils is regulated by proteoglycans (PGs). PGs consist of core proteins to which glycosaminoglycan (GAG) chains are attached. Though many PGs, for example, biglycan, aggrecan, fibromodulin and versican are present in the tendon, the extent of their involvement in tendon organization, and in collagen fibrillogenesis in particular is not well understood. Only the role of decorin, a small leucine-rich proteoglycan, has been described to some detail. Decorin, a major proteoglycan in the tendon, limits collagen fibril growth and thus directs tendon remodeling due to tensile forces. Mechanical tension induces the synthesis of decorin whereas the production of the large PG aggrecan is stimulated in a tendon subjected to compression. Such data underscore the importance of PGs in normal function of tendons and other connective tissues.

We hypothesized that rapid growth and moderate exercise induce changes in PG synthesis in the gastrocnemius tendon of young chickens. Using guanidine HCl and CsCl fractionation we have extracted PGs from gastrocnemius tendons of young chickens, some of which underwent moderate exercise. The presence of specific PGs and GAGs was analyzed with Sepharose CL-2B chromatography, PAGE, HPLC, and immunoblotting for GAGs and core proteins. KS was found in the lower halves of gastrocnemius tendons, however, exercise decreased KS level in 6.5 week old tendons. We have identified decorin in chicken gastrocnemius tendons at all ages studied. Changes in the content of several other GAGs were observed as well. Though chondroitin disaccharide-4-sulfate was identified as the major disaccharide GAG in control and exercised gastrocnemius tendons from 6.5-week-old chickens these tendons also showed differences in the content of other GAGs. Perhaps most significantly, the level of hyaluronic acid, not found in very young tendons, was increased in exercised tendons. Our results suggest that exercise may modify GAG composition in the tendon. It remains to be seen whether such changes would affect collagen fibril formation.

P-20

Differential Effect of Short-term Etidronate Treatment on Three Cancellous Bone Sites in Orchidectomized Adult Rats

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Aim of the study: To compare the effect of short-term etidronate treatment on three cancellous bone sites, the lumbar vertebral body (LBV), the proximal tibia (PT), and the distal tibia (DT), in orchidectomized adult rats.

Materials and methods: Thirty-five male Wistar rats, aged 10 months, were randomly divided into 4 groups: baseline controls (BLC, n=10), age-matched sham-operated controls (AMC, n=9), orchidectomy (ORX, n=9), and ORX + etidronate treatment (n=7). Etidronate treatment (10 mg/kg, daily subcutaneous injection) was initiated 2 weeks after surgery and was continued for 2 weeks. Four weeks after surgery, the 5th LBV, the PT, and the DT were processed for histomorphometry of cancellous bone.

Results: ORX resulted in a decrease in body weight. No significant differences in cancellous bone volume (BV/TV) were found between the BLC

and AMC groups in any skeletal sites. ORX resulted in cancellous BV/TV loss by 19.2 % in the 5th LBV, by 24.5 % in the PT, and by 39.3 % in the DT. The cancellous BV/TV loss was attributable to increased eroded surface (ES/BS) and osteoclast number (N.Oc/BS) with increased mineralizing surface (MS/BS) and no significant alterations in mineral apposition rate (MAR). Etidronate treatment for ORX rats significantly decreased ES/BS and N.Oc/BS to the levels that are not significantly different from the AMC group in all skeletal sites, resulting in a complete prevention of ORX-induced cancellous BV/TV loss. Etidronate treatment markedly decreased MAR in the 5th LVB and the PT, resulting in an increase in osteoid volume (OV/BV), but did not alter significantly either MAR or OV/BV in the DT.

Conclusions: The response of cancellous bone to ORX and short-term etidronate treatment appears to differ among the three skeletal sites. While the magnitude of ORX-induced cancellous bone loss was greater in the tibia than in the LVB and greater in the DT than in the PT, etidronate treatment could completely prevent ORX-induced cancellous bone loss in all skeletal sites by suppressing bone resorption. Skeletal mineralization was inhibited in the PT and the LBV with etidronate treatment but not in the DT. Factors such as mechanical loading and metaphyseal bone architecture may contribute to these site-specific responses of cancellous bone.

P-21

The Effect of Intermittent Administration of 200IU Intranasal Salmon Calcitonin on Bone Mineral Density and Markers of Bone Metabolism in Women With Postemenopausal Osteoporosis

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Aim of the study: We conducted a multicenter prospective open randomized controlled study in order to study the effect of intermittent administration of 200IU intranasal salmon calcitonin on lumbar spine and hip bone mineral density (BMD), and biochemical markers of bone turnover over a period of one year.

Materials and methods: A total of 102 postmenopausal women with osteoporosis (T- score < -2,5) and no history of severe or chronic diseases affecting bone metabolism, were randomly assigned to receive either 200IU nasal salmon calcitonin daily for alternative cycles of one month with one month interval, 500mg elemental calcium and 0,25μg 1α(OH)D3 continuously (n:57 women), or only 500mg calcium and 0.25 μg 1a (OH) D3 (n:45 women). Participants had no treatment interfering with bone turnover for at least 6 months before the beginning of the study. Bone mineral density of the lumbar spine, hip (Lunar DPX-L) and biochemical parameters reflecting calcium metabolism and bone turnover (Ca, P, PTH, total and bone specific alkaline phosphatase and osteocalcin serum levels, urinary Ca24h, Ca/creatinine, and D-pyrilinks/creatinine ratio), were performed at baseline, 6 and 12 months. Statistical analysis was performed using student's t-test for comparison between groups and paired t-test for comparison within groups.

Results: Baseline characteristics of participants including age, body mass index, lumbar and hip BMD, and biochemical markers, were similar among the two groups. Ninety one patients completed the study (50 in the salmon calcitonin nasal spray group and 41 in the other group).

Lumbar bone mineral density was increased significantly in the salmon calcitonin group from baseline (3.02%, p=0.005) and in comparison with the other group (p=0.009). The salmon calcitonin group also had a significant increase in femoral neck BMD compared with baseline values (3.07%, p=0.0005) and in comparison with the other group (p=0.0005), in Ward's triangle BMD (2.92% from baseline values: p=0.009 and in comparison with the other group: p=0.005) and in trochanter BMD (3.38% from baseline values:p=0.007 and in comparison with the other group:p=0.01). Bone specific alkaline phosphatase levels were decreased significantly from baseline in the salmon calcitonin treated group (-3.62% ,p=0.003). In the same group, there was also a significant decrease of PTH serum levels compared to baseline values (-2,52%,p=0.005) .Urinary Ca/creatinine and D-pyrilinks/creatinine levels were also decreased statistically significant from baseline in the salmon calcitonin treated group (-6.13% and –6.30% respectively, p=0.001).

Conclusions: Intermittent treatment with 200IU intranasal salmon calcitonin provides an adequate effect on bone turnover in postmenopausal women with osteoporosis and produce substantial increase of lumbar spine and hip mineral density.

P-22

The Correlation Between the Bone Mineral Density and the Intervertebral Disc Degeneration in Lumbar Spine

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Aim of the study: To evaluate the correlation between the bone mineral density (BMD) and the intervertebral disc degeneration in lumbar spine through the retrospective study.

Materials and methods: The BMDs and the magnetic resonance images (MRI) of the lumbar spine were assessed, from 61 postmenopausal females (age 56.0 ± 3.7, weight 58.9 ± 6.1 kg, height 154.9 ± 3.9 Cm), to evaluate the correlation between the BMD and the intervertebral disc degeneration in lumbar spine. The BMD of the lumbar spine was determined by using dual energy X-ray absorptiometry (DEXA). And the signal intensity of the intervertebral disc, the disc height and the degree of the disc herniation was determined at each lumbar disc level by using the MRI. The correlation between the BMD (mean value of 2nd, 3rd and 4th lumbar spine BMD) and the sum of grading scores of the intervertebral disc degeneration at all patients were assessed.

Results: The mean values of the BMD in lumbar spine body were obtained, (the BMD of L1 + L2)/2 = 0.832±0.135, (L2+L3)/2 = 0.905±0.149, (L3+L4)/2 = 0.956±0.148, (L2+L3+L4)/3 = 0.935±0.141. The mean values of the degeneration degree of the lumbar intervertebral discs were obtained, the L1,2 intervertebral disc =1.475±0.536, L2,3=2.131±0.536, L3,4=2.721±0.521, L4,5=3.279±1.495. These data were analyzed by regression analysis system. There was the positive correlation between the BMD of lumbar spine and the sum of grading scores of the intervertebral disc degeneration (r = 0.415, p value = 0.00087).

Conclusion: The bone mineral density (BMD) has the inverse correlation to the intervertebral disc degeneration and that is the important factor when considering degenerative spinal disease and osteoporosis.

P-23

Molecular Pathways Implicated in Glucocorticoid-induced Osteocyte Apoptosis

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Glucocorticoids lead to decreased bone turnover and osteonecrosis, through various effects on osteoblasts and osteoclasts. At the same time, chronic exposure to glucocorticoids has been shown to affect the viability of osteocytes by increasing their susceptibility to apoptosis (Weinstein, R. et al., 1998). The aim of this study is to investigate the molecular pathways implicated in Dexamethasone-induced apoptosis of osteocytes.

MLO-Y4 osteocyte cells where challenged with dexamethasone (10-6 M) in the presence or absence of a 1hr pre-treatment with a range of bisphosphonate molecules. Apoptotic osteocytes were evidenced at various times after treatment by acridine orange and Nick Translation staining and Annexin-V FITC binding. Molecular pathways were investigated using Western Blot analysis, immunohistochemical techniques and specific inhibitor molecules.

Our results suggest that dexamethasone induces apoptosis through activation of the Fas/CD95 pathway, in a caspase dependent manner. The apoptotic response can be prevented by both N- and non N-containing bisphosphonates as well as by inhibitors of ERK ½ and PKA pathways. Results were confirmed using primary osteocyte cultures. Dex induced apoptosis was associated with a transient increase in phosphorylated ERK

½, followed by an increase in phoshorylated p90rsk. Furthermore, inhibition of Dex-induced apoptosis by the ERK ½ inhibitor, UO126, was associated with decreased Fas activity, suggesting cooperation between the two pathways in the induction of apoptosis. Identification of Dexamethasone activity at the molecular level opens up exciting possibilities for pharmaceutical intervention during age and glucocorticoid hormone related osteocyte loss.

P-24

Densitometric Muscle/Bone Mass Relationships in Dialysed Men and Pre- and Post-Menopausal Women

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We had shown that the DXA-assessed whole-body mineral content (BMC, either crude or adjusted to a common fat mass (FA-BMC) in order to compensate for any interaction of fat in its determination) was linearly correlated to lean mass (LM), showing the same slopes but different intercepts in decreasing order for pre-MP women > men > post-MP women > boys and girls. Being LM linear to muscle mass, this would indicate that 1. bone mechanostat would keep that proportionality homogeneously in the species, but 2. this control is normally affected by nonmechanical factors as sex hormones. This study aims to compare the whole-body BMC or FA-BMC and LM in stable, chronic, peritoneally-dialised (CAPD) and hemodialysed (HD) men (n= 37, age 51.5 ± 12.5 yr) and pre- (24, 36.2 ± 10.6 yr) and post-MP women (47, 55.0 ± 10.8 yr), in which a different metabolic interference with the biomechanical control of bone mass can be proposed to further affect the bone-muscle relationships. Also 600 sex- and age-matched controls were studied.

The dialised patients had a lower BMC or FA-BMC than their corresponding controls. The linear BMC (y) vs LM (x) correlations showed that both CAPD and HD patients plotted indistinctly lower than controls. Curves for men and pre-MP women were similar in slope but showed significantly lower intercepts than controls. Distinctly, post-MP women tended significantly to have lower BMC or FA-BMC per unit of LM than controls in proportion with the reduction observed in LM, reaching very low BMC or FA-BMC values in the extreme cases.

Results suggest that 1. dialysed men and pre-MP women show a normal control of bone mass by muscle mass; 2. however, the metabolic interference from CAPD/HD reduces the BMC/LM proportionality regardless of the patient's fat mass, and 3. this situation is further affected by menopause, after which the BMC control seems to be impaired in proportion with the reduction in mechanical usage (muscle mass reduction). This would confirm that dialysis-induced systemic factors affected the bone mechanostat setpoint over the natural endocrine influences in these patients.

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Effects of Exercise on Bone Labeling at Various Joint Sites

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Aim of the study: The metacarpophalangeal (fetlock) joint of horses is subjected to high stress and prone to overload arthrosis. This study was to look at the effects of exercise on fluochrome labeling of different bone sites in this joint.

Materials and methods: Two yr old horses were run on a treadmill 5d/wk for 6 months. Controls were hand walked. Double labels (10 d apart) of calcein (C) were administered at 3 months and of oxytetracycline (Ox) labels at 6 months. Sagittal sections (120u thick) were used to evaluate labeling in: the subchondral bone of the 3rd metacarpal palmar condyle (Mc3), of the proximal sesamoid bone (PSB), and of the proximal 1st phalanx (P1). In addition, bone at the margin of the P1 articular surface, and a ligament insertion site were studied.

Results: The amount of subchondral bone (B.Ar/T.Ar,%) was consistently higher in the exercise group (significant in MC and PSB sites). Mean values for Ox labeled perimeter (sL+dL.Pm/B.Pm,%) were higher in the exercise group than in controls but the proportion of single and double labels varied with site. At the joint margin and ligament insertion site, the increase due to exercise was somewhat greater.

When the 3 month label (C) was expressed as total length/tissue area and compared to the 6 month label (Ox) there was approximately twice as much label at 3 months in both groups and mean values in the exercise group were higher at all but one site.

Conclusions: 1) The amount of bone formation/labeling is increased with exercise at various sites in the joint. 2) In subchondral bone, the proportion of single and double label varies with site. 3) There was greater labeling at 3 months than at 6 months in these 2 yr old horses. 4) There was a greater increase in labeling due to exercise than there was in final amount of bone. 5) There appeared to be greater labeling at the joint margins and ligament insertion than in subchondral bone.

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Simvastatin Given Twice Daily Increases Cortical Bone Formation and Strength in ovx Rats

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Some of the statins possess bone anabolic properties. Statin increased expression of the BMP-2 gene in osteoblasts resulting in increased cancellous bone volume and a minor decrease in osteoclast number of 3-month-old rats (Mundy et al., Science 286, 1946-1949, 1999). Simvastatin increased the vertebral cancellous bone mass and compressive strength of one-year-old female rats (Oxlund et al., Calcif Tissue Int 69:299-304, 2001). In the present study the effects of statin on tibia cortical bone of OVX (ovariectomized) rats were studied. Sixty Wistar female rats, 4 months old, were allocated to 4 groups: 1. Baseline control, 2. Sham + placebo group, 3. OVX + placebo, 4. OVX + simvastatin. Simvastatin MSD (20 mg/kg) or placebo were given twice daily at 9 a.m. and at 3 p.m. by a gastric tube for 3 months. The rats were injected with tetracycline i.p. at day 11 and calcein i.p. at day 4 before sacrifice of the 7 months old rats. The mechanical properties of the tibia diaphysis were studied by a 3-point-bending test. The breaking strength of the OVX + statin group (111.2 ± 2.1 newton, mean ± SEM) was increased ($2p<0.02$) compared with the OVX group (102.4 ± 2.8 newton), and increased ($2p<0.001$) compared with the sham + placebo group (97.5 ± 2.2 newton). Cross-sections were cut and mineral appositional rates (MAR) at the different aspects and in different regions of the tibia were calculated from the fluorescent labels. In the upper part of the tibia diaphysis for example, the endosteal MAR of the OVX + statin group (1.7 ± 0.1 μm/day) was increased ($2p<0.001$) compared with the OVX group (1.0 ± 0.1 μm/day), and increased ($2p<0.0001$) compared with the sham + placebo group (0.4 ± 0.1 μm/day). In conclusion, simvastatin given perorally twice daily increased the bending strength of the tibia diaphysis of OVX rats. The mineral appositional rates were increased both at the endosteum and periosteum of OVX rats given simvastatin. The new cortical bone exhibited a normal lamellar structure. Simvastatin increased bone formation, but seems to respect the regional pattern of bone resorption, formation and drift.

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Biochemical Processes in the Cortical Bone During the Stress/Strain Changes

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Aim of the study: The bone tissue remodeling is controlled in a dominant way by biomechanical remodeling initiators (i.e. by spherical strain tensors) and by biomechanical speed regulators of metabolic remodeling processes (i.e. by deviators of the stress/strain tensors).

The bone tissue remodeling is relatively a very slow process. In physiologically "normal" conditions, it tends towards the state of remodeling equilibrium in which the coincidence of the first dominant principal stress/strain direction, and the direction of the first principal axis of anisotropy, and the principal directions of the structure (longitudinal axes of osteons) is reached at the point of macrostructure. The presented theory is concerned to the exact formulations of the mechanical (i.e. stress/strain fields) effects on the biochemical metabolic processes.

Materials and methods: On the basis of the up-to-now recognized and available knowledge of biochemical processes related to the creation of a new bone, the kinetics of chemical substances (molecular mixtures) can be expressed by five global stoichiometric equations of bone remodelling.

In regard to the stoichiometric equations, it is necessary to point out that they express global metabolic processes which are initiated by mechanical loading effects in the cases of dominant biomechanical activities. The biochemical reactions related to the cortical bone remodeling and expressed by stoichiometric equations proceed at certain speeds that depend on the speed constants and on the concentrations of individual substrates D_i.

Results: The fundamental kinetic equations of bone remodeling present the relations between the time change and the concentrations of molar substances. The speed remodeling constants with speed remodeling functions depend on the mechanical stress, resp. on the spheric stress tenzor.

Conclusions: The biochemical reactions are iniciated by the deviator of the stress/strain tensor. Shear stresses (the components of stress tensor deviator) deform the microelements of bone tissue and the flow of extracellular liquid mechanochemically (with the direct activity of integrins a, b) starting the production of prostaglandin E_2 which iniciates bone compartment resorption (Klein-Nulend, E.H.Burger).

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Osteoporotic Vertebral Fractures in the Elderly: Does the Anterior Vertebral Body Lose Strength Following Stress Shielding by the Neural Arch?

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Introduction: Osteoporotic vertebral fractures are normally attributed to systemic bone loss caused by age-related hormonal changes and reduced physical activity. However, regional vertebral bone mass and density will also depend on the manner in which the intervertebral disc presses on the vertebral body, and on load-bearing by the neural arch. We hypothesise that age-related degeneration of intervertebral discs increases neural arch compressive load-bearing, and influences the distribution of compressive load on the vertebral body, causing anterior vertebral body bone loss and weakening in the elderly spine.

Materials and methods: Fifteen cadaveric motion segments (aged 72-92 yrs), comprising 2 adjacent vertebral bodies and the intervening disc and ligaments, were compressed to 1.5kN while positioned to simulate erect standing posture and a simulated forward stooped posture. The distribution of intradiscal stress, measured by pulling a miniature pressure transducer along the mid-sagittal diameter of the disc, was integrated over area to give the force acting on the anterior and posterior halves of the vertebral body (1). These were subtracted from the 1.5kN to determine the force on the neural arch. Compressive strength of each motion segment was measured in the stooped posture. Bone mineral density (BMD) of the anterior and whole vertebral body was measured by dual energy x-ray absorptiometry.

Results: In erect posture, the neural arch resisted 48% (STD 26%) of the applied 1.5kN, while the anterior vertebral body resisted only 15% (STD 19%). However, in the stooped posture these values changed to 14% (STD 7%) and 57% (STD 22%) respectively. Compressive strength in flexion correlated negatively with neural arch load-bearing in erect posture ($r2=0.51$, $p=0.006$). Compressive strength correlated with whole vertebral body BMD ($r2 = 0.55$, $p< 0.005$), but was more strongly related to anterior vertebral body BMD ($r2 = 0.72$, $p < 0.001$).

Discussion: Age-related degenerative changes cause intervertebral discs to lose height, increasing compressive load-bearing by the neural arch in erect postures. This unloads the anterior half of the disc, reducing the compressive force on the anterior vertebral body. Habitual unloading causes bone loss that will be pronounced in the anterior vertebral body, making it vulnerable to fracture in flexion, when loading is more concentrated in this region.

1. Pollintine P et al. (2001). The load-bearing function of apophyseal joints increases with age and disc degeneration. Transactions of the Orthopaedic Research Society. San Francisco, USA.

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Musculoskeletal Interactions in Obese, Hyperinsulinemic, Euglycemic Patients

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This study aimed to determine whether the metabolic disturbance caused by obesity-related euglycemic hyperinsulinemia impairs or not the anthropometric and biomechanical relationships between bones and muscles as determined by the bone mechanostat. We have analyzed the relationships between the DEXA-assessed, whole-body BMC and lean and fat masses (LM, FM) in 30 men and 110 pre- and post-MP women with euglycemic hyperinsulinemia classified according to their fasting plasma insulin (FPI) levels lower (i) or higher (I) than 15 uUI/ml, body-mass index values lower (b) or higher (B) than 30, and Insulin Secretion Index (ISI; Matsuda & DeFronzo 1999) values lower (s) or higher (S) than 2.8.

The FPI correlated positively with LM and FM but not with the BMC. Correlations between BMC and LM were parallel to those of some 300 normal age-controls for each group but showed lower intercepts for groups I, B, and S than i, b, and s. After adjusting logarithmically the BMC to a common, 18-kg FM value the groups I, B, and S showed significantly lesser intercepts than controls. Those differences were generally more significant for pre-MP women than for post-MP women or men.

Despite of not affecting plasma glucose, the high FPI condition in these obese patients would have enhanced FM. Assuming that LM reflects muscle mass, they would have also lowered the BMC/muscle proportion, perhaps reducing the mechanical influence of muscles on the skeleton. This would evidence the induction of a shift in the mechanostat setpoint (a typical, ~0.2% bone tissue strain under the maximal customary loads). In addition, the apparent estrogen-dependence of the differences is congruent with the hypothesis that estrogens interact positively with that homeostatic system.

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Symmetry of the Mechanical Properties of the Long Bones: Tomographic (pQCT) and Biomechanical Study in Rats

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Aim of the study: The purpose of the study was to evaluate the symmetry of the properties of the long bones of the rat.

Materials and methods: Fifteen 6 month-old Wistar rats were used. Following euthanasia both femurs and tibiae were removed and cleaned from all soft tissues and the periosteum. All bones were subjected to peripheral Quantitative Computed Tomography (pQCT) at the midshaft of the diaphysis. The parameters that have been assessed were the Cross-sectional moment of inertia (CSMI) and volumetric cortical bone mineral density (vCtBMD). Bone strength index (BSI), the product between CSMI and vCtBMD, was also calculated. The mechanical properties were evaluated using the three point bending test, measuring the load to failure. The paired t-test was used for statistical evaluation.

Results: No statistically significant difference was demonstrated between

the similar bones of the same animal, although the differences between different animals were significant. This applies to the densitometric as well as to the biomechanical properties.

Conclusions: In healthy animals there is no significant divergence of the mechanical and the tomographic properties between the similar long bones of the posterior limbs. The contralateral long bone, femur or tibia, may serve as a control of the similar bone of the same animal.

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The Effect of Venous Stasis on Experimental Fracture Healing: Densitometric, Biomechanical and Histomorphometric Evaluation

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Aim of the study: The purpose of the study was to evaluate the effects of chronic venous stasis on fracture healing using the experimental the model of the rat tibial fracture, employing a variety of techniques.

Materials and methods: Sixty Wistar rats were randomly assigned to four groups, A, B, C and D, consisting each of 15 rats. Groups A and C were subjected to common femoral vein ligation and tibial osteotomy which was stabilized with an 0.8 mm intramedullary nail and Groups B and D were subjected only to tibial osteotomy and nailing. Fracture healing was evaluated 21 and 60 days post-osteotomy using DEXA imaging, to study callus mineralization, and biomechanical testing (three point bending). Additionally 60 rats were randomly assigned to six groups I-VI for the purpose of histomorphometric evaluation. Groups I, II, III were subjected to venous ligation and osteotomy, whereas Groups IV, V, VI were subjected only to osteotomy. The process of fracture healing was studied using decalcified coronal histological sections 10 days (Groups I, IV), 21 days (Groups II, V) and 60 days (Groups III, VI) post-osteotomy. Histomorphometric evaluation of the sections was performed, measuring the quantities of cartilaginous, fibrous and bony tissue.

Results: Histologic study showed that the process of fracture healing in the venous stasis groups was significantly advanced compared to the control groups in the early period of fracture healing, after 10 and 21 days, but there was no difference in the advanced stage of healing (60 days). The area of non-osseous (fibrous tissue and cartilage) callus of the fracture was significantly smaller in the stasis group. By 60 days, there was almost only bony callus between the fracture ends in both the control group and the study group.

Callus bone mineral content (BMC) and bone mineral density were enhanced in the study group by 21 and 60 days. Biomechanically, the ultimate load in the stasis group was significantly higher than in the control group especially in the late phase of healing.

Conclusions: In conclusion, this animal study indicates that chronic venous stasis facilitates early fracture healing, improving callus maturation and increasing the fracture callus biomechanical properties.