Annual Meeting 2002

Venue

The meeting will take place at City Hall, Cardiff, UK.

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CME

The meeting is approved for 18 CME credits for full attendance.

Local Organising Committee

Bronwen Evans (chair), Wil Evans, John Gregory, Jim Martin,
Joanne Plummer, Mike Stone, Rachel Waddington

Meeting Organiser

For further information please contact our Meeting Organiser:
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Next year's Bone and Tooth Society Meeting

9-11 July 2003, Sheffield, UK
Organisers: Dr Aubrey Blumsohn, Dr Nicky Peel

www.batsoc.org.uk

Bone and Tooth Society

The Society is the oldest and largest scientific society in Europe that is dedicated to further research into clinical and basic science problems related to mineralised tissues. The meeting attracts a wide audience from throughout the UK and, increasingly, from continental Europe and further afield. The presentations are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged.

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Programme

Monday 24 June 2002

11.30 Registration and Lunch

13.00 OFFICIAL OPENING

Ms Jane Hutt, Health Minister for Wales

Professor Stephen Tomlinson, Vice-Chancellor, University of Wales College of Medicine

13.15 **ORAL COMMUNICATIONS 1: SEX HORMONES AND BONE**

Chairman: Stavros Manolagas

13.15 OC1 OESTROGEN-INDUCED CANCELLOUS BONE FORMATION IN MICE IS MEDIATED BY OESTROGEN RECEPTOR ALPHA
K E McDougall, M J Perry, R L Gibson, S M Colley, J H Tobias

13.27 OC2 ESTROGEN STIMULATES MEGAKARYOCYTE DIFFERENTIATION AND ESTROGEN RECEPTOR PROTEIN AND MRNA EXPRESSION
S Bord, E Frith, D C Ireland, M A Scott, J I O Craig, J E Compston

13.39 OC3 ISOLATION OF MET, A NOVEL MODULATOR OF OESTROGEN INDUCED TRANSCRIPTION
S M Colley, A Flynn, M Norman, D Wynick, J T Tobias

13.51 OC4 SERUM OSTEOPROTEGERIN IS UNRESPONSIVE TO POSTMENOPAUSAL OESTRADIOL REPLACEMENT
A Rogers, C Pereda, K Naylor, R Eastell, A Blumsohn

14.03 OC5 A COMPARISON OF THE BONE MINERAL DENSITY (BMD) BETWEEN LUTEINISING HORMONE RECEPTOR KNOCKOUT (LURKO) MICE WITH HUMAN CHORIONIC GONADOTROPIN (HCG) OVEREXPRESSING MICE
S J Yarram, S Rulli, F P Zhang, I Huhtaniemi, M J Perry, J R Sandy, J P Mansell

14.15 **SYMPOSIUM: COLLAGEN DISORDERS**

Chairmen: Nigel Loveridge/Marian Young

14.15 IS1 THE COLLAGEN FAMILY AND ASSOCIATED DISORDERS
Vic Duance, Cardiff

14.40 IS2 INHERITED DEFECTS OF COLLAGEN
Mike Pope, London

15.05 IS3 CLINICAL ASPECTS OF COLLAGEN DISORDERS
Nick Bishop, Sheffield

15.30 TEA AND POSTERS (odd-numbered boards attended)

16.45 **PLENARY LECTURE**

Chairman: Juliet Compston

IS4 ACTIVATION OF NONGENOTROPIC ESTROGEN-LIKE SIGNALING (ANGELS): A NOVEL APPROACH TO BONE ANABOLISM AND GENDER-NEUTRAL THERAPY OF OSTEOPOROSIS
Stavros C Manolagas
Little Rock AR, USA

19.00 CIVIC RECEPTION – CITY HALL

Tuesday 25 June

08.30 **ORAL COMMUNICATIONS 2: BONE STRENGTH: CAUSES AND CONSEQUENCES**

Chairman: Tim Chambers

08.30 OC6 DISC DEGENERATION INFLUENCES THE DISTRIBUTION OF LOAD ON THE VERTEBRAL BODY: A CAUSE OF OSTEOPOROTIC VERTEBRAL FRACTURES IN THE ELDERLY
P Pollintine, P Dolan, J H Tobias, M A Adams

08.42 OC7 MECHANICAL INFLUENCES ON SKELETAL DEVELOPMENT IN UTERO
T M Skerry, N M Peet

08.54 OC8 INFLUENCE OF GROWTH RATE ON CORTICAL BONE POROSITY, AND STRENGTH IN THE IMMATURE SKELETON
D H Murray, N Loveridge, B G Williams, D Waddington, C Farquharson

09.06 OC9 REMODELLING CLUSTERS AND REDUCTION IN BENDING LOADS
N Loveridge, J Power, A Lyon, J Reeve, A Goodship

09.18 OC10 PLACE OF RESIDENCE AND RISK OF FRACTURE IN OLDER PEOPLE: A POPULATION BASED STUDY OF PEOPLE AGED OVER 65 LIVING IN CARDIFF
J Saunders, A Johansen, J Butler, M Stone, S Jones, R A Lyons

09:30 **PLENARY LECTURE**

Chairman: Tim Skerry

IS5 RECENT DEVELOPMENTS IN QUANTITATIVE ULTRASOUND
Claus Glüer
Kiel, Germany

10.30 Coffee

11.00 **CLINICAL CASE PRESENTATIONS**

Chairmen: Roger Smith/Mike Stone

11.00 C1 ZOLEDRONATE IN THE MANAGEMENT OF ACTIVE PAGET'S DISEASE
G Chung, R W Keen

11.15 C2 GOSERELIN AND SEVERE SYMPTOMATIC OSTEOPOROSIS
C R Paterson, P A Mole

11.30 C3 MANAGEMENT OF REGIONAL MIGRATORY OSTEOPORORIS
K Moss, J Angel, R W Keen

11.45 C4 MISSING LOOPS - A CASE OF TERTIARY HYPERPARATHYROIDISM
D O'Gradaigh, J E Compston

12.00 **CLINICAL SYMPOSIUM: CURRENT VERSUS FUTURE THERAPIES: WHERE ARE WE NOW?**

Chairmen: Richard Eastell/Stuart Ralston

12.00 IS6 CURRENT THERAPIES FOR OSTEOPOROSIS
Juliet Compston, Cambridge

12.20 IS7 FUTURE THERAPIES
Socrates Papapoulos, Leiden, The Netherlands

12.45 General Discussion

13.00 Lunch

14.00 **SYMPOSIUM: MODELS FOR STUDYING SKELETAL FUNCTION**

Chairmen: Agi Grigoriadis/Gerard Karsenty

14.00 IS8 TRANSGENIC MODELLING IN THE MOUSE
Alan Clarke, Cardiff

14.25 IS9 SMALL LEUCINE RICH PROTEOGLYCANS: ESSENTIAL FOR THE STRUCTURE AND FUNCTION OF MINERALIZED TISSUE
Marian Young, Bethesda MD, USA

14.50 IS10 FLUOROSIS AS A MODEL FOR THE STUDY OF EXTRACELLULAR MATRIX COMPONENTS IN MINERALISED TISSUE FORMATION
Graham Embery, Liverpool

15.15 TEA AND POSTERS (even-numbered boards attended)

16.30 **ORAL COMMUNICATIONS 3: CELL SIGNALLING AND TISSUE ENGINEERING**

Chairman: Jon Tobias

16.30 OC11 NEPHRONECTIN EXPRESSION IS UPREGULATED DURING CHONDROGENESIS IN ATDC5 CELLS
B Houston, E Seawright, C Farquharson

16.42 OC12 THE ROLE OF THE ANTI-ANGIOGENIC FACTOR CHONDROMODULIN-I IN SLOWING DOWN THE RATE OF VASCULAR INVASION AT THE GROWTH PLATE
H I Roach, C Shukunami, Y Hiraki

16.54 OC13 INDUCTION OF BONE FORMATION IN VIVO USING HUMAN OSTEOPROGENITOR AND OSTEOBLAST STIMULATING FACTOR-1 ADSORBED SCAFFOLD CONSTRUCTS
X B Yang, H I Roach, N M P Clarke, S M Howdle, K M Shakesheff, R O C Oreffo

17.06 OC14 FUNCTIONAL CHARACTERISATION OF ISOFORM-SPECIFIC ACETYLCHOLINESTERASES IN BONE.
C A Inkson, T Evron, H Soreq, P Genever

17.18 OC15 GLUTAMATE-DEPENDENT REGULATION OF MEGAKARYOCYTE DIFFERENTIATION THROUGH NMDA RECEPTOR-MEDIATED SIGNALLING CASCADES
I S Hitchcock, M Howard, P G Genever

19.30 DINNER – MILLENNIUM STADIUM

Wednesday 26 June

08.30 **ORAL COMMUNICATIONS 4: OSTEOBLASTS AND BONE FORMATION**

Chairman: Graham Embery

08.30 OC16 ADENOVIRAL BMP-2 GENE TRANSFER IN MESENCHYMAL STEM CELLS - IN VITRO AND IN VIVO BONE FORMATION ON BIODEGRADABLE POLYMER SCAFFOLDS
K A Partridge, X Yang, N M P Clarke, Y Okubo, K Bessho, S M Howdle, K M Shakesheff, R O C Oreffo

08.42 OC17 IDENTIFICATION AND CHARACTERISATION OF PRESENILINS IN OSTEOBLASTS AND THEIR ROLE IN THE CANONICAL WNT SIGNALLING PATHWAY
G J Spencer, E F Shead, M Porter, T S Grewal, P G Genever

08.54 OC18 AMPA TYPE GLUTAMATE RECEPTORS REGULATE OSTEOBLAST/ ADIPOCYTE PLASTICITY AND BONE FORMATION
A F Taylor, S O Odoi, C J Nokes, T M Skerry

09.06 OC19 IDENTIFICATION OF CBFA1-EXPRESSING OSTEOPROGENITOR CELLS BY FLOW CYTOMETRY
K Stewart, K E McDougall, T Whitworth, J N Beresford, J H Tobias, M J Perry

09.18 OC20 EFFECTS OF LEPTIN ON NUMBER AND APOPTOSIS OF OSTEOBLASTS, AND CONTROL OF LEPTIN PRODUCTION BY GLUCOCORTICOIDS
N A Perez, C Elford, J W Gregory, B A J Evans

09.30 **PLENARY LECTURE**

Chairman: Bronwen Evans

IS11 THE CONTROL OF BONE MASS AND REMODELLING BY LEPTIN
Gerard Karsenty
Houston TX, USA

10.30 Coffee

11.00 **POSTER DISCUSSION SESSION**

Chairmen: Agi Grigoriadis/Jon Tobias

11:00 P1 FROM RECEPTOR ACTIVATION TO GENE TRASCRIPTION: FUNCTIONAL WNT SIGNALLING IN OSTEOBLASTS
G J Spencer, S L Etheridge, P G Genever

11:05 P2 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 ACTIVITY DETERMINES THE EFFECTS OF GLUCOCORTICOIDS ON BONE
M S Cooper, A Blumsohn, P E Goddard, W A Bartlett, R Eastell, M Hewison, P M Stewart

11:10 P3 OSTEOBLAST-STIMULATING FACTOR-1 ENHANCES OSTEOGENIC DIFFERENTIATION OF BONE MARROW STROMAL CELLS, BUT IS NOT OSTEOINDUCTIVE
R S Tare, K A Partridge, X B Yang, N M P Clarke, R O C Oreffo, H I Roach

11:15 P4 ESTIMATES OF NET ENDOGENOUS ACID PRODUCTION (NEAP) ARE ASSOCIATED WITH INCREASED BONE TURNOVER IN EARLY POSTMENOPAUSAL WOMEN: FINDINGS FROM APOSS LONGITUDINAL STUDY
H M Macdonald, S A New, W D Fraser, D M Reid

11:20 P5 PATTERNS OF EXPRESSION OF CHEMOKINES AND THEIR RECEPTORS IN OSTEOCLASTS
J M Lean, C Murphy, K Fuller, T J Chambers

11:25 P6 INTERACTIONS BETWEEN ESTROGEN AND GLUTAMATE SIGNALLING PATHWAYS
S O Odoi, A F Taylor, K Lee, L E Lanyon, T M Skerry

11:30 P7 THE OESTROGEN RECEPTOR ACTIVATES BMP-6 IN A NON-LIGAND-DEPENDENT MANNER
S M Colley, D B Ong, S Kitazawa, M Norman, D Wynick, J T Tobias

11:35 P8 EFFECT OF OESTROGEN ON BONE TURNOVER IN CORTICAL BONE IN POSTMENOPAUSAL WOMEN
S Vedi, D W Purdie, N J Garrahan, J E Compston

11:40 P9 SPECIFIC IMMUNOLOCALISATION OF A NOVEL PHOSPHATASE TO OSTEOBLASTS AND MINERALISING GROWTH PLATE CHONDROCYTES OF IMMATURE LONG BONES
C Farquharson, E Seawright, B Houston

11:45 P10 LONGITUDINAL CHANGES IN BONE MINERAL DENSITY AND TURNOVER IN NORMAL PREGNANCY
D Hosking, M Kaur, D Pearson, I Godber, N Lawson, P Baker

11:50 P11 3D MICRO-COMPUTED TOMOGRAPHY (MICRO-CT) OF TRABECULAR BONE FROM GROWTH HORMONE DEFICIENT TRANSGENIC RATS
B A J Evans, A Laib, J T Warner, C Elford, S L Evans, J W Gregory, T Wells

11:55 P12 OSTEONAL MATERIAL PROPERTIES IN THE FRACTURED FEMORAL NECK
E L Follon, N Loveridge, D Stokes, W Bonfield

12.00 **SYMPOSIUM: GROWTH HORMONE AND BONE**

Chairmen: John Gregory/Peter Selby

12.00 IS12 GROWTH HORMONE - PHYSIOLOGICAL ASPECTS AND SIGNALLING MECHANISMS
Peter Clayton, Manchester

12.20 IS13 BONE MARROW ADIPOCYTES: A NEGLECTED TARGET TISSUE FOR GROWTH HORMONE ACTION
Evelien Gevers, London

12.40 IS14 GROWTH HORMONE MODULATION OF BONE METABOLISM AND MINERALISATION- CLINICAL IMPLICATIONS IN HYPOPITUITARISM
Paul Carroll, London

13.00 Lunch

14.00 **ORAL COMMUNICATIONS 5: OSTEOCLAST GENERATION IN HEALTH AND DISEASE**

Chairman: Alan Boyde

14.00 OC21 ZOLEDRONIC ACID PREVENTS THE DEVELOPMENT OF MYELOMA BONE DISEASE AND INCREASES SURVIVAL
C M Shipman, K Vanderkerken, H De Raeve, M Perry, A Hijzen, J Lippitt, J Green, E Van Marck, B Van Camp, P I Croucher

14.12 OC22 EFFECT OF PAMIDRONATE ON PERIPROSTHETIC BONE TURNOVER AND IMPLANT STABILITY AFTER TOTAL HIP ARTHOPLASTY
J M Wilkinson, I Stockley, A J Hamer, R Eastell

14.24 OC23 RANKL-INDEPENDENT INDUCTION OF HUMAN OSTEOCLAST FORMATION AND BONE RESORPTION BY TGF-BETA
I Itonaga, A Sabokbar, O Kudo, L Danks, N A Athanasou

14.36 OC24 POSSIBLE ROLE FOR TGF-BETA-INDUCED SOCS EXPRESSION IN OSTEOCLAST/MACROPHAGE LINEAGE COMMITMENT IN-VITRO
S W Fox, T J Chambers

14.48 OC25 MULTILINEAGE POTENTIAL OF CD34-NEGATIVE UMBILICAL CORD BLOOD CELLS
S J Murant, E L Hutson, C Allen, I S Hitchcock, P G Genever

15.00 Bone and Tooth Society AGM

15.45 Prize giving

16.00 Close of Meeting

IS1

THE COLLAGEN FAMILY AND ASSOCIATED DISORDERS

V Duance, Connective Tissue Biology Research Group, Cardiff School of Biosciences, Cardiff CF10 3US, UK

There are 21 known collagen types coded for by at least 34 different genes. Collagens are expressed in all tissues in the body and therefore it is not surprising that mutations in these genes give rise to diseases affecting many different tissues. To date mutations have been identified in 12 of the 21 collagen types.

Collagen biosynthesis is a complex process with many post-translational steps necessary for the assembly of a fully functional protein. Through the extensive studies on osteogenesis imperfecta, some of the factors that influence the severity of the collagenopathies are known although for most diseases a good genotype/phenotype relationship has yet to be established. In some cases very different diseases can arise through mutations in the same gene, for instance osteogenesis imperfecta and Ehlers Danlos Syndrome (Type VIIA and Type VIIB) both arise from mutations in the COL1A1 and COL1A2 genes. In contrast, because of the complexity of the extracellular matrix and the importance of specific interactions on matrix integrity, diseases with very similar pathologies can arise through mutations in genes coding for different matrix components. For instance, multiple epiphyseal dysplasia (MED) can be caused by mutations in genes coding for type IX collagen, cartilage oligomeric matrix protein (COMP) or matrilin 3.

Studies on such connective tissue diseases are providing new insights into the complexity of the extracellular matrix and providing valuable information for our understanding of the mechanisms of these disorders.

IS2

INHERITED DEFECTS OF COLLAGEN

F M Pope, Division of Life Sciences, Franklin Wilkins Building, Stamford Street, London & West Middlesex University Hospital, Twickenham Road, Isleworth, Middlesex TW7 6AF

Inherited defects of connective tissue are a diverse disease group caused by a variety of collagen mutations, as well as other extracellular matrix proteins. There are 20 collagen proteins, coded by more than 30 genes, of which type O collagen mutations cause ligamentous and bone disease. Collage type II mutants predominantly affect cartilage and/or vitreous, whilst type III collagen defects cause arterial fragility. Type V collagen mutants affect skin, vitreous and cornea and can coassociate with collagen types II and XI.

These various errors are mostly private, with only patchy clustering and over 200 type I mutants are recognised. At present diagnosis is slow and very labour intensive. Faster identification, both at the gene and protein level, should in future lead to more accurate and faster genetic counselling and prevention.

IS3

CLINICAL ASPECTS OF COLLAGEN DISORDERS

N J Bishop, Professor of Paediatric Bone Disease, Academic Unit of Child Health, University of Sheffield

19 types of collagen are listed on the OMIM website. Of these 13 are listed as having disorders associated with mutations in the genes encoding their proteins. The spectrum of human disorders associated with collagen mutations is broad, encompassing diseases of bone, cartilage, muscle, skin, blood vessels, renal tissue, the retina, the anterior chamber of the eye, and neural tube closure. For the majority of the disorders the only available therapy is supportive and symptomatic. For disorders of Type I collagen however the last decade has seen significant changes in management with interventions focussed on the osteoporotic bone phenotype that accompanies the mutations in COL1A1 and COL1A2. Observational studies of children and infants with osteogenesis imperfecta treated with bisphosphonates suggests that major changes in quality of life can be achieved through this form of intervention. Nevertheless the optimal drug, route of administration, dose and duration of therapy remain to be determined. For children and adults with disorders of collagen a multi-disciplinary approach is likely to be needed. The management of complex problems in osteogenesis imperfecta involves not only medical intervention but also surgery to correct limb and spine deformity, neurological assessment for basilar invagination and hydrocephalus; treatment of constipation, deafness and dental problems; assessment of mobility, including the use of aids to walking and activities of daily living by an occupational therapist; range of movement, muscle strength and gait analysis by a physiotherapist; and co-ordination and liaison with the families by a specialist nurse.

Challenges remain in many areas, particularly in respect of repairing tissue damage and reversing structural alterations. Nevertheless the prospects and quality of life for children with one group of collagen disorders has been significantly improved over the last decade and we can hope that other groups will soon follow.

IS4

ACTIVATION OF NONGENOTROPIC ESTROGEN-LIKE SIGNALING (ANGELS): A NOVEL APPROACH TO BONE ANABOLISM AND GENDER-NEUTRAL THERAPY OF OSTEOPOROSIS

S C Manolagas

Division of Endocrinology and Metabolism and the UAMS Center for Osteoporosis and Metabolic Bone Disease, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA

Abnormal timing of death of osteoclasts and osteoblasts by apoptosis is a fundamental problem in osteoporosis, and present (e.g. bisphosphonates) or future (e.g. PTH injections) forms of treatment for osteoporosis work, at least in part, by promoting osteoclast apoptosis and attenuating osteoblast apoptosis. Loss of sex steroids leads to increased rate of remodeling. Nonetheless, increased remodeling alone cannot explain why loss of sex steroids tilts the balance of resorption and formation in favor of the former. Estrogens and androgens also exert effects on the lifespan of mature bone cells: pro-apoptotic effects on osteoclasts, but anti-apoptotic effects on osteoblasts and osteocytes. Both these anti- and pro- apoptotic effects stem from a heretofore unexpected function of the classical "nuclear" sex steroid receptors outside the nucleus and result from activation of a Src/Shc/ERK signal transduction pathway, probably operating within preassembled scaffolds such as caveolae. Rapid activation of the Src/Shc/ERK pathway by estrogens or androgens leads to potent downstream regulation of the transcriptional activation of the serum response element and AP-1 sites, demonstrating a link between nongenotropic and genotropic functions of their classical receptors. Moreover, activation of both the ERK and PI3K pathways by estrogens as well as Bad phosphorylation, by at least one of these pathways, seem indispensable for the anti-apoptotic action of estrogens. The estrogen receptor (ER) alpha or beta or the androgen receptor (AR) can transmit signals through the Src/Shc/ERK signaling pathway with similar efficiency irrespective of whether the ligand is an estrogen or an androgen. More importantly, these nongenotropic, sex non-specific actions are mediated by the ligand binding domain of the receptor and can be functionally dissociated from their classical genotropic transcriptional activity with synthetic ligands. Even more strikingly, administration of synthetic ligands, which can dissociate ER or AR signaling through kinases from the classical transcriptional activity of these receptors in the nucleus, to either female or male gonadectomised mice causes increases in bone mineral density (BMD) and bone strength, significantly more than estrogens or androgens, without affecting reproductive organs.

IS5

RECENT DEVELOPMENTS IN QUANTITATIVE ULTRASOUND

C C Glüer, R Barkmann. Medizinische Physik, Klinik für Diagnostische Radiologie, Universitätsklinikum Kiel, Germany

Quantitative Ultrasound (QUS) are now being used in clinical practice for more than a decade. In recent years, technologies have matured and we now have a better understanding what is being measured by QUS techniques. Ultrasound interaction with bone is strongly affected by bone mass but also by other factors related to elasticity and strength of bone. Bone microstructure and material properties both affect QUS parameters, whereas micro cracks do not. It has been shown that QUS results for transverse transmission through trabecular bone can equally well be predicted by bone mineral density or a combination of microstructural variables. This is not surprising since both features should deteriorate in disease. Simulation studies have helped to understand the wave propagation through cortical bone, both in transverse as well as in axial transmission. The former is primarily affected by bone geometry (e.g. cortical thickness) and by material properties while the latter is affected by the material properties of bone matrix and only for thinner cortices also by cortical thickness. Clinically, the method is particularly well suited to estimate fracture risk. Several questions, however, remain with regard to diagnostic criteria and the ability to monitor treatment response. Current interest focuses on applications in other disorders, e.g. rheumatic diseases or other secondary osteoporoses and also on use in children and adolescents. For clinical practice it is important to recognise error sources, the relevance of thorough operator training, and guidelines how to interpret ultrasound results. If used appropriately, QUS methods can provide valuable insight into bone status; if used carelessly, the risk of misinterpretation is substantial.

IS6

CURRENT THERAPIES FOR OSTEOPOROSIS

J E Compston. Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge CB2 2QQ, UK

In recent years there have been significant advances in the management of osteoporosis and a number of options are now available. Largely for historical reasons, the level of evidence for the effectiveness of different treatments varies considerably; however, robust evidence of anti-fracture efficacy from adequately powered randomised controlled trials is now available for the bisphosphonates, raloxifene, parathyroid hormone peptide and calcium and vitamin D. In contrast, the evidence for anti-fracture efficacy of hormone replacement therapy is largely based on observational studies that are subject to bias and likely to overestimate any benefit.

Potentially, the magnitude of anti-fracture efficacy is an important factor in deciding which therapy to use, but it is difficult to compare this between different agents because of differences in trial design and the populations studied. The issue of site-specificity is also relevant, since anti-fracture efficacy has not been demonstrated for all agents at both vertebral and non-vertebral sites. Adverse effects, both skeletal and non-skeletal, influence the choice of treatment but these have not been clearly defined for all agents; in particular the long-term risks and benefits of hormone replacement therapy and raloxifene require further characterisation. As the number of options increases, safety and tolerability become increasingly important, especially since the treatment offers no symptomatic relief and is generally long-term.

There is now good evidence that anti-resorptive agents have a relatively rapid onset of action, significant fracture reductions being demonstrated within one year of starting therapy. This property, together with the well-documented efficacy of such agents in postmenopausal women with established osteoporosis, has encouraged a move away from long-term preventive strategies towards shorter-term intervention in individuals in whom the absolute risk of fracture is high. The development of accurate risk assessment tools to define criteria for intervention is an important challenge for the future. In addition, the optimal duration of treatment is currently unknown; the rate of offset of treatment effect and long-term bone safety are both relevant in this respect and require further study.

IS7

FUTURE THERAPIES

Abstract not available

IS8

TRANSGENIC MODELLING IN THE MOUSE

A R Clarke, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US

The past two decades have witnessed great advances in our ability to create and study genetic mutations in model systems. During this time the mouse has established itself as the primary mammalian system in which to perform these analyses. The principal reason underlying such dominance almost certainly arises out of our ever-increasing ability to manipulate the murine germline. Thus, we have moved from a position where animal models either occurred spontaneously or were generated through exposure to mutagens, to a position in which it is possible to create and study precise genetic alterations of choice. Essentially, two approaches have been used to achieve this, either utilising pronuclear injection to introduce additional sequences or to use embryonic stem cells (ES cells) in conjunction with gene targeting strategies to introduce defined mutations into the germ line. The ability to add genetic material through pronuclear injection has led to the creation of a series of models in which the target protein is over-expressed, an approach which although tremendously successful has suffered from problems with control of the pattern and level of target gene expression. More recent advances, for example using the tet-on or tet-off systems, have greatly increased the degree of control and versatility of this approach. The advent of ES cell based technologies paved the way for precise engineering of the genome, down to the level of point mutations. Recent advances in inducible and conditional technologies (for example using the Cre-Lox system) in conjunction with an ES based approach have now opened the possibility for both temporal and tissue specific gene manipulation. Each of these technological breakthroughs has facilitated significant steps forward in our understanding of many different genetic programmes, and examples of the use of these will be discussed with specific reference to experiments being undertaken in my laboratory.

IS9

SMALL LEUCINE RICH PROTEOGLYCANS: ESSENTIAL FOR THE STRUCTURE AND FUNCTION OF MINERALIZED TISSUE

M F Young. National Institute of Dental Research, NIH, Bethesda, MD

Small Leucine Rich Proteoglycans (SLRPs) are an expanding family of small proteoglycans that are highly expressed in bones and teeth. To determine the functions of the SLRP BGN in vivo, knockout/KO mice were developed and showed a progressive decrease in bone mass with age due to defective bone formation. Based on these observations, we tested the hypothesis that the osteoporosis-like phenotype was due to defects in cells critical to the process of bone formation. Our data showed that BGN deficient mice have diminished capacity to produce marrow stromal cells (MSC), the bone cell precursors, and that this deficiency increased with age. The cells also had reduced response to TGF-beta, reduced collagen synthesis and relatively more apoptosis than cells from normal littermates. In addition, calvaria cells isolated from BGN deficient mice had reduced expression of late differentiation markers such as bone sialoprotein and osteocalcin and reduced ability to accumulate calcium judged by alizerin red staining. We propose that any one of these skeletal cell defects in could contribute to the osteoporosis observed in the BGN KO mice. To test the hypothesis that functional compensation can occur between SLRPs, we created mice deficient in biglycan and decorin. Decorin deficient mice had normal bone mass while the double BGN/DCN KO mice had more severe osteopenia than the single BGN indicating redundancy in SLRP function in bone tissue. To further determine whether compensation could occur between different classes of SLRPs we made mice deficient in both biglycan (class I) and fibromodulin (FM) which is a class II SLRP highly expressed in mineralizing tissue. The mice had an impaired gait, ectopic calcification of tendons and premature osteoarthritis. TEM analysis showed that they also had severely disturbed collagen fibril structures. Biomechanical analysis of the affected tendons showed they were weaker compared to control animals leading to the conclusion that instability of the joints could be the primary cause of all the skeletal defects observed in the FM/BGN KO mice. These studies present important new models of skeletal diseases and will help elucidate the network of signals that control the integrity of mineralized tissue through SLRP activity.

IS10

FLUOROSIS AS A MODEL FOR THE STUDY OF EXTRACELLULAR MATRIX COMPONENTS IN MINERALISED TISSUE FORMATION

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Ingested fluoride in excess manifests itself as fluorosis, a condition widespread in parts of India, Africa and Italy where changes in skeletal mineralisation are evident. In the dentition the enamel becomes mottled and the dentine demonstrates hypomineralisation with structural changes to the extracellular matrix. Our group has sought to unravel the underlying molecular mechanisms governing these changes in fluorotic dentine and bone. At fluoride levels over 10ppm the glycosaminoglycan (GAG) chains of the constituent small leucine-rich proteoglycans such as decorin/biglycan undergo a reduction in chain length and alteration to the disaccharide sulphated moieties, leading to a reduction in overall mass. The changes are post-translational and are evident both in vivo and using in vitro cell based odontoblast and osteoblast-like systems and do not affect the leucine rich core protein. In an attempt to mimic the influence of these structural changes on mineralisation, crystal growth models have demonstrated differences between fluorotic and non-fluorotic derived GAG in modulating hydroxyapatite crystal growth. In vitro studies have indicated that fluoride reduces the ability of intact GAG and the parent PG to bind to hydroxyapatite via calcium mediated interactions. The fluoride driven reduction in GAG chain length also leads to fewer binding sites for Ca2+, implicating the phenomenon in reduced mineralisation potential. Also notable in fluorotic dentine and bone is an increase in ratio of dermatan sulphate to chondroitin sulphate, the former associated with non-mineralised tissues and where its presence in dentine and bone is associated with non-mineralised zones.

We have also investigated the molecular characteristics of dentine-specific phosphoprotein, with key roles in dentinogenesis. The fluorotic phosphoprotein possessed a reduced phosphate content, further demonstrating influence of fluoride on post-translational events. The enzymes alkaline phosphatase and casein kinase II, which are responsible for phosphate transfer and insertion, were found to be susceptible to fluoride following a series of in vitro enzyme inhibitor assays.

Thus our work has shown that fluoride has the potential to influence the expression and characteristics of extracellular matrices of mineralised tissues, rendering fluorosis a valuable study model.

IS11

THE CONTROL OF BONE MASS AND REMODELING BY LEPTIN

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The observation that bone mass is decreased by gonadal failure and that obesity protects from this phenomenon led us to postulate that bone mass, body weight and reproduction were regulated by the same hormones. The attractiveness of this hypothesis arises, among other things, from the fact that it can be tested using in vivo models. If these three functions have to be linked in their regulation then leptin becomes an excellent hormone to study. Indeed, leptin was already known to control body weight and reproduction, and since mutant mouse strains deficient in leptin signaling were available all one had to do was to study the bone histology of leptin signaling-deficient mice. Results of this objective analysis were surprising and of considerable importance. Mice deficient in leptin or its receptor had a high bone mass despite their hypogonadism. To date these are the only animal models where there was a coexistence of high bone mass and hypogonadism. We have shown that, in the entire animal, leptin inhibits bone formation i.e. is an antiosteogenic molecule, following binding to its receptor a hypothalamus neuron. These results established that in the entire animal there was a central regulation of bone formation. Using the same approaches, namely mouse genetics and bone histology, this concept has been confirmed using another mouse mutant strain. In that respect leptin regulates bone formation using the same general route that it uses to regulate body weight and reproduction. This uncovered a novel concept in bone biology that truly enriches our field. We have shown that insulin levels are not related to bone mass in at least four different models including leptin deficient mice, and have embarked in a systematic study trying to delineate the molecular pathways by which the hypothalamus controls bone formation.

IS12

GROWTH HORMONE - PHYSIOLOGICAL ASPECTS AND SIGNALLING MECHANISMS

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Growth hormone (GH) is a potent anabolic peptide that influences a wide array of physiological systems, including cell growth and differentiation, protein, lipid and glucose metabolism, renal function and immune mechanisms. In childhood, GH action contributes approximately 35-45cms to overall stature, as evidenced by the height of untreated GH deficient adults.

GH is secreted in distinct pulses from anterior pituitary somatotrophs, which are regulated by the combined hypothalamic influences of GH releasing hormone (GHRH), somatostatin and Ghrelin. In the circulation a proportion of GH associates with a binding protein (GHBP), which in humans is derived from proteolytic cleavage of the extracellular domain of the GH receptor (GHR). The GHR is widely expressed and therefore GH has multiple targets. One target is the liver. Here GH's effector peptide insulin-like growth factor-I (IGF-I) is produced along with its principal carrier protein IGF binding protein-3. It was thought that both circulating IGF-I as well as IGF-I generated in local tissues in response to GH contributed to bone growth. More recently transgenic mice, with a specific hepatic knock-out of IGF-I, achieve normal skeletal growth, implying that the peripheral actions of GH are more important to growth than hepatic IGF-I generation.

GH achieves its actions at the cellular level by activation of a complex array of intracellular signalling cascades also used by other peptides and cytokines. An important challenge in GH research is to understand how specificity of signalling is achieved in anyone cell type. GH must bind sequentially to two GH receptors, and it is this dimerisation that leads to the recruitment of signalling molecules to the intracellular domain of the GHR. In the absence of intrinsic receptor kinase activity, Jak2 is bound to the dimerised receptor leading to the activation of Signal Transducers and Activators of Transcription (STATs), the Mapk pathway, either through Shc-Ras-Raf or IRS-I-Pi3K, SH-2 domain phosphatases and suppressors of cytokine signalling. GH can also affect Ca2+ entry, independent of Jak2 activation.

Abnormalities at all levels of the GH-IGF axis have been described in humans, each with distinct clinical, biochemical and molecular characteristics, but all resulting in a severe growth disorder.

IS13

BONE MARROW ADIPOCYTES: A NEGLECTED TARGET TISSUE FOR GROWTH HORMONE ACTION

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Bone marrow contains numerous adipocytes whose function is unclear. A decrease in bone volume is often accompanied by an increase in bone marrow fat. Bone marrow adipocytes share a common mesenchymal precursor with osteoblasts and chondrocytes, but the physiological mechanisms that regulate their differentiation is unknown. In the rat growth plate, growth hormone (GH)-receptors are located in the stem cell zone and in the transition zone between proliferative and hypertrophic chondrocytes. GH-treatment of GH deficient dwarf (dw/dw) rats increases the number of proliferative cells but not their proliferation rate, suggesting that GH stimulates the differentiation into proliferative chondrocytes. We hypothesized that GH also would affect the adipocyte lineage. Adult dw/dw rats have 4 fold more adipocytes in their marrow compared to normal rats, and these adipocytes are somewhat larger. After GH, but not IGF-I, treatment, bone marrow adipocyte number returned towards normal. Adipocyte size was smaller after both GH- and IGF-I treatment. Cancellous bone area and % bone surface covered by osteoblasts are lower in dw/dw rats whereas ALP activity in individual osteoblasts is unchanged. Whilst decreasing the number of marrow adipocytes, GH-treatment increased osteoblast-covered bone surface, but did not affect individual osteoblast ALP activity. Estrogen also has direct effects on chondrocytes, osteoblasts and peripheral fat. Whilst ovariectomy decreased cancellous bone area and osteoblast ALP activity, it had no effect on bone marrow adipocyte number or size. Rats fed a high fat diet, which doubled their renal and ovarian fat pad weights compared to chow-fed rats, had an increased percentage bone marrow fat, primarily due to a larger adipocyte cell size rather than an increase in cell number. When rats were treated continuously with hPTH, percentage marrow fat, adipocyte density and adipocyte size were all unchanged. This suggests that the effect of GH on marrow adipocytes is not secondary to effects on peripheral fat or bone metabolism.

We conclude that GH regulates all 3 lineages (chondrocytes, osteoblasts and adipocytes) derived from mesenchymal precursors and suggest that GH affects lineage choice. The bone marrow adipocyte lineage is an important target tissue for GH.

IS14

GROWTH HORMONE MODULATION OF BONE METABOLISM AND MINERALISATION- CLINICAL IMPLICATIONS IN HYPOPITUITARISM

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Epidemiological data indicates that growth hormone (GH) deficiency (GHD) in human adults is associated with reduced bone mineral density (BMD) and increased fracture risk. Using a variety of techniques, including DXA and quantitative CT, investigators have compared BMD in patients with GHD and healthy controls. These studies indicate reduced bone mass at the femur, forearm and lumbar spine in GHD, with the most striking differences observed in those with childhood-onset GHD.

GH replacement in the adult with GHD results in activation of bone modeling, assessed using BSAP, osteocalcin, cross-linked telopeptide of type I collagen, and urinary deoxypyridinolines. These increases in bone metabolic activity do not alter BMD over periods lasting 6 months, but GH administration for periods greater than 12 months increases BMD, with continuing increases observed up to 5 years following GH commencement. Early evidence indicates that the benefits on BMD may be more marked in males. Interpreting these effects it is clear that introduction of GH to adults with GHD activates general bone remodeling with predominant osteoblastic activity evident only after periods longer than 6 months. This anabolic effect on accrual of BMD has persisted in open studies lasting up to 5 years and has been reported to normalise the reduced BMD associated with long-standing GHD. Increasing evidence indicates that increased BMD related to GH replacement reduces fracture risk in the hypopituitary adult.

Development of osteoporosis with attendant risk of fragility fracture is in part determined by the peak bone mass (PBM) achieved in early adulthood. Hereditary features account for ~80% of the variability in PBM but the importance of hormonal factors is increasingly recognised. Preliminary reports from a trial of GH continuation/ discontinuation in adolescents with GHD who have not yet achieved PBM indicates that cessation of GH for 1 year results in arrest of bone mineralisation. GH continuation resulted in a 6% increase in whole-body bone mineral content (DXA) providing evidence that GH may have an important influence on PBM, with its potential implications for bone health in later life.

In summary investigations in hypopituitarism indicate that GH has influences on bone metabolism and bone mass. GHD is associated with reduced BMD and increased fracture risk. These features improve with GH replacement. Further studies will clarify the role of GH in regulating achievement of PBM and the consequences of GH replacement on fracture rates in hypopituitary adults.

OC1

OESTROGEN-INDUCED CANCELLOUS BONE FORMATION IN MICE IS MEDIATED BY OESTROGEN RECEPTOR ALPHA

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Recent evidence suggests that oestrogen's protective effect on the skeleton is mediated at least in part by stimulation of osteoblasts in trabecular bone. The oestrogen receptor (ER) is expressed in at least two distinct isoforms, ER alpha and ER beta, both of which are expressed at significant levels in bone. To determine whether ER alpha plays a significant role in mediating oestrogen's stimulatory effect on the osteoblast lineage, we examined whether oestrogen-induced cancellous bone formation in mice is impaired in mice with a targeted gene deletion in this isoform. We compared the dose-responsiveness of oestrogen-induced bone formation between fourteen-week-old male mice homozygous for a targeted gene deletion in ER alpha (ERKO mice) (Lubahn et al., 1993 PNAS 90:11162) and age-matched wild-type controls (WT). Animals were administered vehicle or 17beta-oestradiol 40, 400, 4000 microg/kg/day for 28 days (4-7 animals per group). The fluorochromes calcein and tetracycline hydrochloride were administered prior to sacrifice, and dynamic histomorphometry subsequently performed at the distal femoral metaphysis. As expected, wild-type mice showed a marked dose-responsive increase in new cancellous bone formation, as assessed by cancellous bone area (BA/TA; $p < 0.0001$), and the absolute extent of mineralising surface (dlS/BA; $p = 0.0058$) (one-way analysis of variance). In contrast, ERKO mice showed no evidence of an osteogenic response, as confirmed by two-way analysis of variance, which showed a significant difference in response according to genotype for BA/TA ($p < 0.0001$) and dlS/BA ($p < 0.0001$). Based on our observation that oestrogen-induced osteogenesis is abrogated in ERKO mice, we conclude that oestrogen-induced osteogenesis is mediated by ER alpha, which is consistent with our previous finding that in mice lacking ER beta, the cancellous bone response to oestrogen appears to be unaffected.

OC2

ESTROGEN STIMULATES MEGAKARYOCYTE DIFFERENTIATION AND ESTROGEN RECEPTOR PROTEIN AND MRNA EXPRESSION

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Anabolic skeletal effects of high-dose estrogen (E) have been demonstrated in postmenopausal women and are due, at least in part, to increased osteoblastic activity. It has been demonstrated in vivo that the megakaryocyte population in human bone marrow increases with E treatment, suggesting that these cells may contribute to the observed anabolic effect. The aim of the present study was to investigate the hypothesis that E stimulates megakaryocytopoiesis.

CD34+ cells were isolated from cord blood by magnetic bead technology (MACS) and cultured for 6 days in a collagen-based system plus or minus 10nM (low-dose) and 100pM (high-dose) 17beta estradiol. Collagen films were dried and cells immunolocalised for CD61, a marker of early megakaryocyte maturation, CD41, a MK specific antigen expressed by more mature MKs and estrogen receptors (ER) alpha and beta. Protein expression was quantitatively assessed by image analysis. Similar CD34+ cells were cultured in IMDM liquid plus or minus E for 6 days prior to RNA extraction. ER mRNA expression was assessed by RT-PCR.

Cells cultured in the presence of E formed more megakaryocyte colony forming units (CFU-MK) than untreated cells. These were highly CD61 positive with a three-and four-fold increase in expression in the low and high-dose E treated cells ($p<0.05$) respectively compared to untreated cells. CD41 expression was increased dose-dependently in CFU-MKs in the E-treated cultures by 3 and 5-fold ($p<0.05$). E stimulated ER expression with ERalpha mainly confined to CFU-MKs and low-level nuclear ERbeta expression in CD34+ cells. This was increased in the E-treated cells with intense staining in CFU-MKs. Low-dose E stimulated ERalpha and ERbeta expression 2 fold ($p<0.01$) compared to untreated cultures with no further significant increase seen with high-dose E. ERalpha mRNA expression was increased by high-dose E whilst the greatest increase in ERbeta expression was seen with low-dose E.

These results demonstrate that in vitro E stimulates the colony forming potential of CD 34+ cells to a more megakaryocytic phenotype. Together with the observed increase in ER protein and mRNA expression, this finding suggests that megakaryocytes may play a role in mediating E-induced skeletal effects.

OC3

ISOLATION OF MET, A NOVEL MODULATOR OF OESTROGEN INDUCED TRANSCRIPTION

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High-dose oestrogen is known to stimulate osteoblast activity in postmenopausal women as well as rodent models. To investigate the molecular mechanisms underpinning this effect, we compared the gene expression profiles of mRNA isolated from the tibiae of adult mice treated with either oestrogen or vehicle for 4 days by subtractive hybridisation analysis. During the course of this investigation, a gene fragment sharing no significant homology with previously characterised sequences was isolated and found to detect a single 3.8Kb transcript by northern analysis. A full length cDNA homologous to this gene was generated by EST directed Rt-PCR using bone marrow cDNA as the source of template. Sequence analysis of the cloned products revealed it coded for a 1031 amino acid protein. This peptide contains a SAF Box DNA binding motif, an RNA binding domain and shares an overall identity of 34% with the oestrogen suppresser SAF-B/HET/HAP. HET has previously been reported to bind the oestrogen receptor directly, act as a repressor both in breast and bone cell lines and augment the anti-oestrogen effects of the SERM Tamoxifen. When the cDNA we have isolated was expressed as a fusion product with enhanced yellow fluorescent protein, it was found to localise exclusively to the nucleus in a punctate manner. It was further observed that its expression in MCF-7 cells resulted in a dose dependent reduction in oestrogen induced ERE luciferase reporter gene expression. In contrast, its expression in ROS and SMER osteosarcoma cell lines augmented oestrogen induced expression. From these data we conclude that this novel sequence represents a previously undescribed, tissue specific Modulator of oEstrogen induced Transcription, which we refer to as MET, that is likely to play a significant role in regulating oestrogen responses in bone and other tissues.

OC4

SERUM OSTEOPROTEGERIN IS UNRESPONSIVE TO POSTMENOPAUSAL OESTRADIOL REPLACEMENT

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Oestrogen enhances osteoprotegerin (OPG) mRNA expression and production of OPG protein in human osteoblastic cells. The biological source and clinical relevance of OPG in serum is however uncertain. The aim of this study was to determine the effect of oestrogen therapy on serum OPG in postmenopausal women.

Participants were 10 hysterectomised postmenopausal women (ages 60 to 75 years, mean 64 years) receiving a subcutaneous oestradiol implant (25 mg) at baseline and after 6 and 12 months. Serum OPG was determined using a commercial ELISA (Biomedica, Austria) that detects monomeric, dimeric and ligand bound forms of OPG, and using an in-house assay. Serum OPG and oestradiol (E2) were measured at baseline and at 4, 8, 12, 24, 52, 78 and 103 weeks. Markers of bone turnover (bone alkaline phosphatase (bone ALP), procollagen type I-N terminal propeptide (PINP), osteocalcin (OC), free deoxypyridinoline (iFDPD), N-telopeptide of type I collagen (NTX)) were measured at baseline and at 4, 8, 12 and 24 weeks.

Baseline concentrations of E2 (67.4 (3.0) pmol/L) increased fourfold by 4 weeks and sixfold by 103 weeks. PINP, bone ALP and OC increased by 28%, 7% and 9% ($P<0.01$) respectively during the first 4 weeks of treatment and then decreased significantly. NTX decreased by 46% over the first 24 weeks ($P<0.01$) and iFDPD by 17% (P=NS). Despite the marked increase in serum E2 and significant reduction in bone turnover, there were no changes in the mean circulating concentration of OPG from baseline (92.7 (4.6) pg/ml) at any time point ($p=0.98$, ANOVA) using either OPG assay.

Circulating OPG is unlikely to be useful to monitor E2 therapy. The tissue origin of circulating OPG is uncertain, and measurements made in serum are perhaps unlikely to reflect OPG activity within the bone microenvironment.

OC5

A COMPARISON OF THE BONE MINERAL DENSITY (BMD) BETWEEN LUTEINISING HORMONE RECEPTOR KNOCKOUT (LURKO) MICE WITH HUMAN CHORIONIC GONADOTROPIN (HCG) OVEREXPRESSING MICE

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Recently we provided evidence for the presence of functional LH receptors upon primary human osteoblasts and the osteoblast-like cell line MG63. Treatment of these cells with hCG, a natural ligand of the LH receptor, resulted in elevated synthesis of type I collagen accompanied by raised levels of MMP-2 and akaline phosphatase activity. Furthermore treatment of murine calvaria with hCG resulted in a modest but significant increase in bone resorption. Taken together these findings support that LH/hCG has a role in bone turnover in situations where these hormones are significantly elevated, namely puberty, pregnancy and the menopause. However, whether LH/hCG has a role to play in the regulation of bone turnover in vivo still awaits investigation. To this end we examined the bone mineral density (BMD), using the Lunar PIXImus mouse densitometer, of mice lacking the LH receptor (LuRKO) for comparison with wild type littermates. In addition we analysed the BMD of mice that were genetically modified to overexpress hCG. Both models exhibited profound changes in their BMD.

Female mice overexpressing hCG showed a significant increase in tibial and femoral BMD at 2, 3 and 6 months compared to age and sex matched litter mate controls. At two months of age there was a ~25% increase in femoral BMD ($p<0.005$) compared to controls. Female LuRKO mice showed a marked decrease in femoral BMD ($P<0.05$) at three months compared to controls. Five month old male LuRKO mice also showed a significant decrease in femoral ($p<0.005$) and tibial ($p<0.05$) BMD compared to controls. In conclusion, LH/hCG may have a direct anabolic effect on bone turnover, in vivo, however, the role played by the sex steroids in these models requires closer investigation.

OC6

DISC DEGENERATION INFLUENCES THE DISTRIBUTION OF LOAD ON THE VERTEBRAL BODY: A CAUSE OF OSTEOPOROTIC VERTEBRAL FRACTURES IN THE ELDERLY

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Systemic causes of bone loss that can lead to osteoporotic vertebral fracture are well documented. However, recent studies suggest that bone loss may be exaggerated in the anterior half of the vertebral body, leaving this region particularly susceptible to fracture. We hypothesise that age-related degenerative changes in intervertebral discs leads to abnormal load-sharing between the anterior and posterior vertebral body and the neural arch.

Thirty-three cadaveric lumbar motion segments (mean age 50yrs; STD 19yrs), comprising of 2 adjacent vertebral bodies and intervening disc and ligaments, were compressed at 2kN while positioned to simulate either an erect standing posture or a forward bending (flexed) movement. Intradiscal stresses were measured by pulling a miniature pressure transducer, side-mounted in a 1.3mm-diameter needle, along the mid-sagittal diameter of the disc. Profiles of intradiscal stress were integrated over area in order to calculate the force acting on the anterior and posterior halves of the vertebral body. These were subtracted from the 2kN to determine the force on the neural arch. Degree of disc degeneration was assessed on a scale of 1-4.

In non-degenerated discs, intradiscal stresses were distributed evenly in both erect posture and forwards bending, and negligible compressive force was resisted by the neural arch. However, in severely degenerated discs, neural arch load-bearing increased to 40% in erect posture, and the force exerted by the disc was concentrated on the posterior vertebral body. In these degenerated specimens, the proportion of the applied 2kN resisted by the anterior vertebral body increased from 18% in erect posture to 58% in flexion.

Degeneration causes disc height loss, increasing neural arch compressive load-bearing in erect postures. Additionally, the disc loses its ability to distribute stress evenly on the vertebral body, with the result that the anterior vertebral body is heavily loaded in flexion. These two effects combine to ensure that, in the presence of disc degeneration, the anterior vertebral body is relatively unloaded in erect postures, and yet severely loaded in flexion. This could explain why anterior vertebral body fractures are common in elderly people with degenerated discs, and why fracture is often associated with forward bending movements.

OC7

MECHANICAL INFLUENCES ON SKELETAL DEVELOPMENT IN UTERO

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Mechanical loading influences bone strength profoundly, and studies in humans and animals have shown that loading has more potent influences on bone during growth than after skeletal maturity. These experiments were designed to test the hypothesis that fetal movement in utero constitutes a mechanical stimulus for skeletal development.

We crossed mice lacking separately genes for Myo-D (D) and Myf-5 (F) that code for muscle proteins. Double knockout animals (ffdd) lack functional striated muscle and reportedly die because of inability to breathe. Myo-D null mice are phenotypically normal, but Myf-5 null mice do not thrive. To generate double knockout offspring, a scheme was instituted to generate DdFf heterozygotes which were then interbred . Mendelian distribution of the F2s would predict 1:16 of pups to be double knockouts, but the ratio was <1:40. The distribution was not Mendelian in other F2 genotypes, with apparent advantages for Ff over FF with either Dd or dd.

19 day embryos were stained with alizarin red/alcian blue. Double knockouts displayed two classifications of abnormalities. Long bones were featureless and straighter than wild types, lacking traction epiphyses where muscle insertions would normally be expected. They also exhibited severe abnormalities in the ribs (which were either absent or <5% of expected length) and absence of the middle and medial portions of the clavicles. Clear but less pronounced abnormalities were present in ffDd pups.

The unpredicted distribution of genoptypes suggests that there is a non-redundant role for either Myo-D or Myf-5 that affects embryo viability. Changes in long bone morphology show that without active movement in utero, neonatal skeletons exhibit what is presumably merely a genetic phenotype with no superimposed functional adaptation. Finally, the absence of ribs and parts of the clavicle suggests that what are currently considered to be muscle proteins have a critical role in development/patterning of parts of the skeleton. Given the ability of music and voices to stimulate human fetal movement, it may prove possible to induce increased adaptive changes in bones before birth. If skeletal parameters measured at birth reflect bone health in age, such strategies could have long term benefits for future generations!

OC8

INFLUENCE OF GROWTH RATE ON CORTICAL BONE POROSITY, AND STRENGTH IN THE IMMATURE SKELETON

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In response to increased loads during growth, bones circumferentially expand to increase their cross sectional moment of inertia. This occurs through the incorporation of primary osteons which form by enclosing blood vessels on the surface; osteoblasts then infill the resulting canal. However, the influence of growth rate on bone architecture in the immature skeleton is not fully understood. To investigate how this occurs we have compared the morphometric differences between tibiae from chickens with fast (F) and slow (S) growth potentials (Body weight at 42 days; F=2441g and S=1224g).

Bone diameter (F=7.16mm; S=5.36mm, $P<0.001$) and cortical width (F=0.702mm; S=0.479mm, $P<0.01$) were increased in the rapidly growing birds. Cortical porosity was elevated periosteally (F=36%; S=27%, $P<0.01$) due to slower infilling of the primary osteons in the rapidly growing birds (% unfilled; F=42.5%; S=27.0%, $P<0.01$). Although bone stiffness (F=363N/mm: S=285N/mm; $P<0.01$) and breaking strength (F=448N; S=270N, $P<0.001$) were higher in rapidly growing birds, after adjustment for body weight the bones were inherently weaker (stiffness: F=60N/mm; S=220N/mm, $P<0.001$, breaking strength; F=99N; S=181N, $P<0.05$). Osteocyte density within the periosteal interstitial bone was higher in the rapidly growing birds (F=5.3/mm2; S=1.8/mm2, $P<0.01$). Sections reacted for ALP and TRAP activity and stained for cement (reversal) lines indicated the absence of primary osteon remodelling in the periosteal region.

In conclusion, fast growth resulted in the expected circumferential expansion to increase the bending strength. Fast growth was accompanied by increased porosity resulting from rapid formation of primary osteons and the incapacity of osteoblasts to completely infill the resultant canal. However, periosteal interstitial bone of the fast growing birds had a higher osteocyte density suggesting that this was not due to a decrease in osteoblasts. These events suggest that transit through the osteoblast differentiation cascade is more rapid in the fast growing chicks. While osteoblast precursors are locally available, as in the periosteum, this has no effect on bone growth. However, if precursors were less readily available, as in the primary osteons, then this would results in reduced bone growth and a higher osteocyte density.

OC9

REMODELLING CLUSTERS AND REDUCTION IN BENDING LOADS

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Previous reports have indicated that the presence of remodelling clusters within the femoral neck cortex is positively associated with the formation of giant canals; such canals are responsible for the increased cortical porosity in cases of femoral neck fracture. Like the human femoral neck, the ovine os calcis is habitually loaded in bending. This study was designed to investigate whether clustering was affected by a reduction in load.

In two skeletally mature female adult sheep an external fixator was applied across a mid-diaphyseal osteotomy in the right tibia for 10 weeks. This results in an initial reduction in the ground reaction force of the osteotomised limb compared to the intact contra-lateral limb. In the current study ground reaction forces were reduced in both sheep (1006 r/l 70% and 1026 r/l 40% at 2weeks and 14% and 17% respectively at 10weeks). The left calcaneus was used as a control. Cortical mass (pQCT) was reduced in the ossa calces from the under-loaded limbs. The proportion of canals with a crenellated surface was increased in the under-loaded bones (1006: left:- 0.16% right:- 0.48%; 1026: left:- 0.26% right:- 0.66%) as was the proportion of canals bearing an osteoid surface (1006: left:- 0.27%, right:- 1.87%; 1026: left:- 0.70%, right:- 2.03%). Using the mean inter-osteonal distance (0.175mm) as the cluster radius there was significant spatial clustering of both resorbing and forming canals in the under-loaded bones. The density of clusters of forming canals was increased in the under-loaded bones from both animals (1006: left:- 0/mm2 right:- 0.18/mm2; 1026: left:- 0.03/mm2 right:- 0.15/mm2); that for resorbing canals was increased in one animal (1006: left:- 0/mm2, right:- 0.053/ mm2; 1026: left:- 0.031/ mm2 right:- 0.033/mm2). The proportion of forming canals that were clustered was higher in the under-loaded bones (1006: left:- 0% right:- 57%; 1026: left:- 31%, right:- 44%). For resorbing canals, this only occurred in one animal(1006: left:- 0% right:- 55%; 1026: left:- 66%, right:- 29%).

This study, although preliminary, has shown that the density of clusters of remodelling activity increases when bending loads on the os calcis are reduced. This lends support to the hypothesis that reduced loading, through changes in patterns of daily activity, increases the formation of remodelling clusters.

OC10

PLACE OF RESIDENCE AND RISK OF FRACTURE IN OLDER PEOPLE: A POPULATION BASED STUDY OF PEOPLE AGED OVER 65 LIVING IN CARDIFF

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Fracture prevention strategies will be most cost-effective if targeted on groups of frail elderly people who are at particularly high risk of falls and fractures. Elderly people living in care homes are one potential target population, but fracture incidence in this setting remains poorly defined in many countries.

We have used the All Wales Injury Surveillance System (AWISS) in a population based study of people aged over 65 living in the city of Cardiff. We linked a postcode-based register of all sheltered accommodation and all residential and nursing homes in the city with injury data from Cardiff's single Accident and Emergency department.

Cardiff has 47,700 residents aged over 65 with 1,918 (4.0%) living in residential or nursing homes and 1,830 (3.8%) in sheltered accommodation. In 1999 we identified a total of 1,305 fractures, 366 of which were at the hip. This gave a crude incidence of 27.4/1000/year for all fractures, and 7.7/1000/year for hip fractures. Care home residents suffered 213 fractures, 95 of which were of the hip; giving crude fracture incidence figures of 111.1/1000/year and 49.5/1000/year, respectively. People living in sheltered accommodation suffered 94 fractures, 28 being at the hip; giving crude fracture incidence figures of 51.4/1000/year and 15.3/1000/year, respectively.

People in care homes and sheltered accommodation tend to be older than those living in the community, and we adjusted for this by calculating age and sex standardised relative ratios for each setting. Compared with the community dwelling population, care home residents had a total fracture risk of 2.9 (95% CI 2.5-3.3) and a hip fracture risk of 3.3 (95% CI 2.6-4.2). People in sheltered accommodation had a total fracture risk of 1.7 (95% CI, 1.4-2.1), and a hip fracture risk of 1.6 (95% CI, 1.1-2.4).

Such figures support the potential cost-effectiveness of strategies that seek to prevent fractures in care homes and sheltered accommodation, and are of special interest to those planning intervention studies in these settings.

OC11

NEPHRONECTIN EXPRESSION IS UPREGULATED DURING CHONDROGENESIS IN ATDC5 CELLS

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ATDC5 is a murine cell line that can be induced to differentiate into a chondrocyte phenotype in vitro. To gain novel insights into the molecular events associated with chondrogenesis we applied an agarose gel differential display analysis to ATDC5 cells undergoing chondrogenesis. Chondrogenic differentiation was induced by culturing confluent ATDC5 cells in DMEM/5% FCS containing 1% insulin. Chondrogenesis was assessed at days 0, 4 and 10 by alcian blue staining and by the expression of the genes encoding type II and type X collagen and aggrecan. Using this strategy we identified, cloned and sequenced a cDNA derived from a transcript whose expression increased in parallel with that of the marker genes. A database search using the BLAST programme gave a high-scoring match (E=0; 100% identity) to the murine gene encoding nephronectin, a recently identified extracellular matrix protein which plays an essential role in kidney organogenesis. Nephronectin is also expressed in proliferating MC3T3-E1 preosteoblasts although its expression decreases markedly during osteogenic differentiation. Nephronectin is a modular protein containing 5 EGF-like repeats, a mucin-like domain containing an RGD motif, and a meprin, A5 protein, and receptor protein-tyrosine phosphatase mu (MAM) domain. Using a bioinformatics approach we located the murine nephronectin locus on murine chromosome 3 band H1-H2 and subsequently identified the human orthologue on a syntenic region on human chromosome 4q24-25. Using gene specific RT-PCR assays we confirmed that nephronectin expression is upregulated during chondrogenesis in ATDC5 cells. Since nephronectin functions as a ligand for alpha8 beta1 integrin during kidney formation, we next assayed for the expression of these genes during chondrogenesis in ATDC5 cells. Beta1 integrin expression increased dramatically during chondrogenesis, in keeping with its established role as a regulator of chondrocyte differentiation. However, we have been unable to detect alpha8 integrin expression at any stage of chondrogenesis. This suggests that chondrocyte-derived alpha8 beta1 integrin is unlikely to be an endogenous ligand for nephronectin in cartilage. Further studies will determine the role of nephronectin in chondrogenesis and determine the identity of the ligands and cells that it interacts with.

OC12

THE ROLE OF THE ANTI-ANGIOGENIC FACTOR CHONDROMODULIN-I IN SLOWING DOWN THE RATE OF VASCULAR INVASION AT THE GROWTH PLATE

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Chondromodulin-I (ChM-I) is a bifunctional autocrine regulator of cartilage, which stimulates matrix synthesis by chondrocytes, but inhibits vascular endothelial cells[1,2]. In fetal bovine bone, ChM-I was expressed by all chondrocytes except hypertrophic cells and was present in the matrix throughout the epiphysis, except the hypertrophic zone, consistent with its proposed anti-angiogenic role[2,3]. We studied the age-related changes in ChM-I immuno-localization in femoral growth plates from young, mature and aged rats, using a new antibody raised against mature human ChM-I. We also studied the responses to vascular invasion of the epiphyseal chondrocytes of neo-natal rabbits, using culture on the chorio-allantoic membrane (CAM), an experimental model of angiogenesis.

In 2-week old rats, ChM-I was present in all chondrocytes and matrix of the epiphysis, except for the hypertrophic zone, confirming previous findings. However, in 4-16 week old rats, there was a progressive change in the localization of ChM-I. Hypertrophic chondrocytes became positive for ChM-I, while cellular staining gradually disappeared from the other zones. By 12-16 weeks, the strongest immuno-staining was on the inner perimeter of the lacunae of hypertrophic chondrocytes. As lacunae were opened at the vascular front, ChM-I initially remained on the cartilage-side of the lacunae, and then disappeared completely. In aged rat growth plates, ChM-I was only present where chondrocytes had become re-activated.

During CAM culture, CAM derived vascular elements will penetrate any tissue unless prevented by anti-angiogenic factors. Our studies demonstrated that ChM-I was synthesized by chondrocytes on the periphery of explanted rabbit chondro-epiphyses in response to the vascular challenge.

Conclusions: In young rats, the loss of ChM-I in the hypertrophic zone was as expected for an anti-angiogenic factor. The unexpected immuno-localization around hypertrophic chondrocytes in rats with reduced rate of growth suggested that ChM-I might act as "brake" on vascular invasion until degraded by proteases, perhaps MMP-9. The notion that ChM-I was synthesized in response to a vascular challenge, presumably to protect against vascular invasion, was supported by the CAM culture experiments.

[1] *Biochem Biophys Res Comm* (1991) 175:971-977;[2] *J Biol Chem* (1997) 272:32419-26;[3] *Biochem Biophys Res Comm* (1998) 249:885-890

OC13

INDUCTION OF BONE FORMATION IN VIVO USING HUMAN OSTEOPROGENITOR AND OSTEOBLAST STIMULATING FACTOR-1 ADSORBED SCAFFOLD CONSTRUCTS

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The ability to augment bone formation remains a key clinical need. The process of bone growth, regeneration and remodelling is mediated, in part, by the immediate cell-matrix environment. Osteoblast stimulating factor-1 (OSF-1), also known as pleiotrophin, is an extracellular matrix-associated protein, which is present in those matrices that act as targets for the deposition of new bone. Previously we have shown OSF-1 will induce human osteoprogenitor adhesion, chemotaxis, proliferation, differentiation and CFU-F formation in vitro. The aims of this study were to examine the potential of porous poly(lactic-co-glycolic acid) (PLGA) constructs adsorbed with OSF-1 to induce osteogenesis by human osteoprogenitors in vivo using the diffusion chamber and subcutaneous implant models.

PLGA scaffolds of defined porosity (50-200um) were generated using a unique supercritical fluid mixing method. Primary human bone marrow cells derived form 5 patients (59-78 years of age) were impregnated onto PLGA (75:25) porous scaffolds adsorbed with or without recombinant human OSF-1(50ng/ml) in osteogenic conditions. Cell/growth factor constructs were placed subcutaneously or within diffusion chambers before intraperitoneal implantation into athymic mice. In diffusion chambers, no bone formation was observed in human bone marrow/scaffold constructs alone. OSF-1 adsorbed constructs showed morphologic evidence of new bone matrix and cartilage formation within diffusion chambers as evidenced by X-ray analysis, metachromatic staining with toluidine blue, sirius red and alcian blue staining as well as type I collagen and von Kossa histochemistry in 4 of 9 chambers. Evidence of organised new woven bone was confirmed by birefringence of collagen within spicules of new bone. Furthermore, cartilage formation was observed within PLGA scaffolds confirming penetration of human osteoprogenitors through the scaffold constructs. In addition, after 4-6 weeks evidence of new bone formation was observed in subcutaneous implants as detected morphologically by sirius red staining.

These results demonstrate that OSF-1 has the ability to promote human osteoprogenitor differentiation with evidence of cartilage and bone formation within unique biodegradable porous PLGA scaffolds. The successful generation of osteogenic tissue within 3-D biomimetic structures incorporating OSF-1 indicates the potential for the development of protocols for de novo bone formation for skeletal repair.

OC14

FUNCTIONAL CHARACTERISATION OF ISOFORM-SPECIFIC ACETYLCHOLINESTERASES IN BONE.

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Acetylcholinesterase (AChE) is a multifunctional protein with diverse and tissue specific roles unrelated to cholinergic signalling. Alternative splicing and post-translational modification generates three AChE isoforms: AChE-S (synaptic), AChE-E (erythrocytic), and AChE-R (readthrough), which differ in their C-terminal sequences and properties. AChE-S forms tetramers and dimers which may be either membrane bound or secreted, whereas AChE-E interacts with membranes through GPI-linked dimers. AChE-R lacks the residues required for membrane insertion and is secreted as a soluble monomer. We have previously shown that osteotropic stimuli (TGF-beta 1, bFGF and vitamin D3) and mechanical strain increase AChE expression by osteoblasts. Furthermore, osteoblasts secrete AChE and preferentially adhere to AChE substrates, implicating a role for AChE in regulating cell-cell and cell-matrix interactions in bone. We have used in situ hybridisations, immunohistochemistry and ex-vivo cultures from AChE transgenic mice to determine the expression patterning and function of different AChE isoforms during skeletogenesis and osteoblast differentiation. Using biotinylated RNA probes on paraffin wax sections of neonatal rat limbs, prominent expression of mRNA encoding the AChE-R isoform was observed, most specifically in the periosteum, perichondrium and endosteal osteoblasts surrounding bone trabeculae. Positive AChE-R staining was also identified in recently embedded osteocytes and proliferating and pre-hypertrophic chondrocytes. Expression of mRNA for AChE-E and AChE-S isoforms was more sporadic and less abundant compared to AChE-R, but demonstrated similar distributions patterns in bone and cartilage. Immunolocalisations for AChE using antibodies directed to the conserved domain of AChE, or to isoform-specific AChE C-termini confirmed that AChE protein expression patterns corresponded with mRNA distribution. CFU-f assays were performed on marrow extracted from transgenic mice overexpressing the different isoforms of AChE. After 15 days of culture in the presence of L-ascorbic acid, dexamethasone and beta-glycerophosphate, a marked increase in alkaline phosphatase activity was identified in all cultures overexpressing AChE, and most significantly in cells overexpressing the AChE-R isoform, compared to wild-type controls ($P<0.001$). These findings support the functional significance of AChE in bone physiology and suggest strongly that isoform-specific AChE expression profiles significantly influence osteoblast differentiation.

OC15

GLUTAMATE-DEPENDENT REGULATION OF MEGAKARYOCYTE DIFFERENTIATION THROUGH NMDA RECEPTOR-MEDIATED SIGNALLING CASCADES

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Megakaryocytes reside in bone marrow and regulate circulating platelet numbers. However it is also clear that these cells are instrumental in maintaining bone marrow homeostasis by releasing numerous cytokines and growth factors that regulate haematopoietic and mesenchymal stem cell differentiation. Transgenic manipulation of megakaryocyte differentiation in vivo frequently induces skeletal defects, demonstrating the importance of identifying intercellular communication pathways within the bone marrow microenvironment. Here we provide evidence that the neurotransmitter glutamate plays an instrumental role in regulating these interactions. Using a fluorimetric assay, we demonstrated that human bone marrow stromal cells (BMSCs) release glutamate at concentrations sufficient to activate receptors expressed on neighbouring marrow cells and fluorescent parachute assays confirmed that megakaryocytic cells form intimate interactions with BMSCs. Primary human megakaryocytes derived from CD34+ umbilical cord blood cells express the NMDA-type glutamate receptor subunits NMDAR1, NMDAR2A and NMDAR2D as well as proteins associated with NMDA receptor signalling such as Yotiao and PSD-95. Furthermore, blocking these receptors with the selective antagonist MK-801 significantly inhibited proplatelet formation, cell enlargement and expression of the megakaryocyte markers CD61, CD41a and CD42a without affecting cellular proliferation or viability. At the ultrastructural level, MK-801 treatment prevented the formation of multi-lobed nuclei, alpha-granules, expanded demarcation membrane systems and proplatelet structures, which were present in mature control megakaryocytes. Using western blot analyses we demonstrated that addition of MK-801 to megakaryocyte-like Meg-01 cells, treated with the phorbol ester PMA to promote differentiation in the presence of exogenous glutamate, inhibited the phosphorylation of calcium-calmodulin dependent protein kinase II (CaMKII), a specific downstream mediator of NMDA receptor signalling in neurons. Exposure of Meg-01 cells to KN-93, an inhibitor of CaMKII, caused profound alterations in morphology, size and adhesion. MK-801 and KN-93 treatment also inhibited the phosphorylation of Erk1/2, a key regulator of megakaryocyte-specific gene transcription. These findings clearly indicate that paracrine glutamate signalling within the bone marrow compartment can regulate megakaryocyte differentiation and function through NMDA receptor-dependent intracellular signalling pathways, which will have fundamental implications on our understanding of marrow cell communication.

OC16

ADENOVIRAL BMP-2 GENE TRANSFER IN MESENCHYMAL STEM CELLS - IN VITRO AND IN VIVO BONE FORMATION ON BIODEGRADABLE POLYMER SCAFFOLDS

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Development of new bone formation strategies offers therapeutic implications in a variety of musculoskeletal diseases. The aim of this study was to determine the feasibility of adenoviral gene transfer into human bone marrow cells, in combination with a porous biodegradable scaffold to tissue-engineer bone. The ability of primary bone marrow cells to be transduced by adenoviral constructs was examined using AxCALacZ, a replication deficient vector carry the E. coli LacZ gene. Transduced cells showed positive staining for beta-galactosidase using X-Gal with efficiency close to 100% at a multiplicity of infection (MOI) of 6.25 to 100. Uninfected cells showed no beta-galactosidase activity. Additional bone marrow cells were isolated and transduced with AxCAOBMP-2, a vector carrying the human BMP-2 gene. BMP-2 activity from transfected osteoprogenitor cells was assayed using promyoblast C2C12 cells. C2C12 cells are exquisitely sensitive to BMP-2 with induction of alkaline phosphatase activity (ED50 20nM) in a dose dependant manner. The media from bone marrow cells, from 4 patients (14 -72 years of age) transduced with AxCAOBMP-2, contained 10-165 nM BMP-2. Media from uninfected controls failed to produce any activity.

Expression of alkaline phosphatase activity, type I collagen formation and mineralisation as assessed by von Kossa confirmed bone cell differentiation and maintenance of the transduced osteoblast phenotype in extended culture for up to 6 weeks on porous poly(-lactic acid co-glycolic acid) (PLGA) (75:25) scaffolds. Additional adenovirally transfected cells cultured in basal media were injected into diffusion chambers containing PLGA scaffolds and implanted intraperitoneally in 5 nude mice. X-ray analysis indicated the presence of bone tissue after only 4 weeks in 3 of 5 mice. Metachromatic staining with toluidine blue in combination with von Kossa staining as well as alcian blue, Sirius red staining and type I collagen immunohistochemistry confirmed the presence of cartilage and bone. Uninfected control cells alone showed no evidence of bone or cartilage formation.

These results indicate the ability to deliver active BMP-2 using human osteoprogenitors transduced with adenovirus. The maintenance of an osteoblast phenotype in extended culture and ex vivo bone formation on a porous, biodegradable scaffold offers a realistic approach to engineer replacement bone tissue.

OC17

IDENTIFICATION AND CHARACTERISATION OF PRESENILINS IN OSTEOBLASTS AND THEIR ROLE IN THE CANONICAL WNT SIGNALLING PATHWAY

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Presenilins (PS) are polytransmembrane proteins that can be proteolytically processed to generate functional N-terminal (NTF) and C-terminal (CTF) intracellular fragments. Missense mutations in PS1 and PS2 are the primary cause of familial Alzheimer's disease although the normal cellular function of these proteins is unclear. PS1 knockout mice have clear neuronal deformities, however they also exhibit gross skeletal defects, including severely malformed axial skeletons with marked underossification. We have now characterised PS expression, regulation, processing and intracellular interactions in osteoblasts, providing the first evidence of a direct role for PS in bone biology.

On frozen sections of embryonic and neonatal rat bones, PS1 and PS2 immunoreactivity was associated with cartilaginous condensations formed during rib development at embryonic day 16 and on periosteal and endosteal osteoblasts in postnatal tibiae. By northern and western blot analyses we demonstrated that SaOS-2, MG-63 and TE85 osteoblast-like cells expressed full-length PS1 and PS2 and their proteolytic NTF and CTF products. Over a period of 20 days, cultured in the presence of L-ascorbic acid and dexamethasone, PS2 expression by primary rat osteoblasts increased significantly during differentiation, whilst PS1 expression remained constant over the same time period. Fluorescence microscopy revealed that immunoreactive PS1 and PS1 CTF were present in dense plaques located at the periphery of osteoblastic cells, with a more diffuse cytoplasmic distribution that frequently formed filamentous alignments which colocalised with F-actin. In MG-63 osteoblast-like cells, full-length PS1 immunoprecipitations generated three major immune complexes of approximately 70kDa, 120kDa and 200kDa, and endogenous PS1 CTF co-immunoprecipitated with endogenous glycogen synthase kinase-3 beta (GSK-3 beta) and beta-catenin, key downstream mediators of the canonical Wnt signalling pathway. Application of lithium, which mimics Wnt signalling by inhibiting GSK-3 beta, induced an increase in full-length PS1 protein expression by SaOS-2 cells, without affecting actin or beta-catenin levels. Our evidence suggests strongly that PS1 and PS2 and their proteolytic products have prominent functions in normal osteoblast activity, acting as key regulators of Wnt-dependent signal transduction with possibly more pervasive actions in cell adhesion, differentiation and skeletogenesis.

OC18

AMPA TYPE GLUTAMATE RECEPTORS REGULATE OSTEOBLAST/ ADIPOCYTE PLASTICITY AND BONE FORMATION

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We have shown previously that NMDA, AMPA and Kainate type glutamate receptors regulate membrane currents and intracellular free calcium in osteoblasts and osteoclasts in a similar way to their function in neuronal cells. Furthermore, inhibition on NMDA receptor function inhibits bone formation in vitro. The present studies were performed to determine the function in bone of AMPA receptors, whose properties and functions differ from NMDA receptors in the CNS.

Calvarial rat osteoblasts were treated with the non-competitive AMPA antagonists CFM-2, GYKI52466 and SYM2206. At doses over 30 micromolar, these antagonists all caused dose dependent reduction in bone formation, concomitant with a dose dependant increase in numbers of adipocytes present. These observations were confirmed by reduced alkaline phosphatase activity, decreased expression of type 1 collagen, osteopontin and osteocalcin, abolition of mineralisation and a reciprocal increase in numbers of Oil Red O positive cells and increased expression of adipocytic markers, PREF-1, AP-2, PPAR gamma and LPL.

While the effects of the antagonists was to increase adipogenesis at the expense of osteoblastogenesis at moderate and high doses, there was a robust effect of low doses (10-15 micromolar) of CFM-2 and GYKI52466 but not SYM2206 to increase alkaline phosphatase and mineral expression. Collagen, osteopontin and osteocalcin were unchanged.

CFM-2 and GYKI52466 are structurally similar and bind to the thiazide sensitive antidesensitisation site of the AMPA receptors, while SYM2206 is chemically distinct and binds to a different region of the receptor.

These results indicate that AMPA receptor mediated signalling in preosteoblasts present in calvarial cultures has the ability to mediate changes of their lineage specification. The effect of low doses of antidesensitisation site antagonists to induce bone formation may be due to the stabilisation of conformational changes of open ligand-stimulated receptors and prolonged agonist stimulated events. It is known that thiazides induce effects on bone cell activity that has been suggested to be mediated by the thiazide receptor. These data suggest an alternative explanation for those effects, and a possible way to initiate anabolic effects on

OC19

IDENTIFICATION OF CBFA1-EXPRESSING OSTEOPROGENITOR CELLS BY FLOW CYTOMETRY

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Oestrogen-induced osteogenesis is associated with an increase in the number of Cbfa1-positive bone marrow cells in mice made heterozygous for cbfa1 by targeted deletion using a beta-galactosidase (lacZ) reporter construct. The increase in Cbfa-1 positive-cells precedes the oestrogen-induced increase in the number of osteoblasts and their immediate precursors (ALP-positive marrow cells close to bone surfaces). The aim of this study was to determine whether this putative osteoprogenitor population could be isolated from the bones of the mutant mice using flow cytometry. Adult cbfa1+/- mice (C57BL/6) were either treated with vehicle (n=4) or treated with a single dose of oestrogen (0.5mg; n=4). After 4 days, femorae were removed and bone marrow cells were flushed out. LacZ-positive cells were identified using the fluorogenic substrate FDG (FluoReporter lacZ flow cytometry kit, Molecular Probes) and non-viable cells identified using propidium iodide. To quantify non-specific staining, bone marrow was flushed from the femorae of littermate wild-type mice (n=4) treated with vehicle. Flow cytometry was performed using a Becton Dickinson FACS Vantage.

Following exposure to FDG, the majority of cells (>80%) remained viable. The mean numbers (± SEM) of FDG-positive cells were 1.48% (0.12) and 2.03% (0.20) for vehicle- and oestrogen-treated cbfa1+/- mice respectively ($p<0.05$). Non-specific staining represented 1.00% (0.06) of total. Taking this into account, treatment with oestrogen was associated with an approximately two-fold increase in the number of Cbfa1+ bone marrow cells. Preliminary data suggest that the cells in the Cbfa-1-positive fraction also possess unique forward scatter and side scatter characteristics, which may facilitate their isolation from the bones of normal as well as transgenic animals. In conclusion, we have demonstrated the feasibility of using flow cytometry to isolate a rare population of Cbfa1-positive cells from the bone marrow of transgenic cbfa1+/- mice. We anticipate that with further refinement this approach will greatly facilitate the immunochemical and molecular profiling of primitive osteoprogenitor cells.

OC20

EFFECTS OF LEPTIN ON NUMBER AND APOPTOSIS OF OSTEOBLASTS, AND CONTROL OF LEPTIN PRODUCTION BY GLUCOCORTICOIDS

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It has recently been postulated that leptin is a potent regulator of bone growth and mineralisation. Whether its effects on bone are direct or indirect, however, remains controversial.

We have investigated the effect of leptin (1.6 microg/ml) on number and apoptosis of primary human osteoblast-like (HOB) cells, an osteosarcoma cell line (MG63) and an osteoprogenitor cell line (HCC1). Cell number was measured with a haemocytometer, and apoptosis with Annexin V-FITC and flow cytometry. 2-Methoxyestradiol was used as a positive control (45% of cells undergoing apoptosis after 48h). We have also used gene array techniques to study the influence of leptin (1.6 microg/ml; 24h) on gene transcription in MG63 cells. Furthermore, we have measured the amounts of leptin produced by HOB cells prior to, and following incubation (up to 7 days) with dexamethasone, prednisolone, oestradiol, growth hormone, insulin, T3, TSH, testosterone, or 5alpha-dihydrotestosterone.

Cell numbers increased by 20% ($p<0.05$) following 24h culture of HCC1 cells with leptin, but there was no effect following 48h. Furthermore, cell numbers did not change following incubation of HOB or MG63 cells with leptin for 24h. Flow cytometry demonstrated a 37% ($p<0.05$) reduction of early apoptotic events in HCC1 cells incubated with leptin for 24h, but an increase (30%; $p<0.05$) in late apoptotic events at 48h. Furthermore, there were no changes in gene transcription, as assessed by gene array technology, following leptin treatment of MG63 cells. In addition, leptin expression was not detected in 3 HOB cell lines derived from children, although variable levels of leptin were detected following treatment with dexamethasone or prednisolone. The other compounds tested did not stimulate leptin production in these cells.

Our work indicates that leptin modulates osteoprogenitor cell numbers, and that this modulation is partly due to an inhibition (after 24h) or stimulation (after 48h) of apoptosis. This effect was not seen in more differentiated cell types. Also, leptin is not produced by any of the cell types. In the 3 HOB cell lines, however, it was possible to stimulate the production of leptin by glucocorticoids, but not by a range of other protein and steroid hormones.

OC21

ZOLEDRONIC ACID PREVENTS THE DEVELOPMENT OF MYELOMA BONE DISEASE AND INCREASES SURVIVAL

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Multiple myeloma is characterised by the growth of plasma cells in the bone marrow and the development of osteolytic bone disease. Myeloma cells are found closely associated with bone and targeting the local bone environment may therefore have effects on both bone disease and the growth and survival of myeloma cells. In the present study we have investigated the effect of the potent bisphosphonate, zoledronic acid, on the development of bone disease, tumour burden and disease free survival, in vivo, in the 5T2MM murine model of multiple myeloma. 5T2MM murine myeloma cells were injected intravenously into C57BL/KaLwRij mice. The myeloma disease was monitored by measuring serum paraprotein concentrations. After 8 weeks all animals had a detectable paraprotein. Animals were treated with zoledronic acid (120micrograms/kilograms, sub-cutaneously, twice weekly), or vehicle, either from the time of tumor cell injection (continuous) or from paraprotein detection (short-term) for 12 or 4 weeks, respectively. All animals injected with tumour cells developed radiographically detectable, osteolytic bone lesions. The presence of lesions was associated with significant decreases in cancellous bone volume in the tibiae and femora, decreases in bone mineral density and an increase in the number of osteoclasts lining bone surfaces. Continuous and short-term zoledronic acid treatment prevented the formation of lesions, preserved cancellous bone loss and reduced osteoclast perimeter. Zoledronic acid treatment also decreased serum paraprotein concentration (39% and 34%), reduced tumour burden and decreased microvessel density. To determine whether this was associated with an increase in survival mice were injected with 5T2MM myeloma cells, treated with zoledronic acid once a paraprotein was detected and the time to signs of morbidity determined. Kaplan-Meier analysis demonstrated a significant increase in the period of disease free survival in those animals treated with zoledronic acid when compared to control (median = 35.0 days (27.4-42.46) vs 47.0 days (34.0-60.0).

These data demonstrate that zoledronic acid prevents the development of osteolytic bone disease in this model of myeloma and is associated with a decrease in tumour burden and an increase in disease-free survival.

OC22

EFFECT OF PAMIDRONATE ON PERIPROSTHETIC BONE TURNOVER AND IMPLANT STABILITY AFTER TOTAL HIP ARTHOPLASTY

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Aseptic loosening due to periprosthetic bone loss is the main factor limiting implant survival after total hip arthroplasty (THA). We have previously shown that a single 90mg dose of the bisphosphonate pamidronate prevents bone loss over 6 months after THA. In this 2-year randomised trial extension study we assessed the effects of this intervention on bone turnover and implant stability.

Twenty-two patients (mean age 58 years [SD 12]) received 90mg of pamidronate and 22 (57 years [SD 13]) received placebo at randomisation 5 days after surgery. Femoral and pelvic bone mineral density (BMD) were measured by dual energy x-ray absorptiometry. Bone turnover activity was measured by single photon emission computed tomography (SPECT) and biochemical markers. Implant migration was measured using the EBRA-Digital method.

In the placebo group rapid periprosthetic bone loss occurred over the first 6 months, and was followed by a partial recovery in bone mass in most regions by 2 years. Patients in the pamidronate group had significantly less femoral, but not pelvic, bone loss than those give placebo (ANOVA P=0.02). Pamidronate was most effective in preventing bone loss in the proximal medial femur (Gruen zones 6 and 7; ANOVA P=0.004, and P=0.014, respectively). Pamidronate suppressed increases in cross-linked telopeptides of type-I collagen and osteocalcin over 2 years (ANOVA P<0.01), and SPECT activity at the lateral femoral shaft at 1 year (P=0.01). Changes in NTX-I and PINP in the placebo group at week 6 predicted 75% of proximal femoral bone loss at 2 years (P<0.001). Total stem migration at 2 years was 1.77mm [95% CI 0.27] and 1.62mm [0.37] for the placebo and pamidronate groups, respectively (P>0.05). Total cup migration was 0.75mm [0.26] and 0.76mm [0.14], respectively (P>0.05). Implant migration was not significantly related to changes in periprosthetic BMD or bone turnover.

Single dose pamidronate therapy preserves femoral bone mass and reduces bone turnover over 2 years after THA, but does not influence implant stability. Early changes in biochemical markers of bone turnover predict femoral bone loss at 2 years and may have a role in directing anti-resorptive therapy to at-risk individuals.

OC23

RANKL-INDEPENDENT INDUCTION OF HUMAN OSTEOCLAST FORMATION AND BONE RESORPTION BY TGF-BETA

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BACKGROUND; Osteoclast progenitors can differentiate into mature bone resorbing osteoclasts in the presence of macrophage colony stimulating factor (M-CSF) and RANK ligand (RANKL), which is expressed on bone stromal/osteoblastic cells. Osteoprotegerin (OPG) inhibits RANKL-induced osteoclast formation and bone resorption. It has been reported that tumour necrosis factor-alpha (TNF-alpha), a potent cytokine involved in regulation of osteoclast activity via a primary effect on osteoblasts, can directly (in the presence of M-CSF) induce the differentiation of osteoclast progenitors into mature osteoclasts. These studies revealed that TNF- alpha -induced osteoclast formation is independent of RANK/RANKL interaction. In the present study we sought to determine whether transforming growth factor beta (TGF-beta), another powerful modifier of bone resorption, can similarly induce osteoclast formation and bone resorption in vitro.

METHODS; Mononuclear cells were isolated from peripheral blood of healthy volunteers and cultured for up to 24 days on glass coverslips and dentine slices in the presence of: (i) RANKL and M-CSF; (ii) TGF-beta, M-CSF ± OPG; (iii) TGF-beta, M-CSF ± anti TNF-alpha antibody. In some experiments, CD14-positive mononuclear cells isolated using Mini MACS CD14 Micro Beads cell sorter were cultured. The extent of osteoclast formation and bone resorption was determined by generation of TRAP- and VNR-positive multinucleated cells, actin ring formation and lacunar resorption on dentine slices.

RESULTS; We noted that addition of TGF-beta, in the presence of M-CSF, but in the absence of RANKL, was sufficient to induce the formation of TRAP- and VNR-positive multinucleated osteoclast-like cells which were capable of forming actin rings and carrying out lacunar resorption on dentine slice in vitro. This osteoclast formation was also induced in CD14-sorted mononuclear cell cultures The addition of OPG or anti TNF-alpha antibody to the cultures containing TGF-beta did not inhibit osteoclast formation and bone resorption, thus suggesting that TGF-beta induces osteoclast formation in manner independent of the RANK/RANKL or TNF-alpha mechanism.

CONCLUSION; Our results indicate that TGF-beta, which is abundant in bone and thought to have potent effects on bone metabolism, can directly induce osteoclast precursors to differentiate into active bone resorbing osteoclasts, by a RANKL-independent mechanism.

OC24

POSSIBLE ROLE FOR TGF-BETA-INDUCED SOCS EXPRESSION IN OSTEOCLAST/MACROPHAGE LINEAGE COMMITMENT IN-VITRO

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The ligand that induces osteoclast (oc) differentiation has been identified as RANKL, a member of the TNF superfamily. However, only some precursors form oc when incubated with RANKL, suggesting that RANKL alone might not be sufficient to ensure that precursors become oc at sites of resorption. Responses to stimuli from the TNF superfamily are characteristically associated with inputs from other factors such as TGF-beta. Previously, we have shown that TGF-beta augments oc formation. Furthermore, oc formation is abolished by anti-TGF-beta antibodies, suggesting that oc that form without the addition of exogenous TGF-beta are dependent on TGF-beta in the medium or produced by precursors themselves.

The mechanism by which TGF-beta facilitates oc formation is unknown. One possibility is that the environment in-vitro is essentially pro-inflammatory, due to the presence of agents such as interferons, and TGF-beta opposes this. Interferons signal via the JAK-STAT pathway, and TGF-beta might therefore block these signals. Recently, a group of STAT-induced factors, termed SOCS (suppressors of cytokine stimulation), have been isolated which form part of the negative-feedback mechanisms that switch off JAK-STAT signalling after cytokine stimulation. Therefore, we proposed to identify those SOCS expressed in oc, and establish if SOCS expression plays a role in the mechanisms by which TGF-beta enables oc formation.

To assess the pattern of SOCS1-4 expression in oc and their precursors we performed Northern analysis and quantitative PCR on RNA extracted from macrophages, oc and precursor cells incubated with or without TGF-beta-1. Interestingly, we found that while SOCS1-4 mRNA is not detectable in macrophages, oc express SOCS3 mRNA, and TGF-beta further upregulates this expression. Furthermore, TGF-beta induces SOCS3 expression in uncommitted precursors within 1 hour.

To determine if SOCS3 plays a role in the mechanisms by which TGF-beta facilitates oc formation we examined the effect of overexpressing SOCS3 in precursors using retrovirus. We found that oc formation and bone resorption were significantly enhanced in SOCS3-infected cells. This suggests that TGF-beta-induced expression of SOCS3 mRNA may represent a mechanism by which TGF-beta suppresses inhibitory cytokine signalling, priming precursors for oc differentiation.

OC25

MULTILINEAGE POTENTIAL OF CD34-NEGATIVE UMBILICAL CORD BLOOD CELLS

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Bone marrow acts as a reservoir for haematopoietic and mesenchymal progenitor cells, however as a resource both for clinical and research purposes its availability and accessibility often proves to be problematic. Umbilical cord blood (UCB) is routinely used for the extraction of haematopoietic stem cells expressing the CD34 cell surface marker (CD34+), however the use of UCB as a source of non-haematopoietic cells has only recently been investigated. It is also believed that more primitive multi/pluripotent stem cells, which are CD34-negative (CD34-), may also be present in UCB. With these points in mind. we used a range of culture conditions to determine the differentiation potential of cells derived from the CD34- fraction of UCB. Blood was extracted from the cord and placenta following pre-term caesarian sections and via informed consent. UCB was separated through Ficoll and immunomagnetically depleted of CD34+ cells prior to culture. Over a period of 4-7 days, colonies of adherent cells developed with a fibroblast-like appearance, whilst non-adherent cells failed to survive in the culture conditions. In prolonged culture and using appropriate inductive stimuli, the adherent cells were capable of differentiating into oil red O-positive adipocytic cells and alkaline phosphatase-positive osteoblast-like cells that were able to form mineralised nodules in the presence of beta-glycerophosphate. Cells of a neuronal phenotype were also generated directly from CD34- UCB using neurogenic conditions or by switching the differentiated osteoblasts from osteogenic to neurogenic conditions. These cells expressed the neurofilament marker alpha-internexin and adopted a stellate morphology with contracted cell bodies and numerous cytoplasmic extensions. In the absence of dexamethasone, multinucleated TRAP- and CD51-positive cells were also generated from the CD34- fraction. These osteoclast-like cells developed from a heterogeneous cell population over 28 days in culture and were able to form resorption pits in dentine slices in the absence of exogenous RANKL and M-CSF.

We have established conditions by which blood can be used as a source for progenitor cells that are able to differentiate into haematopoietic, mesenchymal and neuronal phenotypes. These findings will significantly advance our understanding of stem cell (trans)differentiation and impact on future research and therapeutic strategies.

C1

ZOLEDRONATE IN THE MANAGEMENT OF ACTIVE PAGET'S DISEASE

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We report the case of a 64 year old West Indian gentleman with a 14 year history of polyostotic Paget's disease. The following skeletal sites were identified as being affected following isotope bone scan and radiographical imaging: skull, mandible, thoraco-lumbar spine, sacrum, pelvis and left femur. There was no history of deafness or fracture. Over the previous 9 years he had evidence of active disease with elevated total serum alkaline phosphatase (ALP) and associated bone pain at various sites. During this time he was treated with various agents including calcitonin injections, oral etidronate and oral tiludronate, and intravenous pamidronate. Previously treatment was associated with a good clinical and biochemical response.

In 2001 he presented with increasing skull and jaw pain, with ALP elevated at 400 IU (normal range 30-130 IU). Despite receiving two repeated courses of pamidronate (3 x 60 mg), his disease remained active. A decision was therefore made to treat him with a single infusion of zoledronate 4 mg. This procedure was well tolerated with no adverse side-effects. In addition, there was no evidence of hypocalaemia or impairment of renal function following the infusion. Over a 6 week period his pain symptoms improved and this was mirrored by a 58% fall in the ALP (from 569 to 285 IU/l). Further follow-up is continuing to determine whether his ALP will decrease into the normal range and to assess the duration of the treatment effect.

This case illustrates the potential for zoledronate to be important in the management of patients with active Paget's disease. The results of further comparative studies are awaited with interest.

C2

GOSERELIN AND SEVERE SYMPTOMATIC OSTEOPOROSIS

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Traditional treatments for prostatic carcinoma have included orchidectomy or 'medical castration' with diethylstilboestrol. Since the 1980s luteinising hormone-releasing hormone (LH-RH) analogues have been used for medical castration. We report three men who were referred to the bone clinic after spinal fractures.

Case 1, aged 84, was referred in 2000 because of compression fractures of T11 and T12. He had previously sustained a sub-capital fracture of the right femur after a fall. Dual energy x-ray absorptiometry of the radius showed severe osteoporosis with mean z-score of minus 2.06. Biochemistry was unremarkable apart from low levels for serum testosterone.

Case 2, aged 67, was referred in 1998 because of increasing kyphosis and difficulty in walking. Mean radial z-score was minus 2.72. Biochemical investigations were negative but, unaccountably, no testosterone assay was done. He was treated with cyclical etidronate. He was re-referred in 2000 because of a continuing increase in the kyphosis. Reinvestigation demonstrated low values for serum testosterone.

Case 3, aged 72, was referred in 1999 because of crush fractures of T11, T12 and L5. He had chronic renal failure, due to polyarteritis and had been on dialysis for the preceding three years. Mean radial z-score was minus 2.83 and his prednisolone therapy, typically in a dose of 7.5 mg/day, was thought to be the likely principal cause. Serum parathyroid hormone at this time was normal. He was treated with cyclical etidronate. He was re-referred in 2000 after a further crush fracture. There was no evidence of metastatic disease.

Each patient was found to have a history of prostatic carcinoma and each had been treated with goserelin for at least five years. We were slow to recognise the significance of this therapy in the likely pathogenesis of the osteoporosis. While we know of no previous case reports of this association it was entirely predictable from the known mode of action of this drug and there have been reports of diminution of bone density in groups of treated patients. We would wish to discuss whether patients on goserelin and similar drugs should be targeted for bone densitometry and if so when.

C3

MANAGEMENT OF REGIONAL MIGRATORY OSTEOPORORIS

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A previously healthy, 56 year old Caucasian lady presented with a 6-month history of pain affecting her right foot. There was no history of trauma. The pain was constant with exacerbation during activity. There was only minimal early morning stiffness. Symptoms had failed to improve with rest and with simple analgesics/NSAIDs. There were no other joint symptoms of note. The patient was postmenopausal and was not taking hormone replacement therapy or calcium supplements. There was no history of bone disease or fracture in the patient or first-degree relatives. General physical examination showed no significant findings.

Initial investigation with plain radiography of the ankle joint showed only degenerative change with periarticular osteophytes. A subsequent isotope bone scan showed increased uptake either side of the tibio-talar joint. Routine blood investigations (FBC, ESR, U&Es, LFTs, Calcium, Phosphate, Rheumatoid factor) at this time were all normal and excluded an active inflammatory joint disease or an infective process. MRI scan, however, demonstrated marked oedema in the right talus.

A clinical diagnosis of regional migratory osteoporosis was made. Formal measurement of her bone mineral density using DXA demonstrated osteopenia at both lumbar spine (L2-L4) and hip, with results normal for an age-matched population. The patient was treated with a 2-month course of risedronate 30 mg. During this time her symptoms improved significantly and she was pain free when assessed at 4 months. The results of further imaging studies to determine whether there has been an associated reduction in oedema are awaited.

This case suggests that risedronate may be useful in the symptomatic management of patients with regional migratory osteoporosis.

C4

MISSING LOOPS - A CASE OF TERTIARY HYPERPARATHYROIDISM

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A 44 year old lady was admitted in June 2001 for assessment prior to small bowel transplant. On admission, she was significantly under weight (BMI 19.5), with bone pain and thoracic kyphosis. In 1979, fulminant colitis had necessitated total colectomy. Further bowel resections followed as remission of her Crohn's disease could not be achieved. By 1986, only 140cm of jejunum remained, resulting in ongoing difficulties with hypocalcemia, hypomagnesaemia and with dehydration that led to chronic renal impairment.

Investigations during assessment revealed urea 16.2mmol/L, creatinine 257mcmol/L; corrected calcium 2.83 mmol/L, serum inorganic phosphate 1.61 mmol/L and alkaline phosphatase 282u/L. On lateral thoracolumbar radiographs, codfish vertebral deformities, endplate sclerosis and trabecular coarsening were seen. DXA demonstrated bone loss at lumbar spine (L1-4, T-2.64) and total hip (T-3.11). To distinguish between osteoporosis (due to hyperparathyroidism) and osteomalacia, a bone biopsy was undertaken. This showed both osteomalacia and changes of secondary hyperparathyroidism, with increased osteoid seam width, granular and diffuse calcification fronts and markedly increased resorption with both woven and lamellar bone formation. Her parathyroid hormone level was grossly elevated at 1591ng/mL, with a normal 25-hydroxyvitamin D3 level (15.3 mcg/mL).

Hypocalcaemia stimulates the parathyroid glands through a short feedback loop to produce PTH, which acts on kidney and bone to increase extracellular calcium. PTH also acts indirectly by stimulating renal 1-alpha hydroxylation of 25-hydroxyvitamin D3 to increase intestinal absorption of calcium. This is crucial if bone mineral is to be preserved, and failed in this lady as a result of her missing bowel, with no negative feedback to the parathyroid gland. Her renal impairment also resulted in hyperphosphataemia, which indirectly stimulates PTH production. A direct effect, independent of calcium and 1,25-dihydroxyvitamin D3, has also been postulated that leads to parathyroid hyperplasia. While 25-hydroxyvitamin D was normal in this case, her renal impairment may also have reduced 1,25-dihydroxyvitamin D3 levels, which is an important suppressor of PTH expression and of parathyroid gland hyperlplasia.

This case illustrates the progression from hypocalcaemia with secondary hyperparathyroidism to autonomous secretion of PTH resulting in hypercalcaemia and in severe hyperparathyroid bone disease.

P1

FROM RECEPTOR ACTIVATION TO GENE TRASCRIPTION: FUNCTIONAL WNT SIGNALLING IN OSTEOBLASTS

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Wnt signalling plays fundamental regulatory roles in embryonic patterning, cell fate determination and tissue pathophysiology. In this study we provide compelling evidence for functional Wnt/beta-catenin signalling in osteoblasts. In the absence of Wnt signals (the 'off' state of the canonical Wnt signalling pathway), glycogen synthase kinase-3-beta (GSK3-beta) phosphorylates beta-catenin through an association with adenomatous polyposis coli (APC) and axin, forming a multi-protein destruction complex which induces constitutive beta-catenin degradation through the ubiquitin/proteasome pathway. In the 'on' state, Wnt binds to cell surface Frizzled (Frz) receptors and activates intracellular dishevelled (Dvl), inhibiting GSK3-beta and stabilising beta-catenin, promoting its translocation to the nucleus where it interacts with T-cell factor/leukaemia enhancing factor (TCF/LEF) to regulate Wnt target gene expression. Using a combination of RT-PCR, immunolocalisations and western blot analyses we have demonstrated expression of secreted Wnt ligands (Wnt2, Wnt3a, Wnt4, Wnt2b2, Wnt5a and Wnt11), GSK3-beta, axin, APC and beta-catenin in primary human osteoblasts (hOBs) and osteoblast-like cell lines. We have also shown that osteoblasts express a wide variety of Frz receptors, Dvl 1 and 2 and several frizzled related peptides (sFRPs), which act as soluble decoy receptors and endogenous antagonists of Wnt signalling. To study the functional consequences of osteoblastic Wnt signalling we inhibited GSK3-beta with LiCl to mimic Frz activation. In hOBs and osteoblast-like cells, LiCl (5-20mM) significantly inhibited GSK3-beta activity and evoked a time and dose-dependent decrease in beta-catenin phosphorylation, which was accompanied by increased nuclear translocation compared to basal levels. We also report that osteoblasts express TCF/LEF transcription factors and using a specific luciferase reporter gene assay we demonstrated that brief inhibition of GSK3-beta significantly increased TCF/LEF dependent gene expression, correlating with increased nuclear beta-catenin translocation. Furthermore in differentiating cultures of hOBs, lithium-induced Wnt activation significantly reduced alkaline phosphatase activity and bone nodule formation compared to controls, supporting a functional role for Wnt signalling in the regulation of osteoblast differentiation. Taken together, these data provide substantial evidence for the existence of a functional osteoblastic Wnt/beta-catenin/TCF signalling pathway, providing a penetrating insight into our understanding of bone physiology and identifying possible new therapeutic targets for the treatment of bone disorders.

P2

11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 ACTIVITY DETERMINES THE EFFECTS OF GLUCOCORTICOIDS ON BONE

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Glucocorticoid excess decreases bone formation and bone mineral density (BMD), and increases fracture risk but these effects are difficult to predict in any given individual. We recently described expression of 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) in osteoblasts where this enzyme generates active cortisol (or prednisolone) from inactive cortisone (or prednisone). We hypothesised that this enzyme modulates the effects of glucocorticoids on bone. We have now defined the determinants of variation in the response of bone markers to glucocorticoid therapy. 20 healthy males (age 31+/-8; mean+/-SD) took 5mg prednisolone twice daily orally for 7 days. 24hr urinary steroid metabolites (by GC/MS), BMD and biochemical bone markers (osteocalcin (OC), N-terminal propeptide of type I collagen (PINP), serum C-telopeptide of collagen cross-links (betaCTx) and PTH) were measured before steroids and repeated at days 4 and 7. Prednisolone absorption, prednisone generation and steroid half-lives were subsequently determined (by HPLC) post oral prednisolone (5mg at 0900). OC and PINP both decreased at days 4 and 7 (29+/-11ng/ml vs 18+/-8 and 19+/-8 for OC; 67+/-24ng/ml vs 52+/-19 and 52+/-16 for PINP; all $p<0.001$) but betaCTx and PTH did not change significantly. There was a negative correlation between 11beta-HSD1 activity (measured as the urinary (THF+alloTHF)/THE ratio) at baseline and OC at days 4 and 7 ($r=-0.58$ and -0.56; both $p<0.01$) and PINP at day 4 ($r=-0.51$; $p<0.05$). There was no correlation with 11beta-HSD2 activity (urinary cortisol/cortisone ratio), total steroid metabolites, BMD, peak prednisolone or prednisone level, or prednisolone half-life. However, the fall in serum prednisolone levels after peak prednisone levels had been achieved negatively correlated with (THF+alloTHF)/THE ($r=-0.7$, $p<0.05$) suggesting an increased circulatory prednisolone half-life due to increased prednisolone regeneration.

Urinary measures of 11beta-HSD1 activity predict the response of bone markers to glucocorticoids. This may partly be through hepatic regeneration of active from inactive glucocorticoid. Additionally, increased 11beta-HSD1 activity in osteoblasts may increase osteoblastic glucocorticoid levels at an autocrine level by local steroid activation. Measures of 11beta-HSD1 activity may predict the skeletal response to glucocorticoids and may form the basis of a test to predict individual susceptibility.

P3

OSTEOBLAST-STIMULATING FACTOR-1 ENHANCES OSTEOGENIC DIFFERENTIATION OF BONE MARROW STROMAL CELLS, BUT IS NOT OSTEOINDUCTIVE

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Osteoblast-stimulating factor-1 (OSF-1) is synthesized during early stages of osteoblast differentiation and secreted into the bone matrix. OSF-1 over-expression in mice maintained bone growth for longer and enhanced bone mineral content. The present study aimed to examine i) whether OSF-1 affected osteogenic differentiation of marrow stromal cells; ii) whether OSF-1 had BMP-like osteoinductive effects, and iii) whether OSF-1 influenced osteoinduction by BMP-2.

Murine bone marrow stromal cells (MSCs) were used to elucidate the effect of OSF-1 on osteogenic differentiation. When MSCs were cultured with 10 pg/ml of OSF-1, specific alkaline phosphatase (ALP) activity was increased by 30-50% in basal and osteogenic media. In addition, proliferation was enhanced, as shown by increased DNA content. C2C12 cells (pre-myoblastic murine cell line) were used to investigate the ability of BMP-2 and OSF-1 to induce osteogenic differentiation of pluripotent cell populations. When C2C12 cells were cultured with 50-100 ng/ml rhBMP-2 for two days, their normal course of myoblastic differentiation was diverted to an osteoblastic lineage, as indicated by appearance of numerous ALP-positive cells. In contrast, OSF-1 (between 5 pg/ml and 100 ng/ml) failed to induce osteogenic differentiation in C2C12 cells. Interactions between BMP-2 and OSF-1 were studied by culturing C2C12 cells for two days in presence of 100 ng/ml rhBMP-2 together with 0.05, 5, and 100 ng/ml of OSF-1. OSF-1 inhibited the osteoinductive action of rhBMP-2, as evidenced by a significant decrease in specific ALP activity. However, once C2C12 cells had been stimulated to differentiate along the osteogenic lineage by rhBMP-2 for two days, their osteogenic phenotype was enhanced if OSF-1 (at 5 - 10 pg/ml) was added during the second phase of culture.

In summary, i) OSF-1 stimulated osteogenic differentiation of murine marrow stromal cells at very low concentrations. ii) Although OSF-1 lacked the osteoinductive capability of rhBMP-2 and antagonized BMP-2 during the initial osteoinduction phase, it was able to enhance the osteogenic phenotype of differentiated pluripotent cells after induction had taken place.

These results suggest that OSF-1 exerts its actions on osteogenic differentiation of committed progenitor populations, and although not osteoinductive, enhances the osteogenic phenotype at appreciably low concentrations.

P4

ESTIMATES OF NET ENDOGENOUS ACID PRODUCTION (NEAP) ARE ASSOCIATED WITH INCREASED BONE TURNOVER IN EARLY POSTMENOPAUSAL WOMEN: FINDINGS FROM APOSS LONGITUDINAL STUDY

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Fruit & vegetable intake appears to be positively linked with markers of bone health. A hypothesis gaining much renewed interest is that, fruit & vegetables, by provision of alkaline salts, balance the acidity generated by eating a mixed diet. A simple way of estimating net endogenous acid production (NEAP) is to use the ratio of dietary protein to potassium (1).

We examined the relationship between bone resorption markers and NEAP in women selected from APOSS, a study on 5119 women who had a first BMD scan in 1990-3, 3883 of whom returned for a second scan in 1997-2000. At the follow-up visit 3239 women (aged 50-63 y) completed a validated food frequency questionnaire. Most women provided a urine sample for measurement of free pyridinoline (fPYD) and deoxypyridinoline (fDPD) cross-links by HPLC (expressed relative to creatinine). Women taking bisphosphonates or suffering from thyroid disease were excluded.

Bone resorption markers were found to increase with increasing quartiles of NEAP. Mean ± SD for fDPD/cre were as follows: q1, 5.1±1.8; q2, 5.2±2.1; q3, 5.3±2.0; q4, 5.5±2.0 (P=0.001) and for fPYD/cre: q1, 18.6±6.1; q2, 18.8±6.9; q3, 19.4± 7.0; q4, 19.8±6.9 (P=0.002). This remained significant after adjusting for age, weight, height, smoking status, socio-economic status, physical activity level and menopausal status/ HRT use (fDPD/cre P=0.010; fPYD/cre P=0.031). Furthermore, analysing HRT users and postmenopausal non-users separately, the relationship between NEAP and fDPD/cre was significant for current HRT users (n 1023, P=0.003) but failed to reach significance for either perimenopausal (n 194, P=0.071) or postmenopausal never users (n 827, P=0.065). As there is increased bone resorption in the perimenopausal/early postmenopausal period, it is interesting to note that for women more than 2 years postmenopausal the relationship between NEAP and fDPD/cre was statistically significant (n 664, P=0.001).

These results support baseline APOSS findings (2) and recent DASH II trial results (3), and provide further evidence that a high consumption of alkali-forming foods may benefit the skeleton because they counterbalance the acid forming foods.

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P5

PATTERNS OF EXPRESSION OF CHEMOKINES AND THEIR RECEPTORS IN OSTEOCLASTS

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Although much has been learned recently of the mechanisms by which osteoclast differentiation and function are regulated, less is known of the factors that regulate their migration and localisation. In related cell types, chemokines play a major role in these processes. We therefore systematically tested the expression of RNA for chemokine receptors and their ligands by osteoclasts. Because bone is the natural substrate for osteoclasts and may influence osteoclast behaviour, we also tested expression on bone slices. Quantitative RT-PCR using real time analysis with sybr green was therefore performed on RNA isolated from bone marrow cells after incubation with M-CSF with-without RANKL, on bone or plastic, and assayed for known murine chemokines and their receptors. TRAP expression was quantified as a measure of osteoclast differentiation. We found that RANKL induced a 100-fold increase in TRAP in cells grown on plastic. This was increased a further 10-fold when grown on bone. RANKL reduced the expression of RNA for the Fc receptor (CD16) and c-fms. The chemokines most highly expressed by bone marrow cells incubated on tissue culture plastic with M-CSF and RANKL were: CX3CL (neurotactin/fractalkine), CCL2 (MCP-1), CCL3 (MIP-1alpha), CCL5 (RANTES), CCL7 (MCP-3), CCL9 (MIP-1gamma) (up to 50-fold), CCL22 (MDC) (up to 10-fold), CCL12 (MCP-5) and CXCL14 (BRAK). The most abundant chemokine receptor in the presence of RANKL was CCR1, followed by CCR3, CCR4, CCR5, CX3LR-1 and CXCR4. Cells incubated on bone showed further upregulation of CCR1, CCR3, CCR6, CCR7, CXCR4 and CX3CR. However, we found that the major effect of RANKL was to reduce RNA for several chemokines strongly associated with inflammation. Thus, CCL2 (MCP-1) and CCL3 (MIP-1alpha) were strongly inhibited by RANKL. Cells incubated on bone showed even greater inhibition, with expression of CCL7 (MCP-3) and CXCL9 (MIG) becoming undetectable.

The expression of CCL9 (MIP-1gamma) and CCL22 (MDC) and their corresponding receptors (CCR1 and CCR3) suggests an autocrine or paracrine role for these chemokines in osteoclasts. Initial studies have shown no effect of these agents on osteoclast differentiation or resorption, but both agents stimulate cytoplasmic motility, suggesting that they may play a role in osteoclastic migration/localisation.

P6

INTERACTIONS BETWEEN ESTROGEN AND GLUTAMATE SIGNALLING PATHWAYS

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Estrogen receptors (ER) are clearly involved bone loss in postmenopausal osteoporosis, but the mechanism by which this occurs is not completely clear. Since it is known that glutamate signalling in bone is involved in both formation and resorption, and that glutamate signalling in the CNS is modified by oestrogen, these studies were designed to test the hypothesis that ER mediate the effect of glutamate on bone cells during osteogenesis.

Bone marrow cells from mice lacking separately genes for ER alpha (ERKO) and ER beta (BERKO), and heterozygotes of each genotype as controls were cultured for 18 days with or without the NMDA receptor antagonist MK801 (100muM). At the end of this period cultures were stained for alkaline phosphatase, calcium and collagen, and total cell and colony numbers were counted.

ERKO cells (+/- and -/-) were more fibroblastic than BERKO cells and formed less discrete colonies, despite the same seeding density. In neither genotype was there a difference between the osteoblastic colonies of +/- and -/- cells, but both BERKO genotypes had lower alkaline phosphatase activity than ERKOs. MK801 reduced the number of colonies in ERKO cultures, but colonies that formed were more normally discrete and more mineralised. In BERKO cultures treated with MK801, there was a profound inhibition of colony formation, numbers of colonies and mineral apposition. ERKO cultures contained many adipocytes and MK801 increased profoundly their numbers. In BERKO cultures MK801 only increased the numbers of adipocytes slightly and almost abolished osteoblastic colony formation.

While ERKO and BERKO cells have different profiles of expression of markers of osteoblast phenotype, treatment with MK801 increases the similarity between colonies from each genotype. The pronounced increase in adipocyte numbers in ERKO colonies, and higher levels of expression of osteoblastic markers suggests that rather than inducing transdifferentiation, MK801 increases the proliferation of adipogenic precursors. In contrast, the effect of MK801 in BERKOs was to inhibit osteoblastic differentiation, while having minimal effects on adipogenesis. These data suggest that the interactions between ER and glutamate signalling pathways are complex, but that ER alpha and ER beta have different roles in lineage specification in marrow precursors.

P7

THE OESTROGEN RECEPTOR ACTIVATES BMP-6 IN A NON-LIGAND-DEPENDENT MANNER

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High dose oestrogen is known to induce osteogenesis in the long bones of adult mice. In searching for factors that play a role in this process, a significant increase in Bone Morphogenetic Protein 6 (BMP-6) transcripts was observed in mRNA isolated from femora of animals treated with 17 beta-oestradiol (E2) compared to vehicle (Plant et al. 2002). Based on evidence that E2 increases BMP-6 expression in osteoblasts in a cell autonomous manner in vitro (Rickard et al 1998), we explored the mechanisms by which oestrogen regulates BMP-6 expression in osteoblasts. Initially, the activity of a 1.2Kb BMP-6 promoter sequence coupled to a luciferase reporter (Tamada et al 1998) was compared in ROS and SMER osteosarcoma cells, the latter representing ROS cells stably transfected with oestrogen receptor (ER) alpha. Basal BMP-6 reporter activity was found to be considerably higher in SMER versus ROS cells, with no further increase after the addition of E2. The ligand independent role of the receptor was further confirmed when we observed that co-transfection of ROS cells with an ER alpha expression construct resulted in a four fold increase in reporter activity irrespective of ligand treatment. Equivalent findings were observed in liver (HepG2) and renal (COS) cell lines, and in cells transfected with an expression construct for ER beta. Taken together, these results indicate that ER acts to increase the level of BMP-6 promoter activity in a non ligand dependent manner irrespective of cell background. In light of recent evidence that oestrogen increases ER alpha expression in osteoblast precursors (Zhou et al 2001), we propose that BMP-6 transcription may rise in response to ligand as a consequence of elevated receptor expression, not due to the direct actions of the ligand.

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P8

EFFECT OF OESTROGEN ON BONE TURNOVER IN CORTICAL BONE IN POSTMENOPAUSAL WOMEN

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Previous histomorphometric studies of the effects of oestrogen on bone turnover have focused on changes in cancellous bone. The aim of this study was to investigate the effects of conventional hormone replacement therapy (HRT) and high-dose oestradiol on cortical bone in postmenopausal women.

Transiliac biopsies were obtained from 9 post-menopausal women aged 54-71yrs before (pre-HRT) and after 2 years conventional HRT (post-HRT) and 6 women aged 55-67 yrs who had received long-term high-dose oestradiol implant therapy (High E). Data from both cortices were averaged. Histomorphometric analysis was performed on 8 µm undecalcified sections using image analysis.

In postmenopausal women bone turnover was highest in the pre-HRT group and lowest in the high E group, values in the post-HRT group being intermediate. Thus bone formation rate (µm2/µm/d) was 0.121 ± 0.072 (mean ± SD) pre-HRT, 0.083 ± 0.015 post-HRT and 0.066 ± 0.045 in the high E group, the difference between pre-HRT and high E being statistically significant (p=0.05). A similar pattern was seen for activation frequency, the difference between pre-HRT and high E and post-HRT and high E both being statistically significant (p=0.008 and 0.034 respectively). Wall width was highest in the high E group (54.3 ± 10.2 µm); values in the pre-HRT and post-HRT groups were 47.5 ± 7.2µm and 52.1 ± 7.7µm respectively.

Our results provide the first histomorphometric evidence in postmenopausal women of dose-dependent oestrogen-induced suppression of bone turnover in iliac crest cortical bone. There was also a trend towards higher wall width with increasing dose of oestrogen, consistent with the previously reported anabolic effect in cancellous bone.

P9

SPECIFIC IMMUNOLOCALISATION OF A NOVEL PHOSPHATASE TO OSTEOBLASTS AND MINERALISING GROWTH PLATE CHONDROCYTES OF IMMATURE LONG BONES.

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Skeletal mineralisation is dependent on the generation of inorganic phosphate (Pi) and traditionally this action has been attributed to the bone/liver/kidney form of alkaline phosphatase. We have, however, previously reported a gene (PHOSPHO1) whose expression is upregulated in both growth plate chondrocytes and mineralising SAOS-2 osteoblast-like cells compared with non-mineralising MG-63 cells. The active site structure of PHOSPHO1 suggests that it is a phosphatase and together with the gene expression data has led us to speculate that PHOSPH01 may generate Pi for matrix mineralisation. The aim of this present study was to obtain experimental evidence that PHOSPHO1 is indeed a phosphatase and to determine its sites of expression in long bones by immunocytochemistry.

Recombinant derived PHOSPHO1 was produced by amplifying the protein coding sequence of chick PHOSPHO1 by PCR followed by cloning into bacterial expression vector pBAD-TOPO-TA. The expression of PHOSPHO1/(His)6 was induced using L-arabinose and the recombinant protein was purified by immobilized metal affinity chromatography. The recombinant protein catalysed the hydrolysis of p-nitrophenyl phosphate (pNPP) and the reaction followed Michealis-Menton kinetics with a Km of 33mM for pNPP. The distribution of PHOSHO1 expression by immunocytochemistry was achieved using an antiserum raised against recombinant-derived chick PHOSPHO1. Tissues including, heart, liver, lung, fat, skeletal muscle, kidney, rib and tibiae from 3-week-old chicks were chilled in n-hexane and cryostat sections were stained using an indirect immunoperoxidase procedure. PHOSPHO1 immunoreactivity was evident as a distinct band corresponding to early mineralising hypertrophic chondrocytes within the growth plate of long bones. Proliferating or terminally differentiated chondrocytes did not stain positively. The cartilage remnants directly underneath the growth plate are without a mineralising osteoid layer and did not show any immunoreactivity, however, strong staining was observed in osteoblasts on the surface of the more distal cartilage remnants within the metaphysis. All soft tissues and control sections in which the primary antibody was substituted with pre-immune serum were negative.

These results confirm that PHOSPHO1 is a phosphatase capable of the generation of Pi and that its protein expression is specific to mineralising chondrocytes and osteoblasts. Its importance in skeletal mineralisation has yet to be determined.

P10

LONGITUDINAL CHANGES IN BONE MINERAL DENSITY AND TURNOVER IN NORMAL PREGNANCY

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There is no consensus whether bone mineral density(BMD)changes during pregnancy and whether bone turnover remains coupled. We made longitudinal measurements during normal pregnancy in an attempt to answer these questions.

We measured BMD By Dual energy x-ray densitometry before conception and after delivery, bone resorption as serum beta-crosslaps(CTX)and bone formation from N-terminal pro-collagen peptide(P1NP)before conception and at 12,24 and 36 weeks of gestation in 46 women.

The main change in BMD was a 3.9% loss at the femoral trochanter with 1% losses at the spine and total hip. There was a good correlation between changes at hip and spine (Trochanter v LS r= 0.68 p , 0.0001)indicating a general loss of bone but no correlation between the change in BMD during pregnancy and the pre-conceptual value.

Pre-coceptual P1NP andCTX were correlated (r=0.51) and both indices fell by 12 weeks (p,0.001)and then rose to term with the rise in CTX preceding that of P1NP. At 36 weeks both P1NP and CTX were increased relative to baseline(P1NP p=0.013,CTX p=0.002).The fall in BMD depended on the rate of bone turnover either as the absolute value or as the change sinc conception.

Bone turnover must be uncoupled during pregnancy to account for the loss of bone but this seems to occur in the trochanter which may have less structural function than other regions of the hip or spine

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P11

3D MICRO-COMPUTED TOMOGRAPHY (MICRO-CT) OF TRABECULAR BONE FROM GROWTH HORMONE DEFICIENT TRANSGENIC RATS

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Childhood growth hormone (GH) deficiency is associated with osteopenia, but little is known about its effects on subsequent adult bone strength and fracture risk. We have previously presented results on femoral strength and bone mineral density in a model of moderate GH deficiency, the transgenic growth retarded (Tgr) rat. More recently, we have utilised micro-CT techniques to evaluate the microarchitecture of the distal femur from male, 15 week old, wild-type (W-T) and Tgr rats.

Bones were scanned with a high resolution micro-CT40 system (SCANCO Medical), with a voxel size of 12 micron in all three spatial dimensions and 178 slices measured for each sample. Trabecular and cortical parts of each distal femur were separated with semi-automatic drawn contours and the secondary spongiosa evaluated. Structural indices of the appearance of trabecular bone were assessed using 3-D techniques without model-assumptions.

Micro-CT analysis of the trabecular region of femurs from 15 week old males revealed that those from Tgr rats (n=3) had significantly less calcified tissue per unit volume (relative bone density) than W-T males (n=3) (14.1 ± 1.8 % vs 20.7 ± 1.9 mean ± SEM, $p<0.01$). This was due primarily to a reduction in both trabecular number (67.7 ± 3.7 connection/mm3 vs 83.3 ± 2.89, $p<0.05$), with a reduction in trabecular thickness (0.075 ± 0.002 mm vs 0.084 ± 0.002, $p<0.05$) and a corresponding increase in trabecular separation (0.4 ± 0.01 mm vs 0.29 ±0.02 mm, $p<0.01$). In addition, trabeculae from Tgr rats had a significantly greater intra-individual range of thicknesses than those in W-T femurs ($p<0.05$), and the degree of trabecular anisotropy (an index of trabecular orientation) was significantly lower in Tgr femurs (1.7 ± 0.04 vs 1.45 ± 0.04, $p<0.01$), indicating that the main axial direction is less pronounced in this model. Cortical bone at the distal femur, however, showed little difference of thickness between W-T and Tgr rats.

These micro-CT analyses reveal significant modifications of the architecture of trabecular bone in Tgr rats. The combination of fewer trabeculae with greater heterogeneity of trabecular thickness and less pronounced main axial direction indicates that the control of trabecular organisation is significantly altered in growth hormone deficient animals.

P12

OSTEONAL MATERIAL PROPERTIES IN THE FRACTURED FEMORAL NECK

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While many studies confirm the importance of bone mass and structure in the aetiology of osteoporotic fractures, little is known about changes in material properties and their relationship to structural features or to the relative "bone age". In the current study, we have combined nanoindentation with Environmental Scanning Electron Microscopy (ESEM) to determine local hardness (h) and Young's modulus (E) in osteoporotic bone and any effect of distance from the bone surface and/or structural features on these properties.

Transverse cross-sections of the femoral neck cortex from a 65 year old female were prepared using metallographic techniques and stained with toluidine blue to enhance cellular detail and assist in "ageing" individual osteons. Nanoindentation in individual osteons was done using a Micromaterials Nanotest 600, with the location of the subsequent indents, relative to osteonal features and the canal centre, determined by ESEM and Scion Image Analysis.

Despite large variations in both E (18 1-44 5Gpa) and hardness (0 39-1 43Gpa), mechanical properties decreased as the distance from the Haversian canal increased. Subsequently both ESEM images and statistical analysis indicated that some of this variation was due to positioning of the indentations. Those near (but not within) osteocyte lacunae and those in dark lamellar gave lower values while higher values were associated with overlapping indentations. For individual osteons, excluding this data improved the regression equations [e.g. hardness: adj r2=0.22, h=1.08-0.0075*distance vs adj r2=0.57, h=1.23-0.0102*distance; E: adj r2=0.18, E=36.2-0.181*distance vs adj r2=0.44, E=39.6-0.249*distance] and reduced the variation (h: 0.49-1.05Gpa; E: 18.1-34.4Gpa). Mean hardness was highly variable between osteons of differing "ages" (n=3, 0.25-0.99Gpa) although Young's modulus was less variable (22-28Gpa).

This study indicates that, like mineralisation, hardness and Young's modulii decrease with distance from the bone surface although both properties are markedly affected by structural features such as osteocyte lacunae and dark lamellae. This suggests that changes in osteocyte density, which is increased in osteoporosis, and lamellar thickness, which is decreased, will have important implications, along with bone age, for the local material properties. Future studies will determine the role of changes in " bone age" and structural features in the differences in the mechanical properties of osteons in osteoporotic and normal bone.

P13

FIBROMYALGIA, BONE DENSITY AND VITAMIN D DEFICIENCY

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Fibromyalgia is a poorly understood chronic musculo-skeletal disorder causing widespread pain and other problems. The disability often leads to loss of employment, depression and reduced physical activity. We were concerned that this could be the cause of osteoporosis and an increased liability to fracture. The literature contains conflicting reports on the association between fibromyalgia and reduced bone density.

Our study was a hospital-based, case-controlled study to examine whether female patients with the fibromyalgia syndrome (FMS) are at risk of osteoporosis. Our patients (n=40) and control subjects (n=37) were pre-menopausal and both groups had a mean age of 42.5 years.

As a group the FMS patients were more likely than the controls to be physically inactive ($p<0.001$), to have a poor calcium intake ($p=0.019$), to be smokers ($p=0.006$), to have been treated with steroids ($p=0.001$) and to have a past history of fractures ($p=0.03$). BMD was lower in FMS than control groups only at the mid-distal radius ($p=0.015$). This site reflects cortical rather than trabecular bone and indicates long-term rather than short-term bone loss. At the ultra-distal radius and spine no difference was found.

We found significantly lower serum 25-hydroxyvitamin D concentrations in the FMS patients compared with the controls ($p=0.007$). Three FMS patients also had raised serum levels of parathyroid hormone. While the serum alkaline phosphatase was higher in the FMS patients ($p=0.02$) this was matched by raised serum gamma-glutamyltransferase ($p=0.017$) and plasma viscosity ($p=0.001$) suggesting a hepatic rather than bony origin and probably an inflammatory process. No significant differences in terms of the disease or lifestyle parameters were apparent between FMS patients who had high and those who had low serum 25-hydroxyvitamin D levels ($p>0.1$).

This study showed a bone density difference at only one site; this probably reflects the lifestyle of the patients. The difference was small and was not seen at other sites. Our findings do not justify routine bone densitometry in premenopausal patients with FMS. Our most significant finding was that vitamin D subnutrition was very common in fibromyalgia patients and could compound the disability. We feel that it is important that this should be detected and treated.

P14

PERIPHERAL BONE MINERAL DENSITY IN PATIENTS WITH DISTAL RADIAL FRACTURES

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There is good evidence that diminished bone density at any site of measurement predicts overall future fracture risk. Some recent surveys have shown that, as a group, fracture patients often had low values for bone density. It has been suggested that women who sustain fractures should be targeted for osteoporosis evaluation and considered for therapeutic intervention. However few patients are. The aim of the current study was to determine the number of patients who would benefit from assessment and treatment in a population of patients with Colles' fractures in whom there was no previous intention to treat.

We attempted to recruit every female patient presenting to a busy fracture clinic with a Colles' fracture over a period of two years. Each patient was interviewed for relevant medical and lifestyle history before undergoing bone densitometry with an OsteoPlan plus pDXA scanner. We measured the bone mineral density (BMD) in the contralateral radius in 235 patients. We then excluded 39 patients from the further analysis of the data because of past fractures of the contralateral forearm (n=23), medical conditions or drug therapy known to affect bone (15), road traffic accident (1). We prepared a separate analysis of the findings in the 46 women who had the menopause before the age of 45.

Of the principal study group of 150 patients 87 percent had ultra-distal z-scores below 0 [mean z-score minus 1.02 (SD 0.94)] and 15 percent were below minus 2. Of those who were premenopausal and aged under 45 (n=33), 98 percent had a z-score less than zero [mean z-score minus 1.51 (SD 0.82)] and 24 percent had z-scores less than minus 2. This result was not due to the inclusion of women with an early menopause who had been eliminated.

Thus, while low values for ultra-distal BMD were found at all ages, these were particularly low in age-matched terms among the pre-menopausal women aged less than 45. This large survey confirms and extends the findings from earlier small studies. It underlines the importance of further investigation of young patients with distal forearm fractures to identify osteoporosis, to seek any underlying cause and to consider therapeutic intervention.

P15

HYPERMOBILITY AND TEMPORARY BRITTLE BONE DISEASE

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Background and objectives. We have earlier reported a variant of osteogenesis imperfecta (OI) causing fractures limited to the first year of life. The cause of this disorder remains unclear. It appears to be more common in twins and in infants born preterm. Heritable factors may also be important; in a majority of cases one parent showed unusual joint laxity but no other features of OI. Our aim was to compare the joint laxity in these parents with that in a randomly selected age-matched reference population.

Methods. Infants with temporary brittle bone disease (TBBD) were identified both from clinical and medico-legal referrals. Criteria for diagnosis included a history of fractures without a corresponding history or physical signs of injury; there were often substantial numbers of symptomless rib and metaphyseal fractures. The routine evaluation of each family included examination of both parents (n=216) where available for joint laxity using the widely used nine-point Beighton scale. A database was created taking the most flexible parent of each child (n=115). Where there was no difference in score, one parent was randomly selected. We created an age-matched control group derived from randomly selected adults attending a local general practice together with a group of medical students and sixth-form school children (n=139).

Results. Since laxity is known to vary with sex and age, both groups were divided into 5-year age-groups. Where there were adequate numbers, comparison by chi square showed that the younger parents particularly had increased laxity, compared with their age-matched controls. When all age groups were analysed together, the TBBD parents, both males and females, differed from the controls ($p<0.001$).

Conclusion. Our findings might be thought to suggest that the heritable factor in TBBD risk was, as in most patients with OI, a mutation related to collagen synthesis. However no abnormality has been identified to date in TBBD parents in collagen from cultured skin fibroblasts or in Col1A1 and Col1A2 genes. Similarly the molecular cause of familial joint laxity without OI remains unknown. When this is discovered one reward could be the identification of an important molecular cause for TBBD.

P16

HARVESTING PATHOLOGY IN A DENSITOMETRY CLINIC

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Over the last 12 years the number of new patients referred to the bone clinic has increased apparently exponentially from 30 per year to about 600. Almost all the rise reflects patients referred because of concern about the possibility of osteoporosis. We recently carried out an audit of all the new patients seen over the four years 1997-2000. Of 1207 patients, 1134 were referred because of this concern. Of these, 141 patients were, as a result of review of the clinical history, densitometric findings and laboratory evaluation, regarded as having osteoporosis of sufficient severity to justify intervention. In the great majority of these (121 patients) one or more risk factors for osteoporosis were identifiable. These patients could not be regarded as having 'idiopathic osteoporosis' and many had more than one risk factor.

During the same period no less than 66 patients had a new diagnosis made other than just osteoporosis. We identified five new patients with coeliac disease, 30 with osteomalacia, five with osteogenesis imperfecta, three with paraproteinaemia, one with lymphoma and 16 men with hypogonadism. Six new patients with hyperparathyroidism were identified from patients referred for densitometry; during the same period 20 others were identified in referrals for the evaluation of hypercalcaemia.

The number of patients and the variety of significant diagnoses highlight one major danger in the uncritical use of open access densitometry. Failure to recognise these disorders may lead to the prescription of drugs for osteoporosis which, in the light of the underlying cause of the osteopenia, are entirely inappropriate. We wish to draw attention to one additional hazard of open access densitometry. Some services provide results solely in terms of t-scores as opposed to z-scores. For identifying patients for further investigation, t-scores are inappropriate; their use in this context is likely to lead to the under-investigation of younger patients and the over-investigation of older patients. Had we limited our evaluation to patients with a t-score of less than minus 2.5, we would have missed 25 patients with significant remediable bone disease other than osteoporosis.

P17

ROLES OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) RECEPTORS IN HUMAN OSTEOBLASTIC CELLS CHEMOTAXIS TO VEGF

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Angiogenesis is a tightly regulated process involved in growth, repair, and bone remodelling. Vascular endothelial growth factor (VEGF) is secreted by both endothelial and osteoblastic cells, it has been shown to have an essential role in angiogenesis and maintaining osteoblastic cells' function.

In this study, a direct-viewing chemotaxis chamber (Dunn chemotaxis chamber) was used to analyze the chemotactic responses of human bone-derived osteoblastic cells (HOBs) and human foetal osteoblastic cells (HFOBs) to recombinant human VEGF. We report the first direct observation of chemotaxis of both HOBs and HFOBs towards a source of 100 ng/ml rhVEGF165. Using immunocytochemistry techniques, VEGF receptors (VEGF-R1/Flt-1 and VEGF-R2/Flk-1) have been locolised in both the HOBs and HFOBs. When the VEGF-R1 or VEGF-R2 were blocked respectively using the specific antibodies to VEGF-R1/Flt-1 or VEGF-R2/Flk-1, the chemotactic effects of rhVEGF165 (100 ng/ml) on both the HOBs and HFOBs were totally abolished.

The findings suggested that the VEGF receptors' pathway is involved in regulating osteoblasts' function and bone metabolism. This study surports the hypothesis that VEGF/VEGF receptor may serve as common mediators between osteogenesis and angiogensis through intercommunication between endothelial cells and osteoblastic cells.

P18

EXTRARENAL SYNTHESIS OF 1,25-DIHYDROXYVITAMIN D IN SUBCUTANEOUS FAT NECROSIS

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Subcutaneous fat necrosis is a rare condition thst occurs primarily in newborn infants after trauma or asphyxia at birth.It may be associated with hypercalcaemia,proposed mechanisms for which include,increased prostaglandin activity,calcium release from necrotic fat cells and increased production of 1,25-dihydroxyvitamin D.Elevated levels of 1,25-dihydroxyvitamin D have been documented in at least two published reports.

Two male infants with subcutaneous fat necrosis presented to a paediatric dermatology department within six years.They were born at term by emergency caesarian section for fetal distress.An abnormal appearance of the skin of the back developed within four days consisting of extensive deep indurated subcutaneous plaques of a reddish purple colour.Punch skin biopsies showed an inflammatory infiltrate within the subcutaneous fat with needle shaped clefts typical of subcutaneous fat necrosis.Hypercalcaemia(4.1 & 3.4 mmol/l)developed four to eight weeks after birth with evidence of nephrocalcinosis on renal ultrasound.Treatment with intravenous fluids,frusemide and oral corticosteroids caused resolution of the hypercalcaemia and skin changes within four weeks.

The skin biopsies were assessed for expression of 25-hydroxyvitamin D-1alpha-hydroxylase using a specific polyclonal antiserum.Immunohistochemistry showed dysregulated expression of 1alpha-hydroxylase compared to normal skin biopsies with strong expression in inflammatory infiltrates.

This report confirms that the hypercalcaemia seen in subcutaneous fat necrosis is due to extrarenal synthesis of 1,25-dihydroxyvitamin D occurring in the granulomatous tissue in the subcutaneous fat.

P19

BIOCHEMICAL MARKERS OF BONE TURNOVER IN RACEHORSES ARE INFLUENCED BY TRAINING AND GENDER

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We hypothesise that training regimens for racehorses differ in their effectiveness in engendering fracture resistant bone architecture. Gender and season are other potential risk factors. The aims of this study were to use biochemical markers of bone cell activity: i) to identify components of the training regimen which influence bone (re)modelling; and ii) to determine the influence of gender and season.

Blood samples were collected monthly from 147 two year old thoroughbreds in 10 racing stables over an 11 month period. Training speeds and distances were recorded daily. Speed catergories were gallop (>14.25m/s), canter (11-14m/s), and slow canter (<11m/s). Bone markers measured were osteocalcin (OC), the carboxy-terminal propeptide of type I collagen (PICP), and the carboxy-terminal cross-linked telopeptide of type I collagen (ICTP). Gender, season and training were included as main effects in the statistical mixed model, with bone markers as outcome variables.

There was significant variation between trainers in the training regimens used. Gallop was the only speed to have a significant effect on OC ($p = 0.017$) and ICTP concentrations ($p = 0.001$). There was an inverse correlation between the cumulative distance trained at a gallop and the concentrations of OC ($p = 0.001$) and ICTP ($p < 0.0001$). In contrast, there was a positive correlation between cumulative distance trained at a canter and concentrations of ICTP ($p = 0.008$). Concentrations of both OC and ICTP were significantly higher in males than in females (OC:$p = 0.04$, ICTP:$p = 0.01$). There was no significant effect of gender on PICP, except during May when concentrations were higher in females ($p = 0.002$). Season had no effect on any marker.

This study suggests that galloping is the main component of a racehorse's training regimen associated with decreased bone turnover. In a previous study we have shown that this pattern of change in bone markers was associated with an increase in bone density. Training for long distances at lower speeds is associated with higher levels of bone resorption and may be detrimental. Gender differences in bone marker concentrations in young horses are likely to reflect differences in bone size.

P20

SITES OF RETINOIC ACID (RA) SYNTHESIS IN REGENERATING BONE DETERMINED BY IMMUNOLOCALISATION OF THE RA SYNTHESISING ENZYME RALDH2

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Retinoic acid (RA) has multiple functions during limb development and regulates key events during limb regeneration in lower vertebrates. We have previously shown that RA also plays a role in the regulation of endochondral ossification in deer antlers, the only complex mammalian structures that regenerate. RA receptor mRNAs show distinct patterns of expression in antler tissues and exogenous RA promotes osteoblast and osteoclast differentiation in vitro while inhibiting expression of the chondrocyte phenotype. The objective of the present study was to identify sites of RA synthesis in antler tissues by immunolocalisation of the enzyme RALDH-2, a major RA generating enzyme that is indispensible for early embryogenesis. The RA content of these tissues was determined by HPLC.

In the rapidly growing fully formed antler, RALDH-2 is expressed in the epidermis of skin (velvet), in perichondrium and mesenchymal progenitor cells. Expression is very low in chondroprogenitors and differentiated chondrocytes (which express RXRbeta mRNA) although cells surrounding the vascular channels in antler cartilage express RALDH-2. These perivascular cells express RARalpha mRNA, type I collagen and RANKL, and are therefore likely to be of the osteoblast lineage. At sites of bone formation, RALDH-2 is expressed in periosteum and in differentiated osteoblasts which express osteocalcin (determined by immunolocalisation). HPLC analysis showed that tissues immunoreactive for RALDH-2 also synthesise and/or release RA. The ligand for RARs, all-trans-RA, is present in skin, perichondrium, periosteum, cartilage and bone. In contrast, the RXR/RAR ligand, 9-cis-RA, is only detected in mineralised cartilage and in bone. RALDH-2 is also expressed in the antler blastema (the healing wound from which the antler develops) which contains RA.

In conclusion, this study is the first to describe the expression of mediators of RA synthesis in bone. These data further define sites at which RAs are likely to play a functional role during antler development since regions of RA synthesis correspond closely with the localisation of cells that respond to RA. This study is also the first to demonstrate the presence of 9-cis-RA in skeletal tissues (it is not detected in embryonic bone), which indicates that it may have a specific function in regenerating bone.

P21

COMPARISON OF 1 YEAR CHANGES IN HAND, HIP AND SPINE BONE MINERAL DENSITY IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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Rheumatoid Arthritis (RA) is associated with bone loss. Peri-articular osteopenia is an early radiological feature, and generalised bone loss is also known to occur early in RA. The objectives of this study were to measure changes in bone mineral density (BMD) in the hand, hip and spine in patients with early RA, and to establish the determinants of BMD changes.

51 patients aged [mean(SD)] 54(10.7) years with early[12.8(5.7)months duration] RA were studied for 1 year. There were 22 men and 29 women[12 premenopausal, 15 postmenopausal [median (IQR): 9(2.5-10.5) years postmenopause], 2 peri-menopausal on HRT]. DXA of the dominant hand, spine and left hip was performed at baseline and after 1 year, as were RA activity assessments. ESR was measured at 0,6 and 12 months, and the area under the curve (AUC-ESR) calculated. At entry, 69% were sero-positive, and 45% erosive on hand/wrist X-rays. 92% were being treated with disease modifying drugs and 12% with oral prednisolone. The ESR, CRP (NR: <5) and HAQ were [median (IQR)] 15(6-30), 1.2(0-10.3) and 0.75(0.375-1.25) respectively. Baseline BMD [mean (SD)] was: lumbar spine (LS) 1.006(0.15) g/cm2; T-score: -0.81(1.39); Z-score: -0.01(1.33), femoral neck (FN) 0.785(0.13) g/cm2; T-score -1.4(1.29); Z-score -0.1(1.15), dominant hand (HAND) 0.393 (0.04) g/cm2.

For the whole population, 1 year BMD changes [mean (SD)] were: +0.2(3.6)%, -0.29(5.6)% and -0.28(4.3)% for LS, FN and HAND respectively (all insignificant). Subgroup analysis based upon greater disease activity [baseline CRP>5 (n=16), AUC-ESR>40 (n=15): ACTIVE group] was performed. No significant differences in LS and FN were found. Within ACTIVE, 1 year HAND changes were: -2.3(3.8)%, and -2.5(4.1)% for CPR>5 and AUC-ESR>40 respectively (both $p<0.05$). Within INACTIVE, 1 year HAND changes were insignificant: +0.6(4.5)% and +0.7(4.1)% for CRP<5 and AUC-ESR<40 respectively. Between subgroups differences in HAND were significant for CRP and AUC-ESR (both $p<0.05$). Stepwise multiple regression showed that AUC-ESR and baseline erosions were important determinants of HAND changes.

Changes in LS and FN BMD were insignificant in this group of patients with early, relatively mild RA. However HAND BMD decreased significantly in the subgroup with elevated inflammatory markers and erosive disease.

P22

EFFECT ON BONE MINERAL CONTENT OF SUPPLEMENTS OF VITAMIN K, VITAMIN D AND CALCIUM IN OLDER WOMEN

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A two-year placebo controlled, randomised double-blind intervention study was designed to determine the influence of habitually high intakes of vitamin K, vitamin D and calcium on the bone health of older women and to investigate nutrient interactions.

We studied 244 women aged over 60 years at baseline, who were allocated to one of four intervention groups; 1: placebo, 2: 200 microgram vitamin K daily, 3: 10 microgram vitamin D plus 1 g calcium daily, and 4: combined vitamin D and vitamin K plus calcium. 209 women completed the six-monthly visits for measurements of bone density at the femur and radius, for assays of markers of bone turnover and for completion of questionnaires on diet and lifestyle.

Analysis of differences between bone mineral content (BMC) at each visit relative to baseline indicated a significant increase in BMC at the ultra-distal radius in group 4 ($p<0.05$). The response in the calcium+vitamin D group did not reach significance; this possibly suggests that additional calcium is not beneficial in older women who are already calcium replete

There was an apparent synergistic effect of combined calcium+vitamin D and vitamin K supplementation on BMC at the ultra-distal radius. The mechanism remains to be determined, but osteocalcin is known to require vitamin D for regulation of synthesis and vitamin K for gamma-carboxylation. This was a relatively healthy, motivated and active population of women who had an adequate intake of calcium (mean 1047 mg/day). There was a low dietary intake of vitamin D but exposure to sunlight brought serum levels of 25-hydroxyvitaminD well into conventional reference ranges. However the response to supplementation in groups 3 and 4 in causing a modest reduction in serum PTH values suggested that there had been on average sub-optimal status. 27% of the whole group had intakes of vitamin K below the UK recommendation. The fact that an effect on reducing bone loss was seen in a relatively fit population suggests that the benefit might be greater in a more disadvantaged group.

P23

BMD IS POORLY PREDICTED BY QUANTITATIVE ULTRASOUND OF THE RADIUS

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Background: Quantitative ultrasound (QUS)devices have become available for evaluating qualitative bone charac-teristics. QUS is less expensive than DXA, portable and radiation free. QUS of the heel bone is associated with fracture risk. It has been suggested that DXA may be replaced by QUS in the future.

Methods: We examined associations between speed of sound (SOS, m/s) of the radius assessed by the Omnisense multi-site ultrasound device and BMD (bone mineral density, g/cm2) measured by DXA at the distal forearm, femoral neck and spine. 62 women were examined (age 67+/-12 years, height 162+/-7 cm, body weight 63+/-9 kg). Duplicate mea-surements of SOS were performed in 59 women.

Results: The CV (RMS) for SOS was 0.7%. SOS was correlated with age ($r = -0.64$, $p < 0.001$) and height ($r = 0.39$, $p < 0.005$), and with BMD of the forearm ($r = 0.67$, $p < 0.001$), femoral neck ($r = 0.39$, $p < 0.001$) and spine ($r = 0.32$, $p < 0.01$), but not with weight ($r = 0.06$). In multiple regression analyses with correction for age, height and weight, BMD of the forearm was only weakly associated with SOS (Rpartial = 0.50, $p < 0.001$, Rmultiple = 0.73, SE = 0.05). SOS did not inde-pendently predict BMD of the femoral neck or spine.

Conclusion: BMD is poorly associated with QUS of the radius. This does not exclude the possibility, however, that QUS may predict fracture risk independently of BMD.

P24

LIFESTYLES AND ACTIVITY LEVELS IN AN ELDERLY POPULATION WITH AND WITHOUT MINIMAL TRAUMA FRACTURES

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Physical activity?s positive effect on bone health is widely accepted. Recent studies have revealed that clinical depression is a risk factor for osteoporosis, and that health benefits derive from social activities. This survey aimed to evaluate differences in lifestyles and pursuits in older individuals with and without recent low trauma fractures.

Ethical permission and informed concent was obtained. An interview-administered questionnaire to 207 clients aged 60 ? 79 years (12.6%; 26 male; 87.4%; 181 female) determined lifestyle behaviours in the three months preceding the fracture or interview. Interviews were conducted in the fracture clinic, the ward, or by telephone. The fracture group (Fg) comprised 120 clients; the non-fracture group (NFg) 87, matched for race, age and gender was obtained from local GP lists. Exclusions: clients with neurological, severe cardiorespiratory or other disease related to inactivity or osteoporosis. Levels of activity were ranked using a 5-point scale.

As expected, the NFg had significantly higher levels of activity than the Fg ($p<0.01$). However, more than a quarter (26%, 32/120) of the Fg had high activity levels, whilst conversely 21% (14/87) of the NFg had low levels of activity. Multidimensional scaling was used to determine differences between the sub-groups. For example, the Fg in each case were unlikely to drive (53% v 72%); less likely ($p<0.01$) to visit a library, have meals out or visit a pub (31% v 72%) or watch TV quiz shows (47% v 83%) or have a hobby; but were more likely to have a recent bereavement (61% v 25%) and have active, unskilled former occupations (50% v 75%). (Bereavement in this context included loss of a family pet, friends, children leaving home or loss of a spouse). The picture that emerged in this study indicates that factors besides physical activity may contribute to increased fracture risk in an otherwise healthy population. Bereavement may cause a reactive depression, which can predict functional decline in older women. Non-participation in social pursuits may indicate isolation with comparable functional decline in function.

P25

RELATIONSHIP BETWEEN VITAMIN D AND NEUROMUSCULAR FUNCTION IN ELDERLY PEOPLE WHO FALL

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Vitamin D supplementation significantly reduces the incidence of fractures. Over 90% of fractures occur as a result of falls. Evidence suggests that vitamin D deficiency impairs neuromuscular function, causing an increase in falls and thereby fractures. The relationships between vitamin D and various aspects of neuromuscular function in elderly people who fall were examined in a cross-sectional study.

Subjects recruited from a falls clinic and stratified according to 25OHD levels (Groups 1 25OHD<12ug/L, Group2 25OHD12-17ug/L, Group3 25OHD>17ug/L). Age-matched volunteers with no history of falls and 25OHD>17ug/L comprised Group 4 (n=20 per group).

Functional performance was assessed using aggregate functional performance time (AFPT), incorporating 50ft walk, get up and go, stairs ascent and descent. Psychomotor function was measured by choice reaction time (CRT), postural sway using a pressure platform and quadriceps strength using maximal voluntary contraction (MVC).

Fallers had impaired AFPT (51.0 v 32.8, $t=4.97$, $p<0.05$), CRT (1.66 v 0.98, $t=2.86$, $p<0.05$) and MVC (223 v 271, $t=2.35$ $p<0.05$) compared to controls. Fallers had worse sway than controls (NS).

Fallers with 25OHD<12ug/L had worse AFPT (66.0 v 44.8, $t=4.15$, $p<0.05$), CRT ($t=3.59$, $p<0.05$) and weaker MVC (196 v 236, $t=1.99$ $p=0.051$) than fallers with 25OHD>12ug/L. Gp1 had the worst sway.

Multiple regression analysis identified 25OHD as an independent variable for AFPT ($r2= 0.54$ $p<0.05$), CRT ($r2=0.21$ $p<0.05$) and sway ($r2=0.17$, $p<0.05$). PTH but not 25OHD was an independent variable for MVC ($r2=0.47$, $p=0.02$).

Elderly people who fall have significantly impaired neuromuscular function compared with controls. Furthermore, vitamin D deficiency is independently associated with impaired functional performance, psychomotor function and sway. Vitamin D supplementation in this group may improve neuromuscular function and thereby reduce falls and fractures.

P26

A RATIONALE FOR VITAMIN D PRESCRIBING IN A FALLS CLINIC POPULATION

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Ninety percent of fractures occur as a result of falls. Elderly people who fall are more likely to be at increased risk of vitamin D insufficiency, a major contributor to fracture risk. At present identification of 25OHD insufficiency relies on venepuncture, which is expensive, time consuming and may be unnecessary if clinical predictors were identified. We examined the prevalence and predictors of 25OHD insufficiency in a prospective observational study of patients attending a falls clinic in order to develop a rationale for vitamin D prescribing in this setting.

Data were recorded for 400 consecutive patients aged >65 attending a falls clinic. This included a medical and social history, an abbreviated mental test and physical examination. Serum bone biochemistry and 25OHD levels were measured by standard techniques.

The population was elderly (mean age 78.3, range 65-97), majority were female (73.3%) community dwelling (96% in their own homes) and cognitively intact (90% AMT >8/10). Only 17% were housebound.

The mean 25OHD level for this population was 16.5ug/L (7.8ug/L). 72.5% of patients had 25OHD levels <20ug/L and 98.7% <40ug/L. These patients did not have evidence of biochemical osteomalacia.

The number of times out per week ($p<0.02$) and serum albumin ($p<0.03$) were identified as independent predictors for 25OHD insufficiency. There was no other significant association between vitamin D and the studied clinical or biochemical factors.

Vitamin D insufficiency is highly prevalent in an elderly falls clinic population. The predictive value of outdoor activity and albumin for vitamin D insufficiency was low (multiple $r2=0.06$). It may be pragmatic to supplement all falls clinic patients, without recourse to 25OHD measurement, in view of the high prevalence, benefits of supplementation, and lack of toxicity at therapeutic doses.

P27

AN EXPERIENCE OF OSTEOPOROSIS EDUCATION SESSIONS

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Education sessions were set up for patients suffering from osteoporosis. The aim to (i) Evaluate existing knowledge of patients newly diagnosed with osteoporosis, as well as to ascertain where they acquired their knowledge from. (ii) Provide information regarding osteoporosis. (iii) Evaluate the increase in patients' knowledge following the education session. (iv) Assess patients' satisfaction, and (v) Provide a vehicle to discharge patients. The session involved the Rheumatology specialist nurse, dietitian, occupational therapist and physiotherapist in a 2½ hour session.

Patients were identified following attendance for DXA scanning. Information was obtained using questionnaires, one issued before the session, one immediately after and one six months later. Currently pre and post session questionnaires of 200 patients have been analysed, 140 have completed questionnaire two. To date only 60 (30%) patients have returned all three questionnaires.

The sample consisted of 160 (80%) females and 40 (20%) males. The mean age was found to be 65.7, (range 34 – 87). 184 (92%) of patients were aware that they had been diagnosed with osteoporosis. 127 (64%) stated that a hospital doctor informed them of this, 56 (28%) were told by their GP, 1 from another source. The remainder 16 (8%) claimed that they had not been given a diagnosis. From the pre session questionnaire it was gleaned that only 73 (37%) patients had received information regarding osteoporosis, rising to 136 (97%)following the education session. When asked where they obtained this information, both questionnaires revealed that a doctor or nurse had provided the information (73 (37%) and 122 (87%) respectively). Patient knowledge of osteoporosis was found to be variable. Within a possible score range of –36 to +36 the mean (SD) score was 9.3 (7.4). These scores increased to 22.0 (7.1) immediately following the education session, falling back to 18.0 (9.1) after six months. An increase in knowledge was noted in all age groups. 122 (61%) patients have been discharged back to primary care.

There appeared to be a lack of information provided to this group of patients. Their knowledge base varied greatly, knowledge scores increased significantly across all age groups and this knowledge was retained. 122 (61%) of patients were discharged. Patient satisfaction was high and reported the sessions as being clear, informative, and beneficial.

P28

BONE MINERAL DENSITY AND BIOCHEMICAL MARKERS OF BONE TURNOVER IN ASEPTIC LOOSENING AFTER TOTAL HIP ARTHROPLASTY

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Aseptic loosening is the main cause of implant failure after total hip arthroplasty (THA). In this process focal bone loss at the implant-bone interface results in mechanical failure of the implant-host construct. Whilst many studies have demonstrated early changes in periprosthetic bone mineral density (BMD) around successful implants after THA, there are no data on periprosthetic BMD change in subjects with failing implants. The aims of this study were to determine whether subjects with aseptic loosening have differences in periprosthetic BMD and in biochemical markers of bone turnover compared subjects with successful implants.

Proximal femoral and pelvic BMD were measured by dual energy x-ray absorptiometry and bone turnover markers were assayed in 49 subjects 12.6[4.3] (mean [SD]) years after THA. Femoral BMD loss was found in Gruen zones 2,5,6, and 7 in subjects with a loose femoral implant (n=17) compared to those (n=32) with fixed femoral implants ($P<0.05$ all comparisons). This BMD difference was greatest (-31%, $P=0.02$) in the proximal, medial region of the femur. Overall there was no difference in bone turnover markers between subjects with a loose implant (femoral or pelvic) versus controls, although subjects with femoral loosening had higher levels of the bone resorption marker N-telopeptides of type-I collagen ($P=0.02$) than those with a fixed femoral implant. No differences in pelvic BMD or bone turnover markers were found between subjects with loose (n=18) versus fixed (n=31) pelvic implants.

Failure of femoral, but not pelvic, components after THA is associated with region-specific decreases in BMD and an increase in urinary excretion of N-telopeptide cross-links of type-I collagen. It remains unclear whether low bone mass predisposes to, or is the result of, the loosening process. Therapeutic strategies aimed at inhibiting femoral bone loss after THA may influence the natural history of aseptic loosening.

P29

THE REGULATION OF CELL MOTILITY BY INTEGRINS IN OSTEOBLASTIC CELLS

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Integrin-matrix interactions are important regulators of osteoblast behaviour. We have previously reported on the pattern of integrins expressed by human osteoblasts (HOB's) in vitro (Bennett et al, 2001 *Arch Oral Biol*. 46. 229). Here, using cell migration as an index, these studies have been extended to include functional studies on the role of integrin mediated cell-matrix interactions in HOB cells cultured on fibronectin (Fn) or collagen type 1 (COL 1). HOB cells were obtained from primary explant culture. Cell surface expression was determined using flow cytometry as previously described (Bennett et al, 2001) or by immunofluoresence with Ab's specific for the beta 1, alpha 2 and alpha 5 integrin subunits . HOB cells expressed each of the integrins studied. Migration assays were performed using a standard Boyden chamber assay (8um pore size) coated with Fn (10ug/ml) or COL 1 (100 ug/ml). After 4 hours, cell migration was observed in wells coated with either substrate but not in uncoated controls. Migration on Fn and COL I was partially blocked in the presence of an RGD peptide. Pre-incubation with alpha 5 (10ug/ml) blocking antibodies partially blocked migration on Fn with no significant effect on COL I. In contrast, the use of alpha 2 blocking antibodies caused a marked reduction in migration on COL I but not Fn. Incubation with a mitogen activated protein kinase kinase (MAPKK) inhibitor (U0126) reduced cell migration by 63% and 76% on Fn and Col 1 respectively.

Cell matrix interactions involving the alpha 2 and alpha 5 integrin subunits participate in the regulation of HOB cell migration in vitro. This may involve the MAPK/ERK signalling pathway.

P30

A QUANTITATIVE 3D ANALYSIS OF BONE RESORPTION USING CONFOCAL MICROSCOPY.

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Osteoclasts are a polarised cell type and exhibit directional resorption into bone (penetrative/tunnelling resorption) and along the bone surface (tracking resorption). Thus, a quantitative 3D analysis of the resorption process is useful to assess these events and this can be done by scanning electron microscopy or reflection confocal microscopy. Herein, an alternative method is described, using immunofluorescent stains and confocal microscopy that allows thousands of resorption sites to be assessed for area, depth and volume.

Rabbit osteoclasts, isolated from limb bones, were cultured on dentine slices (surface-labelled with fluoro-X) for 1, 3, 7 and 10 days. A variety of resorption inhibitors, 0.05 mM E-64 (a protease inhibitor), 0.1 mM alendronate (a bisphosphonate) and 25 nM kistrin (an integrin antagonist) were also assessed in the 3 day cultures. After fixation and permeabilisation, the cultures were analysed by immunofluorescent staining and confocal microscopy for the non-resorbed surface matrix (with fluoro-X) and the resorbed matrix (using Cy5 labelled antibody to type I collagen). The resorption sites were located by a surface loss of the fluoro-X stain together with positive staining of type I collagen exposed in the resorption pits. Using a x16 objective, 16 optical xy sections for each fluorochrome were taken through the resorption sites from 5 areas for each dentine slice. The image data was analysed using an in-house computer programe which yielded pit number, area, depth and volume. All the resorption parameters increased significantly with time in culture ($n=14$, $P < 0.008$). An exception was seen at day 7 in which the resorption area stabilised while the depth and volume continued to increase. These data indicated a phase of tunnelling, rather than tracking, resorption. E-64 and alendronate inhibited all resorption parameters by 41-95% ($n=18$, $P < 0.023$). In contrast, low concentrations of kistrin, although inhibiting bone resorption by 36% ($n=16$, $P < 0.031$), did not alter pit depth, and so, maintained penetrative resorption.

In summary, these techniques provide an alternative, semi-automatic approach to assess in vitro bone resorption by osteoclasts and facilitate a 3D quantitation and analysis of the resorption process. The method could be further developed to increase the throughput of screening for osteoclastic inhibitory drugs.

P31

THE NEUREXINS AND THEIR LIGANDS IN OSTEOBLASTS

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Cell:cell, cell:ECM interactions are pivotal in the regulation of bone cell activity. The neurexin gene family, a highly polymorphic set of neuronally expressed genes are implicated in the formation of cell junctions and neuronal cell recognition. Up until now their expression pattern has been exclusively restricted to neuronal cells. However in this study their expression has been found to be upregulated in ROBs driven to form bone nodules in-vitro.

In neuronal cells, neurexins interact with various ligands, including the neuroligins, neurexophilins and CASK. Neuroligins have been shown to interact with the short beta neurexins, through the extracellular domains of both proteins, forming heterotypic receptor complexes that appear to cause cell adhesion between different neuronal cells. In addition the shorter secreted neurexophilins interact specifically with the long alpha neurexins and may be involved in cell:ECM interactions. The cytoplasmic regions of the alpha and beta neurexins bind CASK, through a PDZ domain. CASK locally recruits actin filaments to the cytoplasmic tail of neurexins and may couple the cell junctions to the actin cytoskeleton. Furthermore the multi domain nature of CASK then allows it to recruit many different proteins, a process which is implicated in the formation of cell junctions.

In this study primary rat osteoblast cell were isolated from rat calvaria cell cultures and driven with dexamethasone, betaglycerophosphate and ascorbic acid to produce bone like nodules in-vitro. RT-PCR showed that neurexin 1 alpha and 2 alpha, neuroligin 2, neurexophilin 1 and 3 and CASK messages were all induced after the addition of the bone forming media. In addition western analysis showed that CASK protein expression was induced and provisional western analysis shows a neuroligin 2 protein product is produced. Furthermore immunocytochemistry with anti-Neurexin 1 alpha and anti-CASK antibodies show that they are localised to rat osteoblasts in-vitro.

In conclusion , the expression of the normally neuronal neurexins along with their associated ligands in rat osteoblasts cells suggest that the cell junction forming role of neurexins in neurones may also be applicable to rat osteobalst cells and may mediate cell:cell and cell:ECM interactions.

P32

A BIO-ASSAY FOR OSTEOCLASTOGENESIS INHIBITORY FACTORS IN SERUM

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Osteoclast differentiation and activation is, to a large extent, controlled by the action of tumour necrosis factor family molecule, receptor activator of nuclear factor-kappa B ligand (RANKL). This molecule is produced on the surface of osteoblastic cells where it interacts directly with a specific receptor on osteoclast precursors to bring about differentiation. RANKL can also be produced in a soluble form by activated T-cells and by some tumour cells. The action of RANKL in the stimulation of bone resorption is counter-acted by several inhibitors of osteoclastogenesis, of which the best characterised is osteoprotogerin (OPG). OPG is a secreted protein and a member of the tumour necrosis factor receptor family, and acts by binding to RANKL. It is present in human serum where its concentration can be estimated by immunoassay. Different immunoassays have led to somewhat variable results presumably because of OPG can exist in different forms in serum, for instance, degradation products, monomeric or dimeric forms and forms with various TNF-like ligands attached. This led us to develop a bio-assay for inhibitory factors of osteoclastogenesis in serum. The assay is based on the production of osteoclast-like cells from mouse bone marrow derived non-adherent monocytes in the presence of recombinant human macrophage colony stimulating factor and RANKL. Human serum is added to media containing monocytes and the above cytokines, and three days later tartrate-resistant acid phosphatase-positive osteoclast-like cells are counted. A range of responses from sera from patients with metabolic bone disease have been recorded ranging from stimulation to almost complete inhibition of osteoclast-like cell formation. The characteristics of this assay and the effects of specific sera will be described.

P33

THE NUMBER OF MESENCHYMAL STEM CELLS CIRCULATING IN THE PERIPHERAL BLOOD OF HUMANS, INCREASES AFTER FRACTURE.

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Recent studies have suggested the existance of small numbers of mesenchymal stem cells (MSCs), circulating in the peripheral blood of guinea pig, mouse,rat and human. There is no data as to whether fracture increases the number of such circulating MSCs.

15mls of peripheral blood was taken from 5 patients (male, age 20-50)at day 1-3 and 14-21 following long bone fractures. Control samples were also taken from 3 matched volunteers. The peripheral blood mononuclear cells (PBMNCs) were isolated by centrifugation over a Lymphoprep density gradient (Nycomed). Some PBMNCs were immediately fixed for immunocytochemistry (ICC); others were cultured in DMEM, supplemented with dexamethasone and glycerophosphate, for 4 weeks prior to ICC.

The PBMNCs from fracture patients, tested prior to cell culture, stained positive for Endoglin, Vimentin, Collagen type 1, BMP-2, BMPR 1+2 and Cbfa 1. In contrast, there was little or no staining for these markers in the controls.

Following culture, the PBMNCs from fracture patients contained numerous single adherent spindle-shaped cells, but no colony formation. Most of these cells were positive for Cbfa-1, BMP-2, BMPR 1+2,Endoglin, type 1 collagen, Osteocalcin and Vimentin but all were completely negative for alkaline phosphatase. The greatest number of cells were found in the blood samples taken at 14-21 days post-fracture. In contrast, we did not observe any fibroblastic cell formation in the control cultures.

This study demonstrates that patients with a recent fracture have an increase in the number of MSCs circulating in their blood. We are conducting further work to establish wheather they leak into the blood from the healing fracture site, or arise from distant sites as part of the response to injury. This phenomenon could provide exciting alternatives for harvesting skeletal progenitor cells or delivering treatment for skeletal disease.

P34

EFFECT OF CALCIUM SUPPLEMENTATION ON THE BONE MINERAL DENSITY OF POST-MENOPAUSAL WOMEN GIVEN HORMONE REPLACEMENT THERAPY

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Calcium supplements have been widely advocated for the prevention of bone loss in post-menopausal women and indeed are a requirement in clinical trials for the prevention of osteoporosis in many countries such as Canada and the United States. We wished to evaluate the effect of calcium supplementation by comparing the changes in bone mineral density (BMD) in post-menopausal women given hormone replacement therapy (HRT) and who were either given calcium supplements or not supplemented. We performed a multi-centre double-blind prospective randomised, placebo-controlled study in apparently healthy post-menopausal women (aged 44-65 years) randomised to either placebo, or continuous oral oestradiol 17 beta 1 mg or 2 mg with sequential dydrogesterone for 2 years. The primary endpoint was the percentage change from baseline in BMD measured by DXA, in the lumbar spine of actively treated groups compared with placebo. Two hundred and four women completed the study in Canada and 165 women in the UK. Canadian women were all given 500mg/day of calcium in adherence to the regulatory requirements of Canada. There was no significant difference in BMD at baseline (1.036g/cm2 in UK women and 1.044 g/cm2 in Canadian women) although the Canadian women were younger (54.4 years vs 57.0 years) and there were a larger number less than 2 years post-menopause (34.7% vs 15.1%). Repeated measures analysis of variance was performed on the dataset. Canadian women showed a significantly smaller change in BMD with HRT treatment irrespective of dose, compared to UK women and this remained significant after adjusting for baseline BMD, age and time since menopause (p=0.033). These data suggest that calcium supplementation does not result in increases in BMD above that provided by HRT alone and calls into question the requirement for calcium supplementation in clinical trials of the prevention of osteoporosis. It remains possible, though unlikely, that there are country-specific differences in the skeletal response to calcium supplementation.

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P2X(7) RECEPTOR-DEFICIENT MICE MAINTAIN THE ABILITY TO FORM MULTINUCLEATED OSTEOCLASTS IN VIVO AND IN VITRO

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The P2X7 receptor is a member of the family of P2X purinergic receptors, which upon sustained activation forms large pores in the plasma membrane. In cells of haematopoietic origin, P2X7 receptor activation has been shown to lead to multiple downstream events including cytokine release, cell permeabilisation, apoptosis and more recently the generation of multinucleated giant cells. We have previously shown that blocking the P2X7 receptor in cultures of human blood monocytes supplemented with MCSF and recombinant RANKL prevented multinucleated osteoclast formation. Whilst it is clear that P2X7 receptor is important in the fusion of these cells the exact mechanism behind P2X7 receptor-induced pore formation and cell fusion is unknown.

We therefore examined mice deficient in the P2X7 receptor to determine whether deletion of this receptor would effect the formation of multinucleted cells in vivo and in vitro. We also examined the bone mass of P2X7R-/- and P2X7R+/+ mice by dual-energy X-ray absorptiometry and performed histomorphometric analysis.

Surprisingly, we found the P2X7R-/- mice to be healthy with no overt skeletal problems. Histochemical examination of longitudinal sections of distal femoral metaphyses from P2X7R-/- mice showed the presence of multinucleated osteoclasts in vivo. MCSF and RANKL-treated P2X7R-/- mice spleen cultures also gave rise to multinucleated osteoclasts in vitro. Furthermore, we have demonstrated the ability of these multinucleated osteoclasts, upon stimulation with maitotoxin, to form pores in the plasma membrane in vitro. These data are consistent with the existence of an endogenous pore structure present in cells that can be activated either by the P2X7 receptor or in its abscence, alternative signals to mediate fusion and pore formation. Further investigations are underway to ellucidate fully the compensatory mechanisms leading to redundancy of this receptor in the osteoclast lineage.

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THE ROLE OF OSTEOPROTEGERIN IN BREAST AND PROSTATE CANCER

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Osteoprotegerin (OPG) is a multifunctional protein that inhibits bone resorption by suppression of osteoclastogenesis and enhances cell survival by acting as a decoy receptor for a member of the TNF family, TRAIL. These activities suggest that OPG may play an important role in the growth and survival of tumour cells in skeletal metastases.

The objective of this study was to measure the relative production of OPG in breast- and prostate cancer cell lines grown in vitro, and to determine whether this tumour derived factor could protect cells from TRAIL induced apoptosis. We also measured OPG levels in serum from breast- and prostate cancer patients and correlated OPG concentrations with disease status.

The following cell lines were used: Prostate, androgen insensitive: DU145 and PC3, androgen sensitive LNCAP. Breast: estrogen insensitive MD-MDA231 and estrogen sensitive, MCF7. Production of OPG by the cell lines was measured in 3-day conditioned medium by an in-house ELISA method. Serum levels of OPG were measured using a commercially available ELISA kit. The effects of endogenously produced and exogenously supplied OPG on TRAIL-induced apoptosis were measured in PC3 and MD-MDA231 cells by evaluation of nuclear morphology after DAPI staining.

We found that hormone-insensitive breast- and prostate cancer cell lines produced substantial amounts of OPG over a 72h period, whereas hormone-sensitive cell lines did not produce detectable levels of OPG when grown under the same conditions. The levels of OPG produced by PC3 and MD-MDA231 cells were sufficient to inhibit TRAIL-induced apoptosis by up to 50%. The anti-apoptotic effect was dose-dependent and could be reversed by addition of sRANKL. In serum from breast- and prostate cancer patients we found increased levels of OPG in patients with advanced disease compared to patients with localised disease.

In conclusion – OPG is produced by hormone-insensitive breast and prostate cancer cell lines in vitro, and may act as a survival factor for these cells by inhibiting TRAIL-induced apoptosis. This data, in combination with the observed elevated serum levels of OPG in breast- and prostate cancer patients with advanced disease, indicate that OPG may be an important factor in tumour metastasis and survival in the bony microenvironment

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THE USE OF TYROSINE KINASE INHIBITORS TO DISSECT THE ROLE OF C-FOS-INDUCED FIBROBLAST GROWTH FACTOR RECEPTOR-1 IN CHONDROCYTES

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We have previously shown, using inducible c-Fos expression in clones of the ATDC5 chondroprogenitor cell line, that exogenous c-Fos inhibits chondrocyte differentiation in vitro. We have presented that expression of ectopic c-Fos, in cells of clone DT12.4, upregulates fibroblast growth factor receptor-1 (FGFR1) expression, and can modulate FGF-dependent effects on cell morphology, anchorage-independent colony formation, proliferation and differentiation. These data suggest a functional role for c-Fos-induced upregulation of FGFR1.

Here, we have begun to dissect these interactions and investigate whether any c-Fos-dependent effects on chondrocytes could be altered by using chemical inhibitors of FGF-signalling. We first used the inhibitor SU5402, which specifically inhibits the ligand activation of FGFR1. Treatment with SU5402 completely abrogated the FGF-2-induced morphological changes in DT12.4 cells. More importantly, the enhancement of FGF-2 effects by exogenous c-Fos was also inhibited by SU5402, suggesting that the c-Fos effects were due to enhanced FGFR1-dependent signalling. In contrast, while SU5402 rescued the FGF-2-dependent inhibition of differentiation, as determined by alkaline phosphatase activity, preliminary results suggest that SU5402 was unable to reverse the c-Fos-induced inhibition of differentiation. We are currently analysing the effects of SU5402 on c-Fos-dependent changes in cartilage nodule formation and chondrocyte gene expression.

Additionally, we have treated DT12.4 cells with the MEK inhibitor PD98059, which inhibits signalling via a number of tyrosine kinase receptors, including FGFR1. Treatment with PD98059 in the absence of exogenous c-Fos leads to a decrease in cell viability. Interestingly, induction of c-Fos expression significantly rescued cell viability in the presence of PD98059, suggesting that c-Fos may mediate the survival effects of growth factors acting via the MAPK pathway. FGF-2 also rescued cell viability in the presence of PD98059, suggesting the involvement of a MEK-independent pathway for FGF signalling in chondrocyte survival. However, induction of exogenous c-Fos did not enhance the FGF-2 effects on cell survival, suggesting that the c-Fos-induced increase in FGFR1 may not be involved in FGF-2 effects on chondrocyte survival.

Thus, specific tyrosine kinase inhibitors provide powerful tools for determining the relative contribution of c-Fos dependent upregulation of FGFR1 to mediating FGF-dependent effects on chondrocyte morphology, growth, survival and differentiation.

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CHONDROCYTES, GROWTH PLATES, AND RANKL-INDUCED SIGNALLING: OBSERVATIONS AND IMPLICATIONS OF OSTEOPETROTIC MUTATIONS

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In the course of studies of osteopetrotic mutations in rats and mice, we and others have noted the appearance, in a subset of mutations, of a progressive, severe growth plate chondrodystrophy. Most notably, this occurs in RANKL knockout mice and in the toothless (tl) osteopetrotic rat, both of which block osteoblast-derived, osteoclastogenic signals. Given the growth plate phenotypes also associated with other RANKL pathway knockouts, i.e., RANK and TRAF6, the simplest hypothesis was that RANKL directly affects chondrocyte differentiation during endochondral ossification. We investigated this hypothesis using several approaches. First, we determined whether the tl rat was a RANKL loss-of-function mutation, but this was ruled out by gene mapping, expression, and other lines of evidence. Next, we looked for the expression of the RANKL receptor, RANK, and its associated intracellular signal mediator, TRAF6, in growth plate chondrocytes by immunohistochemistry. Both were present in pre-hypertrophic zone chondrocytes in rodent long bones, consistent with a direct role for RANKL signalling in chondrocyte differentiation. To pursue these findings, we are currently using cell culture models to assess the timing of expression and the impact of RANKL and its associated factors during chondrocyte differentiation.

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ALTERED BONE MORPHOGENETIC PROTEIN RESPONSIVENESS IN CHONDROCYTES OVEREXPRESSING C-FOS

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The c-fos proto-oncogene, a member of the AP-1 family of transcription factors, plays a critical role in the proliferation and differentiation of chondrocytes. The roles of c-fos in vivo have been demonstrated by the generation of mice which lack c-fos and develop osteopetrosis, or in mice which overexpress c-fos and develop chondrosarcomas and osteosarcomas. We have developed an in vitro c-fos inducible system in ATDC5 chondrocytes and have shown that exogenous c-Fos expression inhibits chondrocyte differentiation. However, the mechanisms through which c-Fos exerts its effects are not known. Bone Morphogenetic Proteins (BMPs) are members of the transforming growth factor (TGF)-beta superfamily, and play critical roles in not only osteogenesis, but also chondrogenesis. Here, we have investigated the effects of BMPs on cartilage differentiation and gene expression in a previously characterised clone of ATDC5 cells (DT12.4) expressing an inducible c-fos gene.

We first investigated the effects of exogenous BMP-2 and BMP-4 on in vitro differentiation of DT12.4 cells in the absence or presence of exogenous c-Fos induction. BMP-2 and -4 caused a dose-dependent increase in cartilage nodule formation although doses greater than 100 ng/ml were inhibitory. Interestingly, exogenous BMP-2 and -4 rescued the c-Fos-dependent inhibition of differentiation. However, higher concentrations of BMPs were required to restore cartilage differentiation in the presence of c-Fos, and the extent of differentiation was greater than in the absence of c-Fos. RT-PCR analysis of type II and type X collagen expression confirmed the rescue of c-Fos inhibition of differentiation at the molecular level. These results suggest that DT12.4 cells overexpressing c-Fos exhibit a decreased responsiveness to BMP-2 and -4. To investigate this further, we next investigated whether ectopic expression of c-Fos altered the expression of endogenous BMPs. Indeed, Northern blot analysis demonstrated that induction of c-Fos inhibited the expression of BMP-4 mRNA during the early phases of DT12.4 cell differentiation.

Taken together, the inhibition in endogenous BMP-4 expression and corresponding decrease in cellular sensitivity to exogenous BMP-4 treatment, suggests that one mechanism by which c-Fos inhibits cartilage differentiation is by disrupting the autocrine regulation of cartilage differentiation by BMP signalling.

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THE EFFECTS OF C-FOS OVEREXPRESSION ON OSTEOCLAST DIFFERENTIATION IN TRANSGENIC MICE

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It is well-established that the c-fos proto-oncogene is an essential gene for osteoclast differentiation. However, c-fos is also important for oncogenic transformation of osteoblasts, since transgenic mice ubiquitously overexpressing c-fos develop bone tumours. Moreover, we have shown that transgenic founder mice overexpressing deregulated c-fos in osteoclasts using a TRAP promoter also develop large bone lesions and tumours. In both cases, the tumours contain large amounts of neoplastic bone which is remodelled by abundant osteoclasts. However, the functional role of the osteoclasts in neoplastic bone remodelling, and the consequences of c-Fos overexpression on osteoclast differentiation and activity in transgenic mice are not well understood.

We have analysed the expression of osteoclast marker genes and osteoclast-inducing cytokines in c-Fos-induced tumours. Histochemical staining and in situ hybridisation analysis revealed high expression of several osteoclast markers, such as TRAP, cathepsin K and MMP-9 present in multinucleated cells and mononuclear precursors. Osteoclast-like cells also expressed high levels of c-fms and RANK mRNA. RANKL and OPG were also expressed in transformed osteoblast-like cells, but also in other stromal/fibroblastic cells present within the tumours at sites not in close proximity to bone tissue. The high number of osteoclasts in c-Fos-induced tumours suggests that exogenous c-Fos expression enhances osteoclast differentiation.

To investigate further whether this observed increase in osteoclasts was due to specific effects of c-Fos on the macrophage/osteoclast lineage, we next examined the in vitro osteoclastogenic capacity of primary M-CSF-dependent bone marrow cells derived from c-Fos transgenic mice cultured in the presence of M-CSF and RANKL. Preliminary experiments confirmed that M-CSF-dependent bone marrow precursors express the c-fos transgene. Dose-response experiments for M-CSF and RANKL revealed a ~50% increase in the number of TRAP-positive multinucleated cells from transgenic bone marrow compared to marrow cells derived from wild-type littermates. Semi-quantitative RT-PCR analysis demonstrated that there were no differences in RANK expression between transgenic and wild-type bone marrow cells, suggesting that the apparent increase in RANKL responsiveness in c-Fos overexpressing cells lies downstream of RANK signalling. These results imply that osteoclast/macrophage precursors overexpressing c-Fos exhibit an increased sensitivity to RANKL and M-CSF signalling, which may lead to enhanced osteoclast formation.

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NEURAL ARCH LOAD-BEARING CAN WEAKEN THE ANTERIOR VERTEBRAL BODY AND PRE-DISPOSE TO OSTEOPOROTIC FRACTURE

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Osteoporosis weakens vertebrae making them prone to fracture, particularly when the spine is flexed. Reduced whole vertebral body bone mineral density (BMD) is conventionally used to diagnose spinal osteoporosis. Recent evidence suggests that bone loss can be more pronounced in the anterior vertebral body. We hypothesise that age-related degeneration of intervertebral discs increases neural arch compressive load-bearing, causing pronounced anterior vertebral body bone loss and weakening.

Fifteen cadaveric motion segments (aged 72-92 yrs), comprising of 2 adjacent vertebral bodies and intervening disc and ligaments, were compressed to 1.5kN while positioned extension (2 degrees) to simulate erect standing posture. Profiles of intradiscal stress, measured by pulling a miniature pressure transducer along the mid-sagittal diameter of the disc, were 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 flexion to simulate forwards stooping motion. BMD of the anterior and whole vertebral body was measured by dual energy x-ray absorptiometry. The degree of disc degeneration was assessed using established criteria.

All discs were mildly or severely degenerated. In erect posture, the neural arch resisted 12–86% of the applied 1.5kN, while the anterior vertebral body resisted only 4–32%. Compressive strength in flexion correlated negatively with load-bearing by the neural arch 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 ($r2 = 0.72$, $p < 0.001$).

Age-related degenerative changes cause intervertebral discs to lose height, increasing the likelihood of neural arch compressive load-bearing in erect postures. This unloads the disc, particularly its anterior half, 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. These observations could explain why conventional BMD measurements of whole vertebrae do not always predict who is at greatest risk of osteoporotic vertebral fracture.

1. Pollintine P et al (2001). *Transactions of the Orthopaedic Research Society*. San Francisco, USA.

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INVESTIGATION INTO THE PLASTICITY OF ADULT HUMAN BONE MARROW ADIPOCYTES

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Osteoblasts and adipocytes are derived from the same stem cell in adult marrow and plasticity exists between the two lineages. In Osteoporosis there are reduced osteoblast numbers and increased adipocyte numbers suggesting a disturbance in the normal balance of the cells' differentiation pathways, leading to defective osteoblastogenesis and favouring adipogenesis. The stage of adipocytic differentiation at which plasticity is lost and commitment is established is not known. We have recently shown that by changing the environment adipocytic precursors can convert to osteoblasts. This study aims to investigate the ability of mature adipocytes to convert to osteoblasts.

Local Ethical Committee approval was obtained. Mature adipocytes were isolated from the bone marrow sample of a young adult male donor by density gradient centrifugation and cultured in vitro in adipogenic medium for 7 days. They were then cultured in dedifferentiation medium for 35 days prior to being switched to osteogenic medium or mineralising medium for 14 days. Morphology and histochemical staining for oil red O, alkaline phosphatase and alizarin red S was noted at regular intervals. Mature adipocytes dedifferentiated to fibroblast-like cells and then redifferentiated to osteoblasts and formed mineralised calcium upon appropriate stimulation. In conclusion, by changing the environment mature adipocytes can convert to osteoblasts. Current work is centred on extending these findings by genotypically typing adipocytes and osteoblasts during the switch between the two lineages. We propose that, if the factors responsible for the switch between adipocytes and osteoblasts were identified, they could be used for the development of novel therapeutic intervention in Osteoporosis.

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VERTEBROPLASTY CAN RESTORE THE MECHANICAL FUNCTION OF THE OSTEOPOROTIC SPINE BY RELIEVING NEURAL ARCH COMPRESSIVE LOADING

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Vertebroplasty, a procedure involving orthopaedic cement augmentation of vertebrae, can alleviate pain associated with osteoporotic vertebral fracture. Vertebroplasty restores the mechanical stiffness of individual fractured vertebrae, but no data is available on the effect of vertebroplasty on the functional spine unit. We investigate how vertebral body fracture and subsequent vertobroplasty affects the compressive load distribution in cadaveric motion segments.

Eight motion segments, comprising two adjacent vertebrae and the intervening discs and ligaments, were dissected from levels T9-L6 of five cadaver spines (72 - 90 yrs). Each specimen was positioned in moderate extension or moderate flexion and compressed at 1.5kN while intradiscal stress profiles were obtained by pulling a miniature pressure transducer along the mid-sagittal diameter of the disc (2). Compressive failure was then induced in moderate flexion, and stress profiles repeated. Vertebroplasty was performed by injecting 7ml of low viscosity polymethylmethacrylate cement through the pedicles into the anterior of the fractured vertebral body. Stress profiles were then repeated.

For each profile, the force on the neural arch was obtained by subtracting the disc compressive force, calculated by integrating intradiscal stress over area, from the applied 1.5kN (1).

Fracture caused the average compressive load resisted by the neural arch to increase from 17.1% to 42.2% in flexion ($p<0.01$) and from 51.8% to 72.9% in extension ($p<0.0005$). Vertebroplasty on fractured veretebrae caused neural arch load-bearing to decrease from 42.2% to 23.68% ($p<0.03$) in flexion, but had no effect in extension ($p=0.2$) There was no significant difference between pre-fracture and post-vertebroplasty status in flexion ($p=0.11$).

Neural arch loading increased to abnormally high levels after fracture, especially in flexion, and this may be a source of pain in living people after vertebral body collapse. Vertebroplasty did not reduce neural arch load bearing in extension, possibly because the anterior, and not the posterior section of the vertebral body was augmented. However, neural arch loading in flexion was reduced to pre-fracture levels following vertebroplasty, and this could contribute to the pain relief reported by patients who undergo this procedure.

1. Pollintine et al (2001). *Transactions of the Orthopaedic Research Society*. San Francisco, USA.

P44

PROLIFERATIVE CAPACITY AND OSTEOGENIC POTENTIAL OF SERIALLY PASSAGED HUMAN BONE MARROW STROMAL CELLS DERIVED FROM NORMAL AND OSTEOPOROTIC MARROW

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Osteoporosis (OP) results from an imbalance between osteoblastic bone formation and osteoclastic bone resorption leading to bone loss. Evidence from our laboratory shows that the cellular disturbances in OP are not uniform and that 84% of women with postmenopausal OP have reduced osteoblast numbers with a subgroup of these patients showing failure of recruitment from progenitor cells. Cellular senescence occurs when normal somatic cells stop dividing. Senescent cells remain viable but often show alterations in phenotype and cellular gene expression that may affect their differentiation potential. This preliminary study aimed to investigate the proliferative capacity and osteogenic potential of serially passaged human bone marrow stromal cells (HBMSCs) in vitro and determine differences in these between cells from normal and osteoporotic marrow.

HBMSCs were isolated from the bone marrow samples of one normal and one osteoporotic patient by density gradient centrifugation, cultured in standard medium and serially passaged. Morphology, number of days in culture, population doublings, population doubling time and histochemical staining for senescence associated (SA) beta galactosidase and alkaline phosphatase (ALP) was noted for each passage. HBMSCs from each passage were cultured in osteogenic medium for 12 days and histochemical staining for ALP before and after stimulation as well as ALP activity over the 12 days was performed. Cells from normal marrow underwent senescence at passage 10 after 103 days and 20 cumulative population doublings whereas cells from osteoporotic marrow underwent senescence at passage 9 after 83 days and 17 cumulative population doublings. In both normal and osteoporotic samples: SA beta galactosidase staining was less than 10% in initial passages but more than 90% in senescent passages; ALP staining increased from less than 10% prior to stimulation to more than 50% after stimulation in initial passages but remained 0 after stimulation for later passages; and ALP activity showed a gradual rise over the 12 days in osteogenic medium in initial passages but remained at basal levels for later passages. In conclusion, HBMSCs have a limited proliferative capacity and osteogenic potential in vitro. Moreover, HBMSCs from osteoporotic marrow demonstrate senescence earlier which may be an important pathogenetic factor in the abnormal osteogenic differentiation seen in OP.

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INCIDENCE OF HIP FRACTURE IN FEMALE STROKE PATIENTS

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Hip fracture has serious consequences for health resources. Stroke patients are reported to have a higher incidence of hip fractures. Time between stroke and fracture are variously observed as early or significantly delayed. As part of a larger study we investigated fracture incidence in female chronic stroke sufferers and fit controls over 60yr.

Data on 126 female chronic stroke sufferers(CS) recruited from stroke units and 81 fit control(FC) female volunteers, were recorded. Information was collected on fracture history, falls within the previous month, and time of stroke, in 'CS'. Fracture history was collected for 'FC'. LREC approved.

Of 56 fractures in 42'CS' patients(33%) and 37 in 27 'FC'(33%), 31(25%) in 'CS' and 18(22%) in 'FC' occurred since age 50yr. 14 hip fractures occurred in 'CS', 9(7%) since age 50yr , compared with 3(4%) since age 50yr in 'FC'.

Hip fracture frequency was increased 3.1 fold in stroke ($p\sim0.05$) and 1.8 fold for ankle fractures(ns). Arm and wrist fracture frequency was the same for both groups.

Six 'CS' fractured a hip post stroke, with a median time since stroke of 3.5yr, with 3 having had evidence of falls in the previous month. Two of these patients had also had wrist fractures post stroke. Half of patients with post stroke hip fractures had them on the stroke side. Mean age at fracture was 85yr. Mean heel BMD z-score was −1.9 on the stroke affected side and −1.8 on the un-affected side .

In 10yr age groups, one 'CS' aged 70-79yr(n=27) had a post stroke hip fracture against none(n=23) in 'FC'. There were 3 post stroke hip fractures in the 80-89yr group(n=68) against 3 in the controls(n=18). After age 90yr there were 2 post stroke hip fractures(n=19) compared with none in controls(n=6). Falls occurred less frequently in 'CS' <79yr compared with >79yr($p<0.05$).

Hip fracture post-stroke affects the elderly and is delayed by 3-4yr. Hip fracture tends to be increased in stroke patients but over half occurred pre-stroke. Half of the 'CS' patients with post-stroke hip fracture had fallen the previous month. Falls increase in stroke subjects >79yr.

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URINARY BONE MARKERS CAN MONITOR TREATMENT FOR OSTEOPOROSIS

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In clinical trials bone marker measurements decline after 3 months, suggesting that they may be useful in following therapy. This is confounded by individual variation, and uncertainty about which marker responds best. We studied variation in 2 breakdown markers over 1 year, calculated the least significant change (LSC) and applied this to treatment of patients with osteoporosis in the community.

Women over 50yr were recruited from medical practices. A mobile densitometer was used to scan their distal forearms locally, and select those with forearm BMD<0.419. 355 took part in the study. 51 were not analysed because of prior medication. 133 had distal forearm BMD<0.34 and formed Group A (osteoporotic). The others had a DEXA scan at lumbar spine (LS) and femoral neck (FN). 85 of these (Group B) had T score >2 at either site (osteoporotic). The rest formed Group C (normal). They were rescanned at 12 months

Treatment was determined by their own doctor, knowing their bone density. Subjects collected and mailed a second morning urine sample from home at baseline and at 3, 6 and 12 months. Samples were stored frozen until assayed for NTx (ELISA, Osteomark), DPD (RIA, IDS) and creatinine (Jaffe).

136 subjects received no treatment before or during the study and form a reference set. Of these, 31 were Group A, 17 Group B and 88 Group C.

LSC was calculated using within-subject variation of the reference set. This was unchanged among BMD groups. Since DPD/Cr and especially NTX/Cr were positively skewed, LSC was also derived for log-transformed values, which were not significantly skewed.

Of 87 taking bisphosphonates, NTx fell >LSC in 5% (50% if log NTx was used) at 3 months. The proportions were 6% and 70% at 12 months, when the proportions for DPD were 20% in both cases. In Groups B and C, BMD at LS fell >LSC in 24% and 20% respectively, but in FN 7%, only in Group B. LogNTx fell >LSC in 9 of 15 HRT-treated women.

Bone markers in mailed urine samples may be useful to follow treatment of individuals, if reference limits are appropriately assessed.

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EFFECTS OF A 15 MONTH CALCIUM AND EXERCISE INTERVENTION ON MARKERS OF BONE AND CALCIUM METABOLISM IN 16-18 YEAR OLD GIRLS.

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The impact of increased calcium (Ca) intake and exercise on bone mineral status in older adolescents has not been previously investigated. To address this, a 15 month randomised, double-blind, placebo-controlled calcium intervention study (1000 mg of Ca/day (as CaCO3)) was carried out in 144 girls aged 17.3 ? 0.3 years. Subjects were stratified by supplement group and were randomly assigned to an exercise or a non-exercise group. At the end of the study, the Ca-supplemented girls showed significant increases in size-adjusted bone mineral content (BMC) ranging from 1-5%. A modest effect of exercise (1-2%) was found at the hip in those attending >50% of the exercise classes. No interaction was found between the response to Ca supplementation and the exercise program. The mechanisms underlying the skeletal changes were further investigated by measurement of markers of bone and Ca metabolism in fasting morning urine and non-fasting blood samples, collected at baseline and final timepoints. Urine was analysed for creatinine (Cr), Ca, phosphate, deoxypyrindinoline (DPD) and N-terminal telopeptides of type I collagen (NTx). Plasma was analysed for parathyroid hormone (PTH), phosphate, albumin-adjusted Ca, osteocalcin and bone-specific alkaline phosphatase (BAP). Plasma ferritin was measured to determine whether iron status was affected by the Ca supplement. In the intention-to-treat regression model, the calcium intervention resulted in significant increases in urinary Ca (+23%, p=0.045). There were significant reductions (adjusted for time of sample collection) in osteocalcin (-24%, p=0.0001) and bone-specific alkaline phosphatase (-10.6%, p=0.017). PTH was also reduced, but this was not significant (-17.3%, p=0.084). No effect of the Ca supplement was found for any of the other bone markers, or ferritin. Restricting the analysis to those with compliance >50%, showed that urinary Ca remained significantly higher (+32%, p=0.012). Only osteocalcin remained significantly lower (-17.4%, p=0.02), with BAP and PTH approaching significance (-9.6%, p=0.06 and –21%, p=0.07, respectively). No interaction was found between the response to the Ca and exercise interventions. The results of this study suggest the increases in size-adjusted BMC may have been due to a bone-remodelling transient. An effect on bone resorption may have been masked by the overall age-related decline in both NTx and DPD.

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CHEMOTHERAPY-INDUCED APOPTOSIS IN HUMAN OSTEOBLAST-LIKE CELLS AND MODULATION OF THEIR EFFECTS BY DEXAMETHASONE

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Osteopenia may occur during treatment for childhood leukaemia with chemotherapeutic agents and dexamethasone. We have previously shown that several chemotherapeutic agents deplete osteoblast numbers and that this effect is reduced by pre-treatment of cells with dexamethasone. Our aims were to determine whether chemotherapeutic agents induce apoptosis in human osteoblast-like cells and to evaluate whether pre-treatment with dexamethasone influences this effect.

The human cell types used represent different stages of osteoblast differentiation; an osteoprogenitor cell line (HCC1), an osteosarcoma cell line (MG63), and primary bone marrow stromal (BMS) cells and osteoblast-like (HOB) cells derived from children. Cells were cultured with a clinically relevant concentration of vincristine, etoposide, asparaginase or methotrexate for up to 72 h, labelled with Annexin V-FITC and subjected to flow cytometry. In other experiments, HCC1 and HOB cells were pre-treated with 0.1 micromolar dexamethasone for 72 h and then cultured for a further 24 h with dexamethasone and either vincristine or etoposide prior to flow cytometry. The frequency of apoptosis within cell populations was determined using WinMDI software.

After 24 h culture with vincristine, there was an increase of up to 400% in cells undergoing apoptosis in HCC1, BMS and HOB cell populations compared to control cultures (all $p<0.05$). Apoptosis was not demonstrated in MG63 cells cultured with vincristine nor with any cell types cultured with the other agents for 24 h. Following 72 h culture with each of the agents, however, increases of up to 470% in apoptosis compared to control were observed in MG63 and HOB cells (all $p<0.05$). Dexamethasone alone reduced apoptosis by 30% and 43% in HCC1 and HOB cells respectively (both $p<0.05$). Furthermore, pre-treatment with dexamethasone resulted in a 26% reduction of HCC1 entering early apoptosis ($p<0.05$), and a 43% reduction of HOB cells entering late apoptosis ($p<0.05$), when cultured with vincristine or etoposide.

These findings show that chemotherapeutic agents deplete osteoblast numbers in vitro by induction of apoptosis. However, dexamethasone reduced chemotherapy-induced apoptosis in these cells. We speculate that osteopenia may be a result of chemotherapy-induced apoptosis in osteoblast populations yet this effect may be attenuated by prior administration of dexamethasone.

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PHYSICAL MEASURES OF LOWER LIMB BONE: DO THEY HAVE THE SAME EPIDEMIOLOGICAL DETERMINANTS?

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There have been many population-based studies of the epidemiological determinants of bone's physical properties using different technologies. Recently, there has been a move towards using X-ray based techniques for estimating the strength of bones. This varied approach reflects our lack of understanding of what aspect of bone mass, density or strength is regulated biologically by the processes reflected in key epidemiological determinants, such as body weight and physical activity.

We adopted the null hypothesis that physical measures of bone (hip BMD, hip strength and calcaneus BUA) would have the same epidemiological determinants. If true, the choice of measurement would be guided by the needs of a particular study for measurement precision, low price and convenience. If false, the choice of measurement would be guided primarily by biological relevance.

We recruited 330 men and 333 women aged >65yrs from a prospective population based cohort. Total hip BMD was measured using DXA(Hologic-1000W) on two occasions and hip structural analysis software used to derive the section modulus(SM) - an index of bending resistance. Calcaneal ultrasound attenuation and VOS was measured twice at the heel(CUBA system). Multivariate repeated measures analysis of covariance was used for modeling.

Body weight was positively associated with all outcomes apart from VOS($P<.0004$). In women, previous fracture was associated with lower BMD, BUA, VOS and trochanteric SM($P<0.008$). Otherwise, individual determinants segregated only with one or two outcomes. Height was positively associated with SM at the neck and trochanter($P<0.016$) and positive in men only at the shaft($P<.0001$). Contrasting with SM, shaft BMD was inversely associated with height($P=0.003$). Past physical activity was associated with increased shaft (men) or neck (women) SM($P<0.010$) and stair climbing positively influenced shaft BMD in women($P=0.027$). Neck and shaft BMD decreased with age($P<0.030$) and grip strength was positively associated with SM at the neck and trochanter($P<0.019$). A model used to investigate strong gender interactions did not reveal any and further suggested that time spent on weight bearing activity was associated with increased SM at the neck($P=0.009$).

In conclusion, apart from effects mediated through body weight and fracture history, there were noteworthy differences in epidemiological determinants of strength and density at various sites.

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EXPRESSION OF OSTEOPROTEGERIN SPLICE VARIANTS IN HUMAN BONE AND OTHER TISSUES

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Osteoprotegerin (OPG) is a soluble protein that antagonises the effect of Receptor activator of NFkB ligand (RANKL), the key osteoclast differentiation factor. The ratio of OPG/RANKL is an important determinant for osteoclast formation. OPG was originally cloned from the human embryonic lung fibroblast cell line IMR-90, where 3 different mRNA species were identified, resulting from alternative splicing. The main transcript (m) results in a mRNA of 2.4 kB, whereas partial splicing of intron 2 results in a 4.2 kB mRNA (v1). A third mRNA species exists (v2) where all of intron 2 is retained. In v1 and v2, translation stops at termination codons in intron 2, producing truncated proteins which only contain 3 of the 4 Cys-rich motifs required for OPG function (Moringa et al., Eur. J. Biochem. 254, 1997) and could therefore result in a reduction in biologically active OPG protein.

It is unclear if OPG splice variants exist in human bone. Here we examined in a qualitative way the presence of OPG splice variants in human bone and peripheral blood cells in vivo and some other human tissues ex vivo.

Primers were designed to identify the 3 transcripts (m, v1 and v2) using a single forward primer and 3 different reverse primers. RNA was obtained from bone biopsies, or peripheral blood mononuclear cells (PBMCs) using Trizol, treated with DNAse to remove any contaminating DNA and reverse transcribed. 125 nanogram of cDNA was used per PCR reaction. In IMR-90 all 3 transcripts were easily detectable and confirmed as OPG transcripts by sequencing. Presence of all 3 transcripts was also seen in RNA from primary osteoblasts and endothelial cells, whereas bone marrow stromal cells and synovial cells only showed m and v2. In pagetic bone biopsies transcripts m and v2 were detected in all 6 patients tested, whereas v1 was seen in one patient only. In PBMCs only v2 was found (7 out of 18 samples tested).

These data indicate that alternative splicing of OPG occurs in vivo and suggests this may represent an alternative way of regulating OPG expression.

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GLUCOCORTICOIDS, CATCH UP GROWTH AND RESPONSE TO RESCUE TREATMENTS IN THE CHONDROCYTE ATDC5 CELL LINE.

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A principal side effect of glucocorticoid (GC) treatment is growth retardation in children, which is, in part, due to direct effects on growth plate chondrocytes. Previous studies by us have shown that dexamethasone and prednisolone alter the proliferation and differentiation rate of the chondrocyte ATDC5 cell line and that these adverse effects were more pronounced during the chondrogenesis period. In this present study we have assessed the ability of the ATDC5 cells to recover from dexamethasone treatment and also the ability of growth hormone (GH) and insulin like growth factor-I (IGF-I) to rescue the cells from dexamethasone side effects.

Dexamethasone was added at day 0 (D0), in quadruplicate at a concentration of 10(superscript)-6 M for 1, 3, 7, 10, or 14 days after which dexamethasone was removed and all cells cultured up to 14 days. In separate plates dexamethasone at 10(superscript)-6 M was added along with GH and IGF-1 both at 50ng/ml. These were stopped at the end of the chondrogenic phase (D4). Alkaline phosphatase (ALP) activity, protein and proteoglycan concentrations were determined.

RT-PCR indicated that GC and IGF-I receptors were expressed from D0 and the GH receptor from D3. Cell number was reduced at all days with dex treatment, but it was only significant at D14 (0.69 ± 0.09mg vs. 0.96 ± 0.08mg, $P<0.05$). Proteoglycan synthesis showed a stepwise reduction from D0 reaching a plateau at D7 (1.09 ± 0.05 units vs. 0.48 ± 0.04 units, $P<0.001$) after which there was no further reduction. Mean ALP activity (nmoles/mg protein) was elevated with all dexamethasone treatments reaching significance from D10 onwards (845 ± 160 vs. 332 ± 19, $P<0.001$). Addition of GH, IGF-I and GH/IGF-I to the Dex treated cells had no beneficial effect on cell number but GH and GH/IGF-I did increase proteoglycan levels to above those of the dexamethasone alone treated cells ($p<0.05$).

In conclusion, short exposure to dexamethasone allows the chondrocytes to exhibit partial catch-up growth to near normal whereas prolonged exposure (longer than 10 days) suppresses catch up growth by reducing cell number and stimulating differentiation. Further studies with the rescue experiments are required, as, under the conditions examined, both GH and IGF-I had limited beneficial effects.

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THE GENETIC DETERMINANTS OF BONE STRENGTH: TOWARDS THE MAPPING OF CANDIDATE GENES FOR BONE TURNOVER

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Bone mineral density is a complex term that includes mineral content, size, trabecular complexity and shape. Bone strength is determined primarily by these variables. In order to characterise the molecular pathways involved in bone formation and resorption it is necessary to determine the genes that contribute to bone strength.

Our approach has involved the selection of lines of chickens with high and low bone strength (high and low lines respectively). After 8 generations of bodyweight restricted selection on bone characteristics these lines have tibial breaking strengths that differ by 92% (303.7±12.5 v 157.9±10.9 Newtons: $P<0.001$), with no significant difference in bodyweight (1625.5±21.14 v 1706.8±36.8 grams). Measurements of mineral apposition rate (12.2±0.7 v 9.6±0.3 microns/day: $P<0.001$) and serum pyridinoline (0.39±0.03 v 0.46±0.021 pmol/ml: $P<0.05$) indicate that bone formation is increased and bone resorption is decreased in the high line compared to the low line. Tibial cortical thickness was also significantly greater in the high line (0.42±0.009 v 0.37±0.007 mm: $P<0.001$). Although the differences were not significant the number of osteoclasts per unit surface area of bone was lower in the high line (1063±101 v 1229±73 per sq. mm) and the serum concentration of carboxylated osteocalcin was higher (285±21.8 v 230±19.1 ng/ml) reinforcing the above trend. No differences were found in the levels of serum ionised or total calcium, or inorganic phosphate.

We are currently using these two lines to discover candidate genes for bone strength using a dual approach: 1) Microarray analysis of gene expression in bone cells (osteoblasts and osteoclasts) of the high and low lines to characterise significant changes in gene expression between the lines. 2) QTL analysis of a classical F2 intercross to identify areas of the genome with significant QTL for bone strength. Genes that show significant differences in expression levels between the lines will be mapped initially using human/mouse/chicken comparative gene maps. Known properties of genes from available functional data will be used to prioritise the most likely candidate genes at each QTL. This dual approach will allow us to focus on a limited number of genes important for bone strength for further study.

P53

VITAMIN D INSUFFICIENCY AND POOR BONE HEALTH IN LACTO-VEGETARIAN WOMEN IN MANCHESTER

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There is still no agreed definition of a sufficient level of vitamin D in terms of serum 25-hydroxyvitamin D (25OHD) concentration. Until recently any level above that associated with osteomalacia was considered to be sufficient but it is now recognised that there is an intermediate state in which vitamin D levels are insufficient but not frankly depleted. Such a state is often associated with increased levels of parathyroid hormone and leads to poor bone health.

We have examined the level of serum 25OHD in white and Asian omnivore and lacto-vegetarian women in Manchester, as part of a larger study of the effect of diet on bone health. Vitamin D deficiency was defined as <5 ng/ml (12 nmol/L) 25OHD in serum, insufficiency as 5-15 ng/ml (12-38 nmol/L) and sufficiency as >15ng/ml (38 nmol/L). These levels may be considered relatively conservative by some non-UK investigators.

Asian women, whether omnivores or vegetarians, were found to have significantly ($p<0.001$) lower levels of serum 25OHD than white women, with 90% of those studied being in the D-deficient or insufficient category. (Mean±sem serum 25OHD (ng/ml): white omnivore [WO] (n=39) 18.6±1.5; white vegetarian [WV] (n=33) 15.5±1.1; Asian omnivore [AO] (n=28) 8.6±1.2; Asian vegetarian [AV] (n=12) 7.4±1.8). No white women were found to be vitamin D deficient although 36% of WO and 56% WV were D-insufficient. Within ethnic groups, these differences could not be explained by differences in life-style, height or weight, although as a group Asian vegetarian women were significantly older (mean±sem age (y): WO 38±2, WV 35±2, AO 35±2, AV 53 ±4).

The poorer vitamin D status in vegetarian women was reflected in a trend towards poorer bone health (Bone mineral density measured by DXA: Mean±sem Total hip Z score: WO 0.08±0.89; WV –0.12±0.77; AO -0.01±0.94; AV –0.79±0.64 ($p=0.014$); Spine L1-L4 Z score WO 0.38±1.21; WV 0.08±0.83; AO –0.36±0.84; AV –0.40±1.00 ($p=0.008$))

The data suggest that a meat-containing diet may protect against vitamin D insufficiency and resultant poor bone health, although the mechanism of this protective effect is unknown. These findings have implications for bone health in the future since more and more young women in the UK are choosing to eat a wholly vegetarian diet.

P54

ONE YEAR CHANGES IN URINARY BONE MARKERS IN OSTEOPOROTIC PATIENTS TREATED WITH INTRAVENOUS PAMIDRONATE

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Urinary pyridinoline(PYD) and deoxypyridinoline(DPD) are breakdown products of type I collagen and are markers of increased osteoclast activity and bone resorption. We present 1 year bone marker data on patients who received intravenous (iv) pamidronate for difficult to treat osteoporosis.

56 patients with osteoporosis of the lumbar spine and/or hip were openly studied. All received 30mg iv pamidronate 3 monthly and 1g calcium and 800iU vitamin D daily. Urinary bone markers were measured at each 3 monthly visit. Bone mineral density (BMD) measurements were made by DEXA scan at the lumbar spine (LS) and hip at 0,6 and 12 months.

Unfortunately some urine samples were lost and we therefore limited our data to patients with bone marker results at both baseline and 12 months (N=24). All bone marker values were corrected for urine creatinine.

Percentage changes [median(range)] for PYD at 3,6,9 and 12 months were [-11.29(-47.04 to 56.92)],[-9.87(-41.25 to 59.91)],[3.29(-31.20 to 21.62)],[-3.73(-42.59 to 192.95)] respectively. For all changes $p>0.05$.

Percentage changes for DPD at 3,6,9 and 12 months were [-19.10(-67.63 to 104.73)],[-18.35(-59.60 to 44.37)],[-10.88(-37.98 to 59.00)],[-14.09(-54.22 to 171.95)] respectively. For all changes $p>0.05$.

A significant decrease (>30%) in PYD and DPD was seen in 23.1% and 30.8% of patients respectively at 3 months and in 12.5% and 20.8% of patients respectively at 12 months.

Percentage changes in BMD at 12 months [median(range)]: LS BMD[4.63(-12.09 to 15.35)];($p=0.017$) and total hip BMD[3.18(-11.42 to 15.47)];($p=0.006$).

Conclusion

Although there was a downward trend in PYD and particularly DPD levels at most time points in relation to baseline levels, these changes did not reach statistical significance. Changes in BMD at 12 months were significant. Confounding factors in our study were missing data and small patient numbers.

P55

GENE EXPRESSION PROFILES DURING CHONDROGENESIS IN ATDC5 CELLS

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During chondrogenesis, mesenchymal-derived cells undergo a number of transitions that includes differentiation from a committed precursor, proliferation and terminal differentiation to a hypertrophic/mineralising phenotype. There is considerable interest in identifying both the signalling cascades that regulate these transitions, and the function of particular gene products expressed specifically by each phenotype. One strategy to identify such genes of interest is to monitor the changes in gene expression that occur throughout chondrogenesis. Recent advances in microarray technology afford the opportunity to measure the levels of expression of many thousands of genes in a single experiment. Here we describe a small-scale microarray analysis designed to determine the feasibility of monitoring global changes in gene expression in an in vitro model of chondrogenesis.

Murine ATDC5 cells were induced to undergo chondrogenic differentiation by culturing in DMEM/5% FCS containing 1% insulin. RNA was extracted from cells harvested at 5 and 10 days, representing cells that were respectively negative and positive for the expression of type II and type X collagen, as judged by RT-PCR assays. Cy3 and Cy5 labeled cDNAs were hybridised to a Genomic Solutions GeneMap mouse array containing 922 elements in duplicate. The array was imaged in both the Cy3 and Cy5 channels and the relative change in expression calculated from the ratio of the fluorescence intensities.

A preliminary analysis of the raw data indicated that, relative to day 5 cells, 68 genes were up-regulated at day 10. Of these 57 exhibited a 2-3 fold increase in abundance, 9 a 3-4 fold increase, while 2 genes exhibited a greater than 4 fold increase in expression. Significantly more genes (480) were down-regulated and of these 87 exhibited a greater than 4 fold decrease in expression. Of the genes identified two were of direct relevance to skeletal biology the transcription factor Cbfa1 (down-regulated 4-fold) and the extra-cellular cell adhesion protein Osf-2 (up-regulated 6.7-fold). It will be necessary to verify the patterns of expression observed in this study using other methodologies such as Northern analysis or RT-PCR. Nevertheless, these preliminary data demonstrates that microarray technology has wide applications in bone research.

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HIDDEN LOSS OF BONE MINERAL DENSITY AND FAT FREE MASS IN ADULTS WITH CYSTIC FIBROSIS

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Improved survival in Cystic Fibrosis (CF) is associated with complications such as diabetes and bone disease. Loss of bone mineral density (BMD) was associated with loss of fat free mass (FFM) and total body weight,a predictor of reduced survival. This occurs despite nutritional advice, aggressive antibiotic use and centralised care. Maintenance of a normal body weight is widely used as an indicator of effective nutritional management in CF.

Hidden loss of FFM has been reported in chronic obstructive pulmonary disease.

We hypothesised that hidden loss of FFM may occur when body mass index (BMI) is preserved and would be associated with reduced BMD, severe impairment of the lung function and greater systemic inflammation.

Fifty six clinically stable adults (mean (95%CI) age 23.0 (20.8, 25.3) years) and 20 healthy subjects (23.6 (22.0, 25.6) years were studied. Fat mass, bone-free FFM and BMD (DXA), lung function, physical activity (questionnaire), the annual frequency of exacerbations of respiratory symptoms and circulating C-reactive protein (CRP) were determined.

BMD at all sites was less in patients than in healthy subjects ($p<0.01$ for all). BMD at all sites was less ($p<0.05$) in patients with an FEV1 <40% predicted compared with those with an FEV1 >60% predicted. Of the 56 patients, 30 had a normal BMI (>20.0 kg/m2), of which 12 had a low FFM (< lower 5th percentile for healthy). The mean total, trochanteric, intertrochanteric and femoral neck BMD were less in those with a normal BMI and low FFM compared to the normal BMI and normal FFM (all $p<0.01$). Patients with hidden loss of FFM had a reduced index at the right arm, leg and trunk (corrected for height) and more severe lung disease (FEV1*), reduced physical activity *, more frequent exacerbations* (*$p<0.05$) and raised CRP ($p<0.001$) compared to those with a normal FFM. Multiple stepwise regression with total BMD as the dependent variable revealed FFM ($p<0.01$), FEV1 ($p<0.05$) and physical activity ($p<0.05$) influenced BMD loss.

Body composition reflects more closely disease severity than BMI, because hidden depletion of FFM occurs.

These findings indicate some patients manifest clinically inapparent complications of combined lung and metabolic disease.

P57

THE OSTEOCYTIC EXPRESSION OF THE GLUTAMATE TRANSPORTER GLAST-1 AND ITS SPLICE VARIANT GLAST-1A.

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Previously we have demonstrated that the glutamate transporter GLAST-1 is expressed by bone in vivo and have also identified a splice variant of this gene (GLAST-1a) in which exon 3 is excluded1. We predict that GLAST-1a will have a reversed topology within the cell membrane which may result in it having different glutamate transport properties to GLAST-1. We hypothesise that osteocytes use glutamate to communicate with each other and that the GLAST isoforms may act to regulate extracellular glutamate concentration.

We have transfected MLO-Y4 osteocyte like cells with expression vectors encoding GLAST-1 and GLAST-1a GFP fusion proteins. Confocal microscopy reveals GLAST-1 expression in the plasma membrane, with accumulation at the ends of the cell processes. However the distribution of GLAST-1a GFP fusion proteins is much less uniform with much of the protein accumulating in cytoplasmic vesicles.

We have demonstrated, using RT-PCR that GLAST-1a is expressed at a lower level than GLAST-1 in rat bone and brain in vivo, the osteoblastic cell line SaOS-2 and MLO-Y4. Furthermore southern blots of these GLAST RT-PCR products reveal that the GLAST-1a:GLAST-1 mRNA ratio is much higher in MLO-Y4 than in SaOS-2 cells. We are investigating the effect of different extracellular glutamate concentration on the pattern and level of expression of the two GLAST isoforms in these two cell lines. The regulation of these GLAST variants may represent a novel mechanism controlling extracellular glutamate-mediated osteogenesis.

P58

ARE ALL FRACTURES IN POSTMENOPAUSAL WOMEN DUE TO OSTEOPOROSIS?

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It is often assumed that all fractures from minor trauma in postmenopausal women are related to osteoporosis. We have therefore measured bone mineral density (BMD) by DEXA at spine, hip and forearm with the Norland XR36 in 514 postmenopausal women, 237 of whom had suffered 365 adult fractures from minor trauma. We used logistic regression to calculate the increase in relative risk (RR) for each SD rise in body weight (BW) and years since menopause (YSM) and fall in BMD in 9 fracture groups using BW and YSM as covariables. Thus each of the three variables is adjusted for the other two in the data that follow:

Wrist fractures (88) were not related to BW; were related to YSM when BMD was measured at spine or hip (RR 1.46 and 1.32); and to BMD when measured at all sites except spine (RR 1.56 to 1.78).

Rib fractures (54) were unrelated to BW; only to YSM when BMD was measured at spine (RR 1.41); and to BMD at all sites (RR 1.72 to 1.87).

Vertebral fractures (61) were not related to BW; related to YSM at all sites (RR 1.89 to 2.32); and to BMD at all sites (RR 1.54 to 2.04).

Foot fractures (38) were unrelated to BW, YSM or BMD.

Ankle fractures (28) were related to BW when BMD was measured at spine or hip (RR 1.74 and 1.52); not to YSM; to BMD only at spine (RR 1.72).

Hip fractures (15) were not related to BW; to YSM when BMD was measured at spine (RR 1.86); to BMD at forearm and hip (RR 2.16 and 3.13).

Tibial fractures (18) were related to BW at spine only (RR 1.77); not to YSM or BMD.

Humerus fractures (20) were related to BW at all sites (RR 1.68 to 1.88); not to YSM; to BMD at all sites (RR 1.83 to 2.77).

Other fractures (43) were not related to BW; were related to YSM at all sites (RR 1.85 to 1.98); not to BMD at any site.

We conclude that not all postmenopausal fractures are related to osteoporosis; that BW is often a positive risk factor; and that some fractures are a function of YSM without being related to BMD.

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ULTRASTRUCTURAL ORGANISATION AND MOLECULAR INTERACTIONS IN THE HYPERTROPHIC CARTILAGE EXTRACELLULAR MATRIX

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Type X collagen is a member of the short chain collagen family, it is comprised of three alpha 1 (X) chains and contains three distinct protein domains. A short triple helical region which is flanked by a small non collagenous region at the amino terminus (NC2), and a larger non collagenous region at the carboxyl terminus (NC1).

Type X collagen is found in the hypertrophic region of growth plate cartilage, and its expression precedes the onset of endochondral ossification. The suggestion that type X collagen forms a hexagonal lattice in the extracellular matrix, supports the idea that type X may serve as a scaffold upon which mineral is deposited during this process.

Mutations in the COL10A1 gene result in an autosomal dominant inherited skeletal disorder called Schmid Metaphyseal Chondrodysplasia (SMCD). The majority of these mutations occur in the NC1 domain of the protein, since NC1 is the initiation site of trimer assembly, the phenotype of SMCD is caused by a depletion of type X in the matrix.

Preliminary work in our laboratory indicates that the type X collagen interacts with a number of other matrix components. Type X collagen was purified from chick hypertrophic chondrocyte cultures, following collagenase digest the NC1 domain was isolated. To define the interactions both ELISA and SPR have been used. We have identified cartilage oligomeric matrix protein, biglycan and decorin as potential interacting partners. These interactions are currently being further characterised and may suggest that type X collagen plays an important role in the integrity of the hypertrophic zone during the process of endochondral ossification.

P60

A NOVEL SIGNALLING PATHWAY IN CARTILAGE

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We have recently identified a number of genes that are differentially regulated in chondrocytes in vivo at the onset of osteoarthritis (OA). One of these genes encodes the protein kinase R-activating protein (PACT). PACT and protein kinase R (PKR) are involved in a number of responses including signal transduction, differentiation, and apoptosis. Both PACT and PKR have been shown to regulate apoptosis by activation of the eukaryotic initiation factor 2-alpha causing an increase in caspase-3 activation. PACT and PKR have not previously been investigated in chondrocytes but recent evidence has implicated PKR in TNF-alpha responsive signalling cascades in a number of other cell types suggesting a novel role for this kinase in cartilage degeneration.

To investigate the role of PACT and PKR in cartilage degeneration, we are using cytokine treatment of bovine cartilage explants and primary chondrocyte cultures. RT-PCR has confirmed mRNA expression of PACT and PKR in human OA and bovine cartilage, in vivo. Bovine cartilage explants were treated with TNF-alpha (100ng/ml), under conditions known to induce cartilage catabolism, and Western blot analysis of explants using an antibody specific to the 65-68kDa phosphorylated PKR showed that TNF-alpha increased activation of PKR. In addition, an increase in caspase activation was seen in primary chondrocytes following 1 hour of stimulation with TNF-alpha (20ng/ml). The role of PKR in this apoptotic response is currently being investigated by treatment of cultures with the PKR inhibitor, 2-aminopurine.

Since PACT is upregulated at the onset of OA and both PACT and PKR are important participants in the signalling pathways activated by pro-inflammatory cytokines, we believe that they may play an important role in the onset and progression of arthritic diseases.

P61

EFFECTS OF INHIBINS AND ACTIVIN ON HUMAN OSTEOBLAST AND OSTEOCLAST DIFFERENTIATION

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It is widely accepted that estrogen plays a critical role in the maintenance of bone homeostasis, and that estrogen deficiency in post-menopausal women results from de-repression of both osteoblast (OBL) and osteoclast (OCL) development, leading to the loss of bone mass. However, in peri-menopausal women, a time when estrogen levels have not yet begun to diminish, urinary N-telopeptide levels (a marker of bone resorption) are already elevated. The increase in bone resorption is correlated with elevated FSH levels, due to de-repression by Inhibin B (InhB). We have previously shown that cells within murine bone marrow directly respond to changes in the activin/inhibin ratio by altering both osteoblast (OBL) and osteoclast (OCL) development. The current study was designed to determine the effects of activin (ActA) and inhibin A (InhA) and InhB on human osteoblastogenesis and osteoclastogenesis. Inhibin and activin effects on OBL development were assessed using human bone marrow-derived mesenchymal stem cell (MSC) cultures. HMSCs were cultured in osteogenic differentiation medium in the presence or absence of InhA, InhB, InhA+ActA, or InhB+ActA. Osteogenic differentiation was determined on day 8 by measuring expression of alkaline phosphatase (AP) and on day 21 by staining mineralized extracellular matrix. Both InhA and InhB suppressed osteoblastogenesis; the effects of InhB were stronger than that of InhA. The suppression of OBL development by InhA and InhB was maintained even in the presence of ActA. Surprisingly, ActA stimulated OCL development in human peripheral blood mononuclear cells, even in the presence of excess soluble RANK-Fc, a potent inhibitor of OCL development. These data indicate that human OBL and OCL progenitors are direct targets of inhibin and activin regulation. We hypothesize that changes in the inhibin/activin ratio detected by these cells may alter both OBL and OCL differentiation, thereby contributing to the increased bone resorption observed in peri-menopausal women.

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ATREMATE BRACHIOPDS: A NOVEL IN VIVO SYSTEM FOR STUDYING THE EFFECTS OF BISPHOSPHONATES ON MINERALISING TISSUE.

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There is currently no effective medical therapy for Osteogenesis Imperfecta (OI). Bisphosphonates are being trialled, but the benefits of bisphosphonates in OI have not yet been fully established. Caution has been called for in the use of these drugs until sufficient clinical and laboratory data has been produced. This study was designed to complement the existing clinical trials and studies in animal models and also to investigate if fluoride and magnesium had any effect on mineral formation. The brachiopod Glottedea pyramidata (lamp shell) forms a calcium-phosphate mineral in the shell and has been shown to produce a periodic fibrillar protein which may be a form of collagen. The validation of this brachiopod model and the studies of the actions of bisphosphonates and ionic moieties on mineral composition may see an acceptable alternative to study such drug and chemical actions. Cost, public awareness and concern about animal experimentation and ethical considerations have driven workers to look for such suitable in vivo models. Pamidronate has been reported to be administered clinically at a dosage of 3mg/kg of body weight. Therefore, Glottidea, housed in a tank environment were subjected to 1. Pamidronate at a dilution of 3mg/Kg of tank water as the closest approximation for dosage of the drug. 2. Fluoride or magnesium at concentrations x2, x5, x10 that of the concentration found in their normal aquatic environment. The Glottidea were maintained in this environment for a period varying from 1 week to 1 month before fixation of the tissue in 4% glutaraldehyde fixative or freezing at –400C. Under scanning electron microscopy, the Glottidea treated with Pamidronate demonstrated gross changes in the shell. Large, fractures were observed across the shell surface and X-ray microanalysis demonstrated an altered calcium phosphate mineral ratio when compared with untreated animals. Magnesium had the effect of increasing the mineral ratio, whilst fluoride decreased the mineral ratio. The ‘real-time’ changes to mineral formation caused by ionic moieties and the profound effects of the bisphosphonate on the mineral formed in this novel in vivo system requires further study in the light of these preliminary data.