Abstracts from the
Bone and Tooth Society
Autumn Meeting

*September 18th and 19th 1996*

************

*Venue:* Imperial College, London
*Local Organiser:* Dr Caje Moniz
*Department of Clinical Biochemistry*
*King's College Hospital, Denmark Hill, London*

************

*President*
Dr Mike Horton, Division of Medicine, Bone and Mineral Centre, 5th Floor, Sir Jules Thorn Institute, The Middlesex Hospital, Mortimer Street, London W1N 8AA Tel: 44-171-380-9152 Fax: 44-171-636-3151

*President-Elect*
Dr Stuart Ralston, Department of Medicine and Therapeutics, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD Tel: 44-1224-681818 Fax: 44-1224-699884

*Secretary*
Dr Juliet Compston, University of Cambridge, Department of Medicine Box 157 Level 5, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QO Tel: 44-1223-336867 Fax: 44-1223-336846

*Treasurer*
Dr Cyrus Cooper, MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO16 6YD Tel: 44-1703-777624 Fax: 44-1703-704021

*Committee*
Dr Tim Arnett, Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT Tel: 44-171-387-7050 Fax: 44-171-380-7349
Dr Brian Ashton, Department of Rheumatology, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire SY10 7AG Tel: 44-1691-655311 Fax: 44-1
Dr Jon Beresford, Bath Institute for Rheumatic Diseases, One Trim Bridge, Bath BA1 1HD Tel: 44-1225-448444 Fax: 44-1225-336809
Dr Isobel Braidman, Department of Medicine, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT Tel: 44-161-275-5179 Fax: 44-161-275-5272
Dr Jim Gallagher, Department of Human Anatomy and Cell Biology, University of Liverpool, PO Box 147, Liverpool L69 3BX Tel: 44-151-594-5505 Fax: 44-151-794-5517
Professor Lance Lanyon, Royal Veterinary College, Royal College Street, London NW1 0TU Tel: 44-171-468-5000 Fax: 44-171-387-7386

The Society gratefully acknowledge the assistance of: Aura Scientific, Brights Instruments Ltd, Hybaid Ltd, Kabi Pharmacia, Leica, Merck Sharpe & Dohme, Novo Nordisk, Organon, Procter and Gamble Pharmaceuticals, Sandoz, Sanofi Winthrop, Smith and Nephew, Vertec Scientific, The Wellcome Trust, Zeneca Pharmaceuticals
Contents

| Page | Title                                                                                           |
|------|-------------------------------------------------------------------------------------------------|
| O1   | The death of osteocytes by apoptosis in human bone is observed following oestrogen withdrawal by GiRH analogues A Tomkinson, J Reeve, RW Shaw*, JS Noble |
| O2   | Paget's disease of bone: evidence of linkage to chromosome 18q21-22 SI Hardam, NE Hailes, JMG Thompson, SH Rabson |
| O3   | A double-blind, placebo-controlled study to determine the effects of risedronate on bone loss in glucocorticoid-treated rheumatoid arthritis patients R Eastell, JP Devogelaer, NFA Peel, C Gill, DE Bax, C Nagant de Douschasnes, RGG Russell |
| O4   | Estradiol (E2)-induced reduction of osteoclast parameters in human bone marrow cultures if reversed by macrophase-colony stimulating factor (M-CSF) U Salma, M Edwards, AM Flanagan |
| O5   | Mechanical strain versus wall shear stress as the stimulus to bone cells in mechanical loading R Small, JT Mitchell, RL Howard*, TJ Chambers |
| O6   | Osteoclastic cells required for osteoclastic resorption but not for osteoclastic differentiation JM Owens, AC Gallagher, TJ Chambers. |
| O7   | A role for molecular chaperones in bone turnover? SP Nair, S Meghji, M Wilson*, B Henderson |
| O8   | Clodronate and liposome-encapsulated clodronate can be metabolised in a non-hydrolysable ATP analogue by mammalian cells in vitro JC Frith, J Monksson*, RGG Russell, GM Blackburn, DJ Watts, MJ Rogers |
| O9   | The localisation of androgen receptors in human bone EO Abu, V Kucse*, JT Triffitt*, JE Compston |
| O10  | Hip geometry and bone mineral redistribution in European women and men. the EPOS study NJ Crabtree, H Kroger, J Stepan, S Grazio, J Nis, R Lorenc, J Reeve |
| O11  | The effect of high dose testosterone on bone turnover in healthy men C-Y Guo, A Bellin*, R Eastell, FCW Wu* |
| O12  | Mutations in the PEX gene: evidence for alterations in gene function, and implications for X-linked rickets (HYP) PSM Rowe, JN Goulding*, P Francis†, C Oudit †, T Strom †, M Econs†, A Mokryczki †, AF Read ††, JLM O'Riordan |
| O13  | Parathyroid hormone induces PTHrP mRNA expression in human osteoblasts with the kinetics of an immediate early gene CA Walsh, WB Bowler, JA Gallacher |
| O14  | Bone cell specific proteins interact with the 5' region of rat insulin-like growth factor I transcripts CA West, SM Farrow |
| O15  | Localisation of cathepsin K in skeletal tissues by immunohistochemistry and in situ hybridisation A Littlewood-Evans, G Bilbe, T Kokubo*, O Ishibashi*, T Inaoka*, B Wodarski**, JA Gallacher** |
| P1   | Lactational changes in bone mineral of the lumbar spine are influenced by breast-milk output but not calcium intake breast-milk calcium concentration or vitamin-D receptor genotype MA Luskey, A Prentice, LMA Jarjour, S Beavan |
| P2   | Differential patterns of altered bone formation in different bone compartments in osteoporotic RJ Byers, JA Hoyland, AJ Freemont |
| P3   | Differential display PCR analysis of gene expression in cells with variable vitamin D receptor expression M Dabrowski*, SV Hughes, R Bland, JLM O'Riordan**, M Hewison |
| P4   | Stromelysin (MMP-3) synthesis is upregulated in oestrogen deficient mouse osteoblasts in vitro and in vitro JW Breckon, S Papaioannou, I Kon, G Murphy*, RM Hembry*, JJ Reynolds, MC Meikle |
| P5   | Human osteoblast-like cells express members of a novel family of membrane-linked metalloproteinases DJ Dallas, T Edwards, RGG Russell, N McKay, PJ Croucher |
| P6   | Localisation of nitric oxide synthesis in bone derived cells DE Evans, PS Grabowski, M Helfrich, SH Rabson |
| P7   | Comparison of changes in ultrasound parameters and bone mineral density in women on hormone replacement therapy B Lees, SW Garland, JC Stevenson |
| P8   | A possible role for carbon monoxide in the adaptive response of bone cells to dynamic mechanical loading SCF Rawlinson, G Zamani, AA Pistolesi, LE Lanyon |
| P9   | Estrogen's role in regulating the response of bone cells to mechanical strain E Damien, JS Price, RFI Saswillo, LE Lanyon |
| P10  | Limb fracture risk among men and women with vertebral fractures C Cooper, M Kotowicz, EJ Atkinson, WM O'Fallon, B L Riggs, LJ Melton III |
| P11  | Effects of hormone replacement therapy on cancellous bone microstructure in postmenopausal women S Vedi, PJ Croucher*, NJ Garraharn**, JE Compston |
| P12  | Programmed cell death and cell survival in the osteoblast lineage PA Hill*, A Tumbler, MC Meikle |
| P13  | Human mesenchymal stem cell numbers do not decrease with age ROC Orefio, A Bennett, JT Triffitt |
| P14  | Ethnic differences in vitamin D receptor haplotype S Beavan, A Prentice, L Yan, B Dibba*, S Rabston** |
| P15  | Bone turnover and its relationship to menarche RA Hammon, A Blumlohn, RM Wrate, J Barton, R Eastell |
| P16  | Measurement of periarticular bone mass around the knee using fan-beam daf energy X-ray absorptiometry (DXA): preliminary results in rheumatoid arthritis and osteoarthritis M Rashid, A England, A Stewart, DM Reid |
| P17  | Effect of recombinant human bone morphogenetic protein-2 incorporated into a collagen sponge membrane on Periodontal regeneration in vivo GN King, N King, PJ Hughes |
| P18  | Nitric oxide inhibits IL-1 induced c-fos expression in MC3T3 osteoblast-like cells F Bourke, RT van 't Hof, SH Rabson |

1809
P19. Osteogenin suppresses activation but enhances formation of osteogenic response to mechanical stimulation of rat bone
CJ Jagger, JWM Chow, TJ Chambers

P20. Ultrasound provides superior sensitivity and specificity to clinical referral criteria for bone densitometry by DEXA
CM Langton, PA Ballard, SA Steel, DW Pardoe

P21. Sequential induction of the osteoblast phenotype and estrogen receptor expression in human bone marrow progenitors in vitro
ROC Orefe, V Kuscs, AS Virdi, A Bennett, JT Triffitt

P22. Bisphosphonates induce apoptosis in human myeloma cells in vitro
CM Shipman, MJ Rogers, RGG Russell, PI Croucher

P23. Synovial fluid fibroblasts regulate the concentration of 1,25-dihydroxyvitamin D made by macrophages in coculture
SJ Fowler, ME Hayes*, EB Mawer

P24. Regulation of 25-hydroxyvitamin D3, 24-hydroxylase (24-OHase) activity and expression in renal cells by 1,25-dihydroxyvitamin D3 (1,25D) and phorbol ester (PMA)
SE Heys, JJ Berry, AP Mee, EB Mawer

P25. Post's disease: a molecular explanation
S Meghji, B Henderson, K Heron, S Nair, P Mascagni*, ARM Coates**

P26. Expression of tenasin-C in bones responding to mechanical load
CMB Webb, G Zaman, JR Mosley, RP Tucker*, LE Lanyon, EJ Mackie

P27. Cytokine production by mechanically deformed mouse osteoclasts and obstruction with Herbuxmycin
K Armneman, MC Mckee, F McDonald

P28. Differential patterns of osteoblast dysfunction in trabecular bone osteoporosis
RI Byers, JA Hoyland, AJ Freemont

P29. Androstenedione (adione) reduces loss of trabecular bone volume in ovariectomised (ovx) rats
CK Lea, AM Flanagan

P30. Chondrocytes of the fracture callus directly replace the cartilaginous soft callus with bone matrix
R Clay, HJ Rouch, BE Scammell*

P31. Changes in bone turnover and bone mineral density with age
D Fatayerji, AM Cooper, R Eastell

P32. NMDA and AMPA type glutamate receptors in bone
PG Genever, NM Peet, AJ Patton, TM Skerry

P33. Aromatase cytochrome P450 (P450 arom) transcripts are detected in fractured bone but not in adjacent normal tissue
C Lea, AM Flanagan

P34. Expression of vitamin D receptor polymorphisms in type I diabetics
V Lilley, M Webb, SB C Bain, M C Sheppard, M Hewison

P35. Production of the 13(3a) isoform of PTHrP by DU145 prostate cancer cells is associated with autocrine growth stimulation and is down-regulated by 1,25(3H)D3
JA Carron, N Cross*, NJ Hundley**, MJ Diver*, WD Fraser*, JA Gallagher

P36. 1,25-dihydroxyvitamin D3, inhibits expression and secretion of PTHrP by human osteoblasts in vitro
CA Walsh, WB Bowler, WD Fraser, P McGrady, JA Gallagher

P37. Ca2+ release from cultured mouse calvaria is very sensitive to ambient pH
S Meghji, B Henderson, MS Morrison, TR Arnett

P38. Indomethacin and ibuprofen stimulate pit formation by rat osteoclasts
MS Morrison, TR Arnett

P39. Structure/function relationship of the osteolytic activity of chaperonin
S Meghji, K Reddi, S Nair, P Mascagni*, P White, ARM Coates**, B Henderson

P40. Changes in localisation of oestrogen target cells may be key to skeletal function in laying hens
C Baris, J Hoijland*, DH Carter**, BH Thorp*, AJ Freemont*, IP Braidman

P41. Age-related changes in serum levels of bone markers in immature horses are influenced by the season
B Jackson*, CW McBlairthite**, R Eastell*, RGG Russell*, LE Lanyon, JS Price

P42. The evaluation of serum galactosyl hydroxylysine in postmenopausal women with primary hyperparathyroidism
C-Y Guo, WEG Thomas*, AW Al-Dehaiami, A Colwell, B Jackson, R Eastell

P43. The expression of cementoblast-specific genes by periodontal ligament cells in vivo
D Tenorio, M Shamsfer, EJ Hughes

P44. Intravenous clodronate as treatment for Paget's disease of bone
CG Ooi, WD Fraser

P45. Application of defined uniform dynamic strain to human bone cells in vitro
B Ferron, M Emerton, J Urban, R Gundle, D Murray

P46. 1,25-dihydroxyvitamin D receptor and chromogranin A levels are inversely correlated in abnormal human parathyroid tissue
LK Davenport, N Parrott*, EB Mawer

P47. Isolation of LPS-inhibiting proteins from the surface of gram-positive bacteria
SJ Crean, S Nair, K Reddi*, S Meghji, M Harris, B Henderson

P48. Differences in the ability of 2 quantitative ultrasound scanners to predict hip fractures
A Stewart, DM Reid

P49. Functional analysis of membrane aminopeptidase N (CD13) in osteoclasts
G Down, MA Horton

P50. Culture of a novel cell type from flexor tendons
CE Evans, G Schofield, I Trail

P51. Bone mineral density and turnover in blacks and whites
YM Henry, R Eastell

P52. Renal clearance of free and conjugated pyridinium crosslinks of collagen
A Colwell, R Eastell

P53. Apoptosis in bone regeneration during leg-lengthening
G Li, AS Virdi, JT Triffitt

P54. A transgenic animal model for Paget's disease and osteosarcoma
S Forbes-Robinson, EF Wagner, PT Sharpe, A E Grigoriadis

P55. Are adults with learning disability at greater risk of fracture compared with community controls?
TJ Aspray, RM Francis, D Rawlings, SJ Quilliam*, SP Tyer**

P56. A prospective study of fracture prediction using heel ultrasound in postmenopausal women
P Thompson, J Taylor, A Fisher
P57. The role of the c-fos / AP-1 transcription factor in osteoclast and macrophage differentiation
J McCluskey, AE Grigoriadis

P58. The cytokines TNF-alpha and beta cause dose-dependent detachment of osteoprogenitor cells
CE Evans, C Ward, M Davies, CSB Galasko

P59. Quantitative ultrasound measurements: short and long term precision
BM Ingle, R Eastell

P60. The molecular mechanisms underlying c-fos induced bone tumour formation in transgenic mice
E El-Emir, AE Grigoriadis

P61. The development of odonotomes in the incisors of the obese mouse
C Johnstone, MW Roberts

P62. Approaches to the measurement of cytokines in peripheral blood in patients with rheumatoid arthritis
A Rogers, S Rahman, RGG Russell, R Eastell

P63. Are risk factors for vertebral deformities associated with reduced bone density? The IVOS study
P Masaryk, M Lunt, K Weber, C Schreit-Nave, D Reid, H Pols, T O'Neill, D Feltschberg, C Dodenhof, J Dequeker, J Cannata, L Benevolenskaya, G Poor, J Falch, A Stiman, J Reeve

P64. Induction of osteoblastic commitment in mesenchymal stem cells by bone morphogenetic proteins (BMPs)
W Turner, FJ Hughes

P65. Assessment of bone mass in multiple myeloma by quantitative ultrasound
J Dillon, A Stewart, D M Reid, R Souta*

P66. Cellular characteristics of osteoblasts derived from patients with craniosynostosis
RD Evans, K Arneman, MC Meikle, F McDonald

P67. An education package that increases calcium intake in schoolgirls
C Jones, L Nixon, P Thompson

P68. Osteoporosis in postmenopausal women with impaired mobility
D Wright, MB Barnes, D Williams, TJ Daymond

P69. Early proliferative responses following tibial ostectomy
G Li, AHRW Simpson, J Kenwright, JT Triffitt

P70. CGRP neuropeptide expression during secondary fracture repair of the rodent femur
HJ Clingen, GR Dickson, C Shaw*, G Jordan**

P71. Expression of the integrin subunits αV and β3 during osteoclast regulation
I Holt, MJ Davie, MJ Marshall
O1. The death of osteocytes by apoptosis in human bone is observed following oestrogen withdrawal by GnRH analogues

A Tomkinson, J Reeve, RW Shaw*, BS Noble
Bone Group (MRC), Department of Medicine, Cambridge University, and *Royal Free Hospital, London

Post-menopausal oestrogen loss plays a key role in the development of the osteoporotic state. Treatment of endometriosis in young women by gonadotrophin releasing hormone (GnRH) analogues is accomplished by a large reduction in oestrogen levels. We have determined whether oestrogen status affects osteocyte death following GnRH treatment. Trans-iliac crest biopsies were obtained from 11 premenopausal women diagnosed as having endometriosis, aged between 25-43 years. Biopsies were taken six months apart, before and after GnRH therapy. Viable osteocytes were identified using a combination of in situ hybridisation, gel electrophoresis of extracted DNA and morphologically by chromatin condensation. After oestrogen withdrawal cancellous and cortical bone showed an increase in the proportion of dead osteocytes. This was accompanied by a higher percentage of apoptotic osteocytes in all individuals studied. DNA extracted from post-treatment samples and subjected to gel electrophoresis displayed DNA ladders consistent with the presence of apoptosis whilst pre-treatment samples did not.

In summary, GnRH therapy caused a higher incidence of dead osteocytes, probably due to increased levels of apoptosis. A reduction in osteoclast activity might contribute to increased bone fragility and a decreased ability to respond to loading in these bones. These data have important implications for the pathophysiology of the post-menopausal osteoporotic state.

O2. Paget's disease of bone: evidence of linkage to chromosome 18q21-22

SI Haslam, NE Huites, JMG Thompson, SH Ralston
Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD

Inherited factors are known to be important in the pathogenesis of Paget's disease, but the molecular basis of the disease is unclear. In order to clarify the genetic contribution to Paget's disease, we have studied 37 pedigrees (36 Caucasian, 1 Japanese) each containing 2 or more affected individuals. In pedigrees where sufficient details were available, the pattern of inheritance was consistent with that of an autosomal dominant trait. A series of affected sibling pairs have been identified and genotyping performed using microsatellite markers from 18q21-22. Using the SPLINK program, preliminary evidence of linkage was found to D18S42 (LOD=1) and D1XS465 (LOD=1.4) in 38 affected sib pairs analysed so far. In these pedigrees appear to rule out the possibility of a Paget's disease locus in this area thereby raising the possibility of other susceptibility loci. In summary, our data confirm the importance of genetic factors in the pathogenesis of Paget's disease, provides strong evidence of an important susceptibility locus on chromosome 18q and raises the possibility of genetic heterogeneity.

O3. A double-blind, placebo-controlled study to determine the effects of risedronate on bone loss in glucocorticoid-treated rheumatoid arthritis patients

R Eastell, JP Devogelaer, NFA Peel, C Gill, DE Bax, C Nagant de Dieuchais, RGG Russell
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield, UK and Cliniques Universitaires Saint-Luc, Brussels, Belgium

Glucocorticoid therapy and rheumatoid arthritis are two independent risk factors for osteoporosis. We performed a double-blind, placebo controlled study of risedronate in 120 postmenopausal women with rheumatoid arthritis taking prednisolone. The women were in the age range 40 to 79 years and were treated with risedronate 2.5 mg/day, risedronate 15 mg/day for two weeks every twelve weeks (cyclic group), or with placebo (40 per group) for 2 years. The mean change in BMD at week 97 expressed as a percentage in patients on daily risedronate, cyclic risedronate, and placebo are shown in the Table.

At the lumbar spine, the difference between the daily and placebo group was significantly different (P<0.009, Fisher's Least Significant Difference). At the trochanter, the differences between the cyclic and the placebo group (P=0.008) were significant. Bone loss is particularly marked at the proximal femur in patients with rheumatoid arthritis treated with glucocorticoids and this was prevented by risedronate given either daily or cyclically.

O4. Estradiol (E2)-induced reduction of osteoclast parameters in human bone marrow cultures if reversed by macrophage-colony stimulating factor (M-CSF)

U Salima, M Edwards, AM Flanagan
St. Mary's Hospital Medical School, London

E2-deficient states result in increased bone resorption and M-CSF plays a central role in osteoclast formation. We tested the hypothesis that E2 mediates its suppressive effect on osteoclasts through M-CSF. Adult human bone marrow cells were cultured, in ± E2 (10^{-11}-10^{-7}M) for 10 days. The cells were then plated on bone slices in the presence of M-CSF or for a further 10 days. We found that bone resorption and 23ch-positive cells (88.5% of which labelled with 125I cailcitiom (CTR-positive cells)) were reduced in a dose-responsive manner when the cultures were exposed to E2 prior to M-CSF treatment. (% resorption: E2(10^{-7}M, 15.42±3.09) vs control 31.39±3.95; 23ch-positive cells): E2 (942±5.01 vs control 3,424±2.79). Reduction of osteoclast parameters were also found if E2 were added to the cultures only at the beginning of the exp but not if it were only added in the second phase. Addition of M-CSF (0.005-500 ng/ml) with E2 (10^{-7}M) throughout the exp. to the bone marrow cells resulted in a dose-responsive reversal of the inhibition of resorption and reduction of 23ch- and CTR-positive cells. In contrast, addition of IL-1α, IL-6 and TNFα (0.05-50 ng/ml) failed to show any trend in reversing the inhibitory effects (resorption or 23ch- and CTR-positive cells) of E2. Bone resorption and 23ch-positive cells were correlated; the correlation coefficient (r) for M-CSF was 0.943, the gradient was 0.009; r for E2 (10^{-7}M) + M-CSF was 0.946 and the gradient was 0.017 (6 exps.). This implies that E2 did not reduce the rate of bone resorption and did not reduce osteoclast life-span. The data therefore implies that E2 mediates its effect on osteoclast precursors. ELISA measurements showed no significant reduction in M-CSF levels in response to E2 treatment in culture supernatants. This implies that E2 mediates its effects on the osteoclast predominantly through membrane-bound M-CSF, rather than the soluble form.
O5. Mechanical strain versus wall shear stress as the stimulus to bone cells in mechanical loading
R Smith, FT Mitchell, RL Howard*, TJ Chambers
Dept of Histopathology, St. George’s Hospital Medical School, Canner Terrace, London SW17 ORE and *Department of Medical Physics, Atkinson Morley’s Hospital, 31 Copse Hill, London SW20

The nature of the stimulus sensed by bone cells during mechanical loading in vivo has not yet been determined. Previous in vitro loading devices have often used supraphysiological strains, applied strains in a non-uniform and multidirectional manner, and have not separated mechanical stretch from fluid flow. In this study we have used two in vitro loading systems to investigate the effects of strain and shear stress independently. Growing cells on polyethylene film allowed for the use of intermittent unidirectional linear strains in the range of 500-7000 µε (covering the physiological range) without exposure to fluid flow. We did not find any significant increase in NO or PGE production after loading (5 minutes, 1 Hz trapezoidal) of rat calvarium- and long bone-derived cells. MCF7 E1, UMR 106.01 or ROS 1728 cells. However, exposure of these cells to continuous wall shear stresses (parallel plate flow chamber, 1 minute stimulation) in the range of 0.6 to 47 dyn cm⁻² (thought to cover the physiological range) (1) induced an immediate and transient increase in NO production, shortly followed by an increase in PGE. Shear stresses of 0.3 dyn cm⁻² or less did not induce this response. Rat skin fibroblasts did not respond to any of the wall shear stresses used. The NO response was insensitive to glucocorticoid pretreatment, known to inhibit eNOS expression, which is now a cNOS isoform. The mechanism of mechanoreception proposed here bears strong resemblances to the shear stress response of vascular endothelial cells. We intend to use this model to determine the involvement in mechanoreception of factors known to be involved in the endothelial response to shear stress (eNOS, c-fos, shear stress responsive promoter elements), as well as factors involved in response to bone to mechanical loading in vivo.

O6. Osteoblastic cells required for osteoclastic resorption but not for osteoclastic differentiation
JM Owens, AC Gallagher, TJ Chambers, Department of Histopathology, St George’s Hospital Medical School, Canner Terrace, London SW17 ORE

It is generally considered that osteoblastic cells are essential for osteoclastic formation. This view is based largely on the inability of cells from haemopoietic spleen to form resorptive cells without addition of osteoblastic cells. However, since osteoblastic cells stimulate osteoclasts, this observation does not exclude the possibility that spleen cells might be able to support osteoclastic differentiation, while osteoblasts are required to stimulate resorptive activity. We therefore tested the ability of haemopoietic tissue to differentiate osteoclastic precursors into mature osteoclasts in vitro. Both MC3T3 E1, UMR 106.01 or ROS 1728 cells were non-resorptive, when osteoblastic cells were added, however, exposure of these cells to continuous wall shear stresses (parallel plate flow chamber, 1 minute stimulation) in the range of 0.6 to 47 dyn cm⁻² (thought to cover the physiological range) (1) induced an immediate and transient increase in NO production, shortly followed by an increase in PGE. Shear stresses of 0.3 dyn cm⁻² or less did not induce this response. Rat skin fibroblasts did not respond to any of the wall shear stresses used. The NO response was insensitive to glucocorticoid pretreatment, known to inhibit eNOS expression, which is now a cNOS isoform. The mechanism of mechanoreception proposed here bears strong resemblances to the shear stress response of vascular endothelial cells. We intend to use this model to determine the involvement in mechanoreception of factors known to be involved in the endothelial response to shear stress (eNOS, c-fos, shear stress responsive promoter elements), as well as factors involved in response to bone to mechanical loading in vivo.

O7. A role for molecular chaperones in bone turnover?
SP Nair, S Meghji, M Wilson*, B Henderson
Maxillofacial Surgery Research Unit and *Department of Microbiology, Eastman Dental Institute, UCL, London

We have established that the 56kDa molecular chaperones (cpn60) from two gram negative bacteria have potent bone resorbing activity. The molecular chaperones are highly conserved group of proteins and this has led us to investigate the possibility that other members of this family have bone resorbing activity.

The ability of the bacterial molecular chaperones, Cpn60 and Cpn10, and mammalian molecular chaperones, Hsp27, Hsp47, Hsp70 and Hsp90, to stimulate bone resorption was assessed using the calvarial bone resorption assay. The E. coli molecular chaperones, Cpn60 at 16µM, P<0.005, groEL (cpn60) at 169µM, P<0.005 and groES (cpn10) at 2µM, P<0.05 were all active at stimulating bone resorption. However the cpn60 from Mycobacterium tuberculosis and M. leprae were ineffective at stimulating bone resorption. The mammalian molecular chaperones, Hsp27 at 4µM, P<0.005, Hsp47 at 14µM, P<0.05 and Hsp90 at 11µM, P<0.005 also stimulated bone resorption, whilst Hsp72 was inactive in this assay.

These data demonstrate that some bacterial and mammalian molecular chaperones have the ability to cause bone resorption and as such these molecules may play a role in pathological bone destruction. Indeed we have previously suggested that the cpn 60 from Actinobacillus actinomycetemcomitans is a causative agent of the bone destruction associated with the periodontal disease. Could the molecular chaperones also be involved in idiopathic bone diseases such as osteoporosis and if so would they be useful therapeutic targets?

O8. Clodronate and liposome-encapsulated clodronate can be metabolised in a non-hydrolysable ATP analogue in mammalian cells in vitro
JC Frith, J Jönsson*, RGG Russell, GM Blackburn, DJ Watts, MJ Rogers
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK and *Department of Pharmacology, University of Kuopio, Finland

The bisphosphonate clodronate (dichloromethylene-bisphosphonate) is widely used as an anti-resorptive agent in the treatment of Paget’s disease and hypercalcaemia of malignancy and metastatic bone disease. The exact mechanism by which clodronate, and other more potent bisphosphonates (BPs) such as naltrexon, inhibit bone resorption have not been identified but may involve a toxic effect on osteoclasts. Liposome-encapsulated clodronate is also toxic to macrophages in vivo and may be useful in the treatment of rheumatoid arthritis.

It is generally accepted that BPs are not metabolised. However, we have previously shown that clodronate, but not more potent BPs, can be incorporated into a methylene containing, non-hydrolysable ATP analogue p,y-dichloromethylene ATP by Actinohacillus actinomycetemcomitans and may be useful in the treatment of rheumatoid arthritis.

We have now used anion-exchange f.p.l.c. analysis to demonstrate that intact mammalian cells in vitro can metabolise clodronate at an ATP analogue. This metabolite can be detected by f.p.l.c. analysis of extracts prepared from approximately 10⁵ 1774 or MG63 cells that had been incubated for 48 hours with 25µM or 50µM clodronate respectively. These concentrations of clodronate significantly inhibit cell proliferation. The additional peak of absorbance at 254nm on the f.p.l.c. elution profile, corresponding to the clodronate metabolite, co-eluted with synthetic p,y-dichloromethylene ATP. This is the first conclusive evidence that clodronate can be metabolised by mammalian cells. Similarly, 15µM liposome-encapsulated clodronate was metabolised to p,y-dichloromethylene ATP by 1774, but not MG63 cells, which cannot phagocytose liposomes. Neither alendronate nor liposomal alendronate was metabolised by either cell type. The toxic or anti-proliferative effects of clodronate on osteoclasts, macrophages and other cells may therefore be due to accumulation of a non-hydrolysable ATP metabolite. Alendronate is not metabolised and appears to act by a different mechanism.
Androgens have important effects on bone and are necessary for the attainment of peak bone mass in men. Their actions are primarily mediated via specific nuclear receptors; these have been demonstrated in osteoblasts and osteoclasts in vivo but their presence in human bone in situ has not been reported. We have used specific monoclonal antibodies to the androgen receptor to investigate its expression in sections of human osteophytes in femoral and iliac growth plates obtained from three males (11, 15 and 15 years) and two females (9, 12 years). Sections of adenocarcinoma of the prostate were used as positive control.

In the osteophytes, androgen receptors were widely expressed by proliferating, mature and hypertrophic chondrocytes, osteoblasts, osteoclasts and osteocytes. In the growth plate, androgen receptors were observed in hypertrophic chondrocytes, and highly expressed by osteoblasts, osteoclasts and newly formed osteocytes. The receptor was also detected in endothelial cells in blood vessels in the bone. There was no apparent difference in the pattern of receptor expression in males and females.

Our results show for the first time, the presence and distribution of androgen receptors in human bone in situ. Furthermore, they indicate that androgens play a role in bone development in both sexes.

O10. Hip geometry and bone mineral redistribution in European women and men: the EPOS study
NJ Crabtree, H Kruger, J Stepnan, S Orizio, J Nijs, R Lorenc, J Reeve
Bone Research Group (MBRC) Department of Medicine, University of Cambridge CB2 0QX

Hip fracture risk has shown to relate independently to hip geometry and bone mineral density (BMD). This study examines the effects of gender and geography and their relationships to hip fracture rates on a European population. 351 (236 female, 115 male) subjects over 50 years were taken from four centres participating in the European Prospective Osteoporosis Study (EPOS). Using Lunar DPX ‘beta’ version of hip strength analysis (HSA) and hip axis length (HAL). The following quantities were investigated: Femoral neck (FN) cross-sectional moment of inertia (CSMI), upper femoral and tibial growth plates obtained from three males (11, 15 and 15 years) and two females (9, 12 years). Sections of adenocarcinoma of the prostate were used as positive control.

In the osteophytes, androgen receptors were widely expressed by proliferating, mature and hypertrophic chondrocytes, osteoblasts, osteoclasts and osteocytes. In the growth plate, androgen receptors were observed in hypertrophic chondrocytes, and highly expressed by osteoblasts, osteoclasts and newly formed osteocytes. The receptor was also detected in endothelial cells in blood vessels in the bone. There was no apparent difference in the pattern of receptor expression in males and females.

Our results show for the first time, the presence and distribution of androgen receptors in human bone in situ. Furthermore, they indicate that androgens play a role in bone development in both sexes.

O11. The effect of high dose testosterone on bone turnover in healthy men
C Y Guo, A Bells, R Eastell, FCW Wu
Department of Human Metabolism and Clinical Biochemistry, Northern General Hospital, Sheffield. SS 7AU and *Department of Andrology, St Mary’s Hospital, Manchester M13 OHH

The role of testosterone in the relation of bone remodelling is unclear. We evaluated the effect of high dose exogenous testosterone (used as a male contraceptive) on bone remodelling. Twenty healthy men, ages 23 to 42 years (mean ± SD, 32 ± 5), received a weekly injection of 200 mg testosterone enanthate (TE) for 37 to 53 weeks. Sex hormones and biochemical markers of bone turnover were measured. Plasma osteocalcin and testosterone levels increased significantly to 2-3 times above the upper limit of the reference range during treatment. Bone formation, as assessed by plasma bone alkaline phosphatase (BAP) and osteocalcin levels, increased slightly by 11 to 15 weeks and then decreased compared with baseline (repeated measures ANOVA and one sample t-test corrected by Bonferroni). Bone resorption, as assessed by urinary N-terminal telopeptide type I collagen crosslinks (NTX), decreased significantly as did 24 hour urinary calcium excretion (repeated measures ANOVA and one sample t-test corrected by Bonferroni). All the above biochemical markers of bone turnover returned to the reference range after stopping TE treatment. Changes of plasma testosterone was negatively correlated with those in plasma BAP (r = 0.51, p = 0.02). Changes of plasma osteocalcin was negatively correlated with those in plasma osteocalcin (r = 0.52, p = 0.02) and 24 hour urinary Ca (r = 0.75, p = 0.01), respectively. We conclude that high dose testosterone treatment results in increased levels of testosterone and oestriadiol which induce a decrease in bone resorption and formation in healthy young men.

O12. Mutations in the PEX gene: evidence for alterations in gene function, and implications for X-linked rickets (HYP)
PSM Rowe, IN Goulding, F Francisc, C Oudier, T Strom, M Econs, A Mokrzycki, AP Read
JLH O’Riordan
University College London, Midland Medical School, London, *Max Planck Germans, Munich Germany, † Duke University USA, ‡ Manchester University, CNRS, France

Introduction and objectives: The recently cloned PEX gene is defective in HYP, and has homology to type II integral membrane glycoprotein zinc metalloproteinas. These include nephriophysin (NEP), endoglobin converting enzyme-1 (ECE-1), and Kell antigen. Our aim was to characterise the mutations from 39 different HYP families, and to deduce possible effects on PEX function and activity.

Methods: Mutations were detected by PCR based single stranded conformation polymorphism analysis (PCRSSCP), southern blotting and long-range PCR. Primers were derived from intron sequence that flanked exons. Exon intron genomic structure was deduced using computer analysis (grail), of composite long-range sequence from cosmids and cDNA clones.

Results: Twenty exons were analysed, and the following 32 mutations found: 1; four missense, 2; two insertions (8bp and 8bp), 3; four stop codons, 4; six splice donor, 5; two splice acceptor; 6; two small deletions (5 and 104 bp), 7; twelve larger deletions; 8; six intron polymorphisms and one conservative change.

Conclusion: PEX gene mutations are associated with HYP. Some missense mutations replace highly conserved cysine residues important for the folding of the protein. Other missense mutations are in regions important for protease function, and in particular a mutation adjacent to the consensus zinc binding motif may well affect zinc sequestration and enzyme substrate specificitiy. The wide range of mutations found in regions required for protease activity in NEP, suggest that PEX also functions as a protease,
O13. Parathyroid hormone induces PTHrP mRNA expression in human osteoblasts with the kinetics of an immediate early gene
CA Walit, WB Bowler, JA Gallagher
Human Bone Cell Research Group, Department of Orthopaedics, The University of Liverpool, Liverpool L69 3BX

Parathyroid hormone-related protein (PTHrP) is produced by human osteoblasts in vitro and may be an autocrine/paracrine regulator of bone cell function. Expression of the PTHrP gene is controlled by a number of factors including glucocorticoids, peptide hormones and the steroid hormones, 1,25(OH)2D3 and oestriol. In this study we have investigated the kinetics of induction of PTHrP mRNA in cells derived from human bone following PTH stimulation. Cells were serum starved and cultured in phenol red-free medium for 24 hour before stimulation with 100 ng/ml human PTH(1-34). RNA was extracted at specific time points ranging from 15 minutes to 24 hour following treatment with PTH. RT-PCR followed by Southern blotting was used to monitor the expression of PTHrP mRNA by the cells. Specific primers were used to amplify cDNA encoding the three isoforms of PTHrP (1-139, 1-141 and 1-173). We detected signals for transcripts encoding (1-139) and (1-141) 45 minutes after treatment with PTH, whilst (1-173) transcripts were detected 90 minutes after treatment. PTHrP transcripts for all three isoforms were still detectable 24 hours post PTH treatment. These data indicate that PTH has a dual effect on the expression of PTHrP in osteoblasts consisting of an initial rapid induction with the kinetics of an immediate early gene followed by sustained expression PTH-induced PTHrP early gene expression may be an important signalling mechanism in the autocrine/paracrine regulation of bone turnover.

O14. Bone cell specific proteins interact with the 5′ region of rat insulin-like growth factor-I transcripts
CA West, SM Farrow
Department of Medicine, University College London Medical School, Middlesex Hospital, London W1N 8AA

The heterogeneity of insulin-like growth factor-I (IGF-I) transcripts has been well characterised. The length of the 5′-untranslated region (UTR) produced affects translatability, possibly through RNA-protein interactions. We have demonstrated that bone cells preferentially express transcripts which are poorly translated. Therefore, the aim of this study was to examine the interactions of RNA with cytosolic proteins extracted from rat osteoblast-like cell lines. Fragments corresponding to exon 1-derived transcripts were generated by RT-PCR of rat liver RNA. Radiolabelled RNA was synthesised and incubated with cytosolic proteins extracted from osteoblast-like cells and liver. Proteins binding to RNA were detected by UV-cross-linking experiments. RNA from liver and bone cells bound proteins of ~65kDa, 95kDa and 81kDa. These data suggest that proteins which interact with the 5′-UTR of IGF-I are present within osteoblast-like cells and that these may, in turn, exert both general and tissue-specific effects in post-transcriptional regulation of IGF-I synthesis in bone.

P1. Lactational changes in bone mineral of the lumbar spine are influenced by breast-milk output but not calcium intake breast-milk calcium concentration or vitamin-D receptor genotype
MA Laskey, A Prentice, LMA Jarjou, S Beavan
MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ

We have investigated determinants of change in spinal bone mineral during 3 months of lactation. Bone mineral content (BMC) and bone area of the lumbar spine (L1-L4) were measured using an Hologic QDR/1000w at 0.5 and 3 months postpartum in 47 mothers who breast-fed for at least 3 months. Weight and height was also measured. Control data were obtained from 12 mothers who breast-fed for less than 3 months, 11 non-breast-feeding mothers and 23 non-pregnant, non-lactating women. Breast-milk output (deuterium oxide dilution) and breast-milk calcium concentration were measured at 6-8 weeks. Maternal calcium intake was assessed by food frequency questionnaire and 7-day prospective record. Vitamin-D receptor genotype was determined by BuM 1 restriction on blood white cells. Mothers who breast-fed for more than 3 months had lower spinal BMC at 3 months than at 0.5 months (change ($) = 4.0 (0.3%), P<0.0001). There was a wide variation in response, ranging from -9.4% to +2.4%. No significant change in BMC was noted for any of the control groups. Change in BMC was positively correlated with change in bone area (r = -0.001), and negatively with breast-milk output (P = 0.001) and maternal height (P = 0.087). This model accounted for 58% of the variance in change in BMC. The change in spinal bone mineral was not influenced by breast-milk calcium concentration, maternal calcium intake or vitamin D receptor genotype.

Supported by the Sainsbury Charitable Fund
P2. Differential patterns of altered bone formation in different bone compartments in osteoporosis
RJ Byers, JA Hoyland, AJ Freemont
University of Manchester Bone Disease Research Centre, Department of Pathological Sciences, Stopford Building, Oxford Road, Manchester, M13 9PT

Most studies of osteoporosis concentrate on trabecular bone, though most of the bone mass is in other compartments. We investigated bone formation in different bone compartments in osteoporosis using histomorphometric data from iliac crest biopsies of 159 osteoporotic patients. Trabecular apposition rate (TAR) and the cortical apposition rate (CAR) were standardised by expression as Z scores, peristeal and subcortical bone formation were assessed semiquantitatively and differential patterns analysed using a combinatorial matrix. TAR was normal in 26 (16%) and reduced (Z score in 131 (82%), and CAR was normal in 31 (19%) and reduced in 126 (79%). Both TAR and CAR were increased (Z score in 2. Postosteal bone formation (PBF) and subcortical bone formation (SBF) were normal in 63 (52%) and 91 (57%) and reduced or absent in 76 (48%) and 68 (43%) Changes in TAR and CAR were parallel in 134 (84%), whilst those in PBF and SBF were parallel in 98 (61%). Furthermore, 54 of the 134 (40%) in which the changes in TAR and CAR were equivalent showed a difference in the change in PBF or SBF, of which changes in PBF and SBF were equivalent in 44. This confirms that trabecular bone is most frequently affected but shows that trabecular changes are often accompanied by equivalent changes in cortical bone. Different bone compartments are affected more frequently than previously thought and asynchrony of trabecular/cortical and periosteal/subcortical compartments suggests different mechanisms of bone loss.

P3. Differential display PCR analysis of gene expression in cells with variable vitamin D receptor expression
M Dabrowski*, SV Hughes, R Bland, JLIH O'Riordan**, M Hewison
Department of Medicine, The University of Birmingham, Bl 2TH. **Sienicki Institute for Experimental Biology, Warsaw 02 053, Poland and "Department of Medicine, The Middlesex Hospital, London W1A

Regulation of VDR levels plays a crucial role in regulating the actions of 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$). To study the effects of variable VDR expression we carried out differential display PCR (DD-PCR) analysis of monocyte transfecteds that expressed different numbers of VDR. U937 cells received either a control plasmid (MEP - 6,000VDR/cell), sense VDR cDNA (DH13 - 5,000VDR/cell) or antisense VDR cDNA (DH14 - 3,000VDR/cell). All three variants had similar monocyte characteristics to the parental U937 but DH14 showed a higher rate of DNA synthesis leading to extensive apoptosis. DH14 were also resistant to the antiproliferative effects of DH3 whilst DH19 cells were hypersensitive to the hormone. RNA from each of these cell lines was analysed by DD-PCR. Duplicate analysis of fresh RNA samples identified 17 differentially expressed mRNA species in DH14. DNA sequencing showed that two of the bands corresponded to known cDNAs: DNA-dependent protein kinase (DNA-PK) and 17$eta$-hydroxysteroid dehydrogenase IV (17$eta$-HSD IV). DNA-PK is known to be involved in control of cell cycling and may be part of the mechanism by which antiserone inhibition of VDR expression induces apoptosis. Regulation of 17$eta$-HSD expression represents a new potential pathway for the interaction of vitamin D and estrogen signalling pathways. These data highlight the power of DD-PCR as a tool for identifying differentially expressed genes and furthermore, suggest that relatively small decreases in VDR expression may have profound effects on gene expression.

P4. Stromelysin (MMP-3) synthesis is upregulated in oestrogen deficient mouse osteoblasts in vivo and in vitro
JJW Breckon, S Papasavvomou, J. Kon, G Murphy*, RM Hembry*, JJ Reynolds, MC Mckie
Bone Research Unit, Department of Orthodontics, UMDS of Guy’s and St. Thomas’ Hospitals and Strongwra Research Laboratory, Cambridge

A key event in the initiation of bone loss is the removal of surface osteoid by osteoblast derived matrix metalloproteinases (MMPs). The distribution of MMPs, collagenase-I, gelatinase-A, gelatinase-B and stromelysin in mono-ensin treated distal femoral epilasts from ovariectomized (OVX) and sham-operated mice were detected by indirect immunolocalization. Bright intra-cellular staining for MMP-3 was seen in endosteal bone lining cells in OVX samples but not in sham operated controls. No significant differences were demonstrated for collagenase-I, gelatinase-A and -B. Osteoblasts from 1-day-old mouse calvariae were cultured in phenol red-free RPMI 1640 medium supplemented with charcoal stripped fetal calf serum for 96 h either oestrogen-free, oestrogen replete or oestrogen withdrawal after 48 h. Cultures were stimulated for the final 48 hours with eitherPTH, IL-1 or IL-6 or unstimulated. Semi-quantitive indirect immunohistochemistry showed 55% of cells cultured with 17$eta$-estradiol stained for MMP-3, this was increased to 80% in oestrogen withdrawal. Stimulated cultures showed a similar increase from about 70% cells stained with continuous 17$eta$-estradiol to 95% following oestrogen withdrawal.

These findings suggest MMP-3 (stromelysin) synthesis is upregulated in oestrogen deficient mouse osteoblasts. It is hypothesised that MMP-3 may have an important role in the initiation of bone loss in osteoporosis. Furthermore, MMP-3 appears to be continually synthesised by endosteal bone cells following surgically induced osteoporesis in mice.

P5. Human osteoblast-like cells express members of a novel family of membrane-linked metalloproteinases
DJ Dallas, T Edwards, RGG Russell, N McKie, PI Croucher
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Sheffield

Activation of bone remodelling may be initiated by the removal of the unmineralised matrix that protects bone surfaces from uncontrolled resorption. Members of the matrix metalloproteinase family have been implicated in this degradative process, although evidence for activity in vivo remains to be demonstrated, raising the possibility that other enzymes may also be involved. We have identified a novel human cDNA with identity to the reproxoin family of metalloproteininases present in snake-venom (McKie et al., Biochem. J. In Press). The inferred protein sequence demonstrates the presence of a matrixproteinase domain, a disintegrin-like domain, a cysteine-rich region with an EGF-like domain and a transmembrane region. This characteristic domain structure defines this as a novel member of the ADAM family (A Disintegrin And Metalloproteinase) and has therefore been named ADAM-12.

We have used RT-PCR to investigate the expression of ADAM-12 and other ADAM family members by the M6G osteoblast-like cell line. Amplified PCR products were cloned and sequenced to establish their identity. M6G cells were shown to express both ADAM-10 and ADAM-12 and this was confirmed by Northern blot analysis. Furthermore, using this technique ADAM-12 gene expression was shown to be up-regulated by interleukin-1B. Although implicated in extracellular matrix degradation, cell-cell and cell-matrix interactions the function of this novel family of proteinases in human osteoblast-like cells remains to be determined.
Nitric oxide (NO) is an important mediator of bone cell function but little is known about the sites of NO synthesis in bone or isoforms of NO synthase (NOS) that are expressed in bone. Here we studied NO regulation and NOS expression in rat bone marrow cultures and rat osteoblasts (ROB's) by immunocytochemical, in-situ hybridisation (ISH) and RT-PCR techniques. ROB's did not produce NO spontaneously (medium nitrogen, undetectable) but 24h after stimulation with cytokines (IL-1+TNF+IFN) NO production increased dramatically to 30-40 nM, coinciding with detection of inducible (iNOS) mRNA and iNOS immunoreactivity. In contrast, unstimulated bone marrow cultures (containing osteoclasts, stromal cells and leukocytes) did produce variable amounts (1.5-6.0 nM nitrite) of NO spontaneously and this was increased, approximately 4-fold, by cytokine stimulation. RT-PCR analysis showed basal expression of eNOS and iNOS mRNA and immunocytochemical studies showed expression of endothelial NOS in all cell types (including resorbing osteoclasts) whereas iNOS expression was predominately found in macrophages; importantly, <1% of osteoclasts were positive for iNOS even after cytokine stimulation and this was confirmed by ISH using an iNOS specific cRNA probe.

We conclude that eNOS is widely expressed in the bone microenvironment. The significance of eNOS expression in actively resorbing osteoclasts is unknown, but may indicate a role for eNOS in regulation of normal osteoclast function as previously suggested. In contrast, the generation of high levels of NO by a subpopulation of macrophages and osteoblasts cells via iNOS may serve to down regulate or inhibit osteoclast activity in vivo.

P8. A possible role for carbon monoxide in the adaptive response of bone cells to dynamic mechanical loading
SCF Rawlinson, G Gaman, AA Pitsilides, LE Lanyon
Department of Veterinary Basic Sciences, The Royal Veterinary College, London NW1 0TU

Regulation of bone mass in response to mechanical loading requires the controlled modelling and remodelling of bone architecture. Bone cell-derived prostaglandins (PGs) and nitric oxide (NO) have been shown to be likely mediators of this adaptive mechanism. However, as in brain and endothelial tissue, NO may not be the only gaseous metabolite to affect local adaptive responses. Carbon monoxide (CO), like NO, stimulates guanylate cyclase activity and is produced by inducible and constitutive isoforms (haem oxygenase, HO-1 and HO-2) both of which are known to be expressed in various tissues. We have, therefore, investigated the potential role of CO as a candidate in the mechanical regulation of bone cell activity. Immunohistochemistry revealed HO-1 in perilosteal and endosteal osteoblasts and higher levels of HO-2 in these cells and osteocytes. Semi-quantitative RT-PCR indicated similar differences in the levels of HO mRNAs. Zinc protoporphyrin-IX (a blocker of HO activity) abrogated the strain magnitude-related increases in osteocyte G6PD activity associated with dynamic mechanical loading and reversed those increases in osteoblasts. These data suggest a role for endogenously produced CO in the adaptive response of bone cells to mechanical loading.
P10. Limb fracture risk among men and women with vertebral fractures
C Cooper, M Kotsiwicz, E J Atkinson, W M O’Fallon, B L Rigg, L J Melton III
MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO16 6YD, UK, and Mayo Clinic, Rochester, MN 55905, USA

Vertebral deformities are a cardinal manifestation of osteoporosis. However, little is known of the relationship between vertebral deformity and the risk of other age-related fractures. We examined this issue in a retrospective cohort study in Rochester, MN. Using the medical record linkage system of the Rochester Epidemiology Project, we identified all 861 Rochester residents <70 years of age who were radiologically diagnosed for the first time with one or more vertebral deformities over the period 1950-1998. The 196 men and 466 women were followed for the development of subsequent fractures of the hip, distal forearm, proximal humerus and pelvis. Over 8,342 person-years of follow-up, we identified 293 subjects (249 women, 44 men) with a first incident limb fracture, 58 of which involved the proximal femur, and 48 the distal forearm. The standardised morbidity ratios (SMR) of observed to expected fractures were: hip 1.7 (95% CI 1.3-2.2), distal forearm 1.4 (95% CI 1.0-1.8) and any limb 1.5 (95% CI 1.3-1.8). The increased risk was apparent among men and women, and was more marked in subjects with vertebral deformities associated with moderate or minimal trauma than in those with fracture due to severe trauma. These data suggest that the risk of limb fractures is increased among men and women with vertebral deformities. However, the increase is smaller than that previously reported for subsequent incident vertebral deformities. The data are consistent with heterogeneity in the pathogenesis of different osteoporotic fractures.

P11. Effects of hormone replacement therapy on cancellous bone microstructure in postmenopausal women
S Vedi, P Croucher*, NJ Garralha**, JE Compston University of Cambridge School of Clinical Medicine Department of Medicine, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QF, *Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, **Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN

Postmenopausal bone loss is associated with disruption of cancellous bone architecture which has adverse mechanical effects and is believed to be irreversible. The aim of this study was to examine the effects of long-term hormone replacement therapy on cancellous bone structure in women with postmenopausal osteoporosis. Iliac crest biopsies from 22 women with osteopenia or osteoporosis were obtained before and after hormone replacement therapy (mean duration 23.5 months). Cancellous bone architecture was assessed by strut analysis, trabecular bone factor and narrow star volume. Post-treatment biopsies showed no significant changes in any of the structural indices assessed. Our results suggest that hormone replacement therapy preserves existing cancellous bone structure but provides no evidence that this treatment is able to reverse structural disruption in women with postmenopausal osteopenia or osteoporosis.

P12. Programmed cell death and cell survival in the osteoblast lineage
PA Hill*, A Tumbler, MC Meikle Department of Craniofacial Biology and Orthodontics, United Medical and Dental Schools of Guy’s and St Thomas’ London

Apoptosis or cell death is now recognized as the normal mechanism of cell clearance in a variety of physiological and pathological processes. Here we show that tumour necrosis factor-α (TNF-α), a cytokine with powerful bone resorbing activity released by activated macrophages, causes a dose-dependent increase in cell death in both primary murine osteoblasts and human osteogenic sarcoma cells. The TNF-α induced cell death had the morphological features of apoptosis rather than necrosis demonstrated by TEM, in situ DNA labelling and live/dead fluorescent staining. In contrast the trophic factors, insulin and insulin like growth factor (IGF) I or II promote osteoblast survival in vitro, an effect mediated via an interaction with the type I IGF receptor. A variety of other growth factors including interleukin (IL)-1, IL-3, IL-6, IL-11, transforming growth factor-β, macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor and epidermal growth factor had no effect on osteoblast survival. However, platelet derived growth factor and basic fibroblast growth factor potentiated the effects of insulin and IGFs on osteoblast survival. These findings show that specific growth factors produced by osteoblasts and sequestered in bone matrix suppress cell death in osteoblasts and may play an important role in the bone remodelling process whilst the bone loss associated with TNF-α production in pathological conditions may be mediated in part by the induction of osteoblast apoptosis. Furthermore the regulation of growth factor levels may be important in maintaining the viability of osteoblasts susceptible to undergoing apoptosis.

P13. Human mesenchymal stem cell numbers do not decrease with age
ROC Oreffo, A Bennett, JT Triffitt MRC Bone Research Laboratory, University of Oxford, Nuffield Orthopaedic Centre, Oxford OX3 7LD

The decrease in skeletal bone formation and fracture repair observed with ageing has been suggested to be due to a decrease in CFU-F number but human studies are limited. We have examined the variation in CFU-F number in 58 patients by colony counting. Human bone marrow was obtained from patients (15-87 years of age) undergoing total hip replacement or corrective spinal surgery and cultured at 2 x 10^6 cells/T25cm^2 flask. Discrete alkaline phosphatase positive colonies were observed at day 12 and analysed by colony counting. The mean fibroblast colony forming efficiency from the whole patient group was found to be 2.2 x 10^-5 ± 1.28 x 10^-5 (SD). There was no correlation between colony formation and age as detailed below:

| Age Group | Patient no. | Correlation (r value) | Age Group | Patient no. | Correlation (r value) |
|-----------|-------------|-----------------------|-----------|-------------|-----------------------|
| 16-87     | 58          | -0.197                | 15-87     | 36          | -0.290                |
| <60       | 24          | -0.189                | <60       | 15          | -0.282                |
| >60       | 34          | +0.252                | >60       | 21          | +0.121                |
| >70       | 21          | +0.242                | >70       | 14          | +0.251                |

No clear differences in colony size or intensity of alkaline phosphatase staining between colonies were observed in any group. These results demonstrate that CFU-F number does not decrease with age in the patient population studied. Rather, the known reduction in capacity for fracture repair with ageing may be due to reduced responsiveness of osteoprogenitor cells to biological factors. Unravelling these issues should provide new approaches for the modulation of osteogenesis.
P14. Ethnic differences in vitamin D receptor haplotype
S Beavan, A Prentice, L Yan, B Dibba*, S Rashid**
MRC Dunn Nutrition Unit, Cambridge, CB2 2XJ, and Kebba. The Gambia; *Department of Preventive Medicine, Shenyang Medical College, Shenyang 110001, People's Republic of China; **Department of Medicine and Therapeutics, University of Aberdeen, AB2 2ZD

Vitamin D receptor (VDR) genotype has been related to indices of bone health in several populations of North European ancestry, but little is known about allelic distribution in countries where osteoporosis is rare. We have investigated VDR allelic frequency using PCR followed by BstMI and TaqI RFLP in The Gambia (Kebba, Westiang; n=88), North-east China (Shenyang; n=64) and England (Cambridge n=76).

|        | China | Gambia | UK | China | Gambia | UK |
|--------|-------|--------|----|-------|--------|----|
| bb     | 1.1   | 1.0    | 1.0 | 13.6  | 0      | 85.3 |
| Bb     | 2.3   | 1.1    | 1.1 | 13.4  | 47.4   | 37.2 |
| BB     | 0.0   | 15.4   | 0   | 0     | 47.4   | 37.2 |

Previous studies have reported near 100% concordance between Bf and B alleles. We found substantial discordance (26%) in Gambian samples. This is consistent with an older population as indicated by the "out of Africa" model for human origin. There were significant ethnic differences in genotype frequency (p<0.001). Distribution in Cambridge (BB=15.4%; Bb=47.4%; bb=37.2%) was compared to other White populations. The incidence of the b allele was higher in China (BB=0%; Bb=13.1%; bb=37.2%) was compared to other White populations. The high frequency of Bb/Tt genotype in The Gambia and China may be associated with their lower fracture incidence.

P15. Bone turnover and its relationship to menarche
RA Hannon, A Blumsohn, RM Wrage, B Barton, R Eastell
Department Human Metabolism and Clinical Biochemistry, University of Sheffield, Young Peoples Unit, Royal Edinburgh Hospital, Edinburgh

The onset of menarche may mark the end of accelerated bone accretion in pubertal girls. The aim of this study was to relate changes in levels of biochemical markers of bone turnover to the time of menarche. Girls, mean age 12.1 years (range 11.6 to 12.8 years), who were participating in a prospective study of growth and nutrition provided spot urine samples at each of 4 annual time points (n = 50) and at least one pair of serum samples at consecutive annual time points (n = 43). Free immunoreactive deoxypyridinidoline were measured by ELISA (Metra) and expressed as a ratio to urine creatinine (iFPdCr/Cr), bone specific alkaline phosphatase (BAP) was measured by a wheat germ lectin precipitation method and serum estradiol divided by RIA (DPC). For individual girls each study year was defined in terms of years pre or post menarche. Median annual percent change in iFPdCr/Cr, BAP and estradiol for 2 years pre menarche to 2 years post menarche are shown below.

| Year 2 | Year 1 | Year 0 | Year 0 | Year 0 | Year 0 |
|--------|--------|--------|--------|--------|--------|
| Year 1 to 2 | Year 1 to 0 | Year 0 | Year 0 | Year 0 | Year 0 |
| iFPdCr/Cr | 6 (n=8) | 12 (n=22) | -26 (n=36) | -27 (n=27) | -15 (n=14) |
| BAP | 13 (n=3) | -17 (n=10) | -17 (n=19) | -40 (n=14) | -9 (n=6) |
| Estradiol | 140 (n=3) | 75 (n=11) | 64 (n=18) | 57 (n=14) | 44 (n=7) |

*p<0.05; **p<0.001 Wilcoxon sign rank test. Year 0 is year of menarche

We conclude that once estradiol levels are sufficient to trigger the onset of menarche there is a significant rapid decrease in the rate of bone turnover.

P16. Measurement of periarticular bone mass around the knee using fan-beam dual energy X-ray absorptiometry (DXA): preliminary results in rheumatoid arthritis and osteoarthritis
M Rashid, A England, A Stewart, DM Reid
Osteoporosis Research Unit, Department of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD

Rheumatoid arthritis (RA) is associated with marked periarticular osteoporosis but as yet this has only been quantified successfully in the hand. Osteoarthritis is thought to be associated with increased bone mass. We have developed a method to assess bone mass around the knee using 2nd generation fan-beam DXA scanning (LUNAR Expert). Regions of interest (ROIs) were identified and placed semi-manually on AP and lateral scans of the knee acquired using 1.52 or 1.56 forearm software. Repeated scans of a phantom gave an in vivo precision of femoral and tibial ROIs of <1.1% and <1.4% respectively. Lateral phantom scans gave similar or better precision. In 10 volunteers (7 males) short-term precision was similar for AP scans (0.4 to 1.2% depending on the ROI) but poorer for lateral scans (2.6 to 2.7%). In a preliminary clinical study 11 patients with RA involving the knee were compared with 11 patients with OA of the knee and 12 age matched controls. RA patients had marked reductions in BMD around the knee ranging compared with controls 19.28% (p<0.05 to p<0.005). OA patients tended to have greater BMD values than controls (up to 4.7%) but the changes were not significant.

Our new method will allow the quantification of bone loss around the knee in RA and enable a study of the pathogenesis of the changes in bone mass in RA – OA to be undertaken.

P17. Effect of recombinant human bone morphogenetic protein-2 incorporated into a collagen sponge membrane on Periodontal regeneration in vivo
GN King, N King, FJ Hughes
Department of Periodontology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London E1 2AD

The aim of this study was to investigate the effect of recombinant human bone morphogenetic protein-2 (rHBMP-2) on tooth periodontal wound healing when incorporated into a collagen sponge membrane. Surgical defects were created in the molar roots of 30 Wistar rats under general anaesthesia and treated with either, 10 µl of 50 µg/ml rHBMP-2 in a collagen sponge membrane (BMP), or collagen sponge only (COL) or defects were left untreated (UN). Animals were killed 10 days post surgery and the tissues processed for histological examination.

New bone formation was significantly greater in the BMP group compared with COL and UN groups (p=0.05) (4.9±0.1±0.8 µm², 1.6±0.1±0.4 µm² and 2.2±1±0.3 µm² respectively). Furthermore, significantly more bone formation was observed at some distance anterior to the defect in the test group (p=0.01). New cementum formation was also significantly greater in the BMP test group (p=0.02) (72±1±112 µm², 190±3±42 µm² and 149±33 µm² respectively.

These results demonstrated that dental cementum as well as bone may be a target tissue for the action of BMPs without causing ankylosis between bone and tooth. However, the finding that bone formation developed distant to the defect suggests a limitation for the collagen sponge as a carrier system in the therapeutic application of rHBMP-2.

rHBMP-2 was a generous gift from Dr J Wiesner, Genetics Institute, Cambridge, MA, USA
P18. Nitric oxide inhibits IL-1 induced c-fos expression in MC3T3 osteoblast-like cells
P Boucart, RT van’t Hof, SH Rabot
Department of Medicine and Therapeutics, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD

High concentrations of nitric oxide (NO) are known to inhibit osteoclast formation and activity but the molecular mechanisms which underlie this inhibition are unclear. Previous data has shown that the transcription factor c-fos plays a key role in regulating osteoclast formation and that NO can modulate c-fos expression in neurons. Here, we studied the effects of NO on regulation of c-fos in MC3T3 osteoblast-like cells. The NO donor S-nitrosoacetil penicillamine (SNAP; 50μM) strongly inhibited c-fos mRNA accumulation induced by IL-1 (80μM) in MC3T3 cells as assessed by semi-quantitative PCR. Since c-fos transcription is regulated by the serum response (SRE) and CAMP response (CRE) elements, we went on to study the effects of SNAP on binding of nuclear extracts to these elements in eukaryotic mobility shift assays (EMSA). Nuclear extracts were prepared from osteoblasts 4h after stimulation with serum. IL-1 or SNAP using standard methods and used in EMSA with 32P-labelled double stranded oligonucleotides corresponding to the c-fos SRE and CRE elements. SNAP abolished both serum- and IL-1-induced SRE binding in the EMSA and strongly inhibited IL-1-induced CRE binding. In conclusion, NO inhibits IL-1 induced c-fos mRNA levels in osteoblasts, suggesting this as a possible mechanism for the inhibitory effect of NO on bone resorption. The inhibition of c-fos may in turn be due to inhibition by NO of nuclear protein binding to the SRE and/or CRE elements in the c-fos promoter.

P19. Oestrogen suppresses activation but enhances formation of osteogenic response to mechanical stimulation of rat bone
CJ Jagger, JWM Chow, TJ Chambers
Department of Histopathology, St George’s Hospital Medical School, Cranmer Terrace, London SW17 ORE

It is generally held that oestrogen maintains bone mass through altering the set point for mechanical adaptation. We used a model whereby mechanical stimulation induces bone formation in rat caudal vertebrae to test the effect of oestrogen on the response of cancellous bone to mechanical stimulation. Unexpectedly, oestrogen administered daily throughout the experiments (8-11 days) suppressed, and ovariectomy enhanced the mechanical response. We could not attribute this suppression of activation of new bone formation surfaces by oestrogen to suppression of resorption. Osteocytic expression of c-fos and IGF-1 mRNA in response to mechanical stimulation was unaffected by oestrogen; and oestrogen suppressed mechanical responsiveness to a similar extent whether administration commenced 24 h before or 24 h after loading. This suggests that oestrogen was not acting on the strain-sensing mechanism itself, but on an early component of the osteocytic response to signals generated by strain-sensitive cells. However, when oestrogen administration was delayed until osteogenesis was established (3 days), oestrogen augmented the osteogenic response. This data is consistent with in vivo evidence for oestrogen responsiveness in two phenotypically distinct bone cell types: stromal cells, whose functional activities are suppressed, and osteoblasts, which are stimulated, by oestrogen.

P20. Ultrasound provides superior sensitivity and specificity to clinical referral criteria for bone densitometry by DEXA
CM Langton, PA Ballard, SA Steel, DW Purdie
Centre for Metabolic Bone Disease, NIHR Biomedical Research Centre for Metabolic Bone Disease, H S Blackith Building, Hull Royal Infirmary, Anlaby Road, Hull, HU3 2RW

Provision of bone densitometry is generally based upon clinical referral criteria as part of a selective screening programme. There is increasing interest in determining the role of ultrasound measurements of velocity (VOS) and broadband attenuation (BUA) in the diagnosis of osteoporosis. The sensitivity and specificity of ultrasound measurements were compared with clinical criteria (CC) for referral for bone densitometry by DEXA in 107 women aged 66-69 years. VOS and BUA measurements were undertaken on the calcaneus. An extensive medical and reproductive history was taken to select those with at least one of the eight clinical referral criteria agreed by our Health Authority. Osteoporosis was defined by the WHO definition. 49 women were eligible for bone densitometry by the clinical referral criteria. The threshold values for the lowest 49 subjects by ultrasound readings were 65 dB MHz⁻¹ and 1600 m s⁻¹ for BUA and VOS respectively.

| Osteoporosis | Spine | Femoral Neck |
|-------------|------|--------------|
| Specificity | Sensitivity | Specificity | Sensitivity |
| CC          | 57   | 45           | 65          | 40          |
| BUA         | 75   | 79           | 74          | 66          |
| VOS         | 61   | 64           | 54          | 79          |

In conclusion, ultrasound measurements, particular BUA, have higher sensitivity and specificity for BMD referral by DEXA than currently achieved with clinical criteria. This study provides strong evidence for the incorporation of ultrasound bone densitometry in primary healthcare for referral for BMD by DEXA.

P21. Sequential induction of the osteoblast phenotype and estrogen receptor expression in human bone marrow progenitors in vitro
ROC Oreto, V Kusec, SA Virdi, A Bennett, JT Triffitt
MRC Bone Research Laboratory, University of Oxford Nuffield Orthopaedic Centre, Oxford OX3 7LD

To further understand osteoblast differentiation, we have examined the differentiation of human bone marrow fibroblastic cells at defined time points using in situ hybridisation with digoxigenin-labelled riboprobes for Type I collagen, osteocalcin and the estrogen receptor, together with immunocytochemical analysis of estrogen receptor expression and alkaline phosphatase staining. Bone marrow was obtained from 19 patients (29-81 years of age) undergoing hip replacement and cultured in the presence or absence of 10⁻⁶M dexamethasone and 50μg/ml ascorbate for 6, 14 and 21 days. In the absence of dexamethasone and ascorbate, alkaline phosphatase activity and collagen expression was detected at day 6, and reached a maximum from 14 to 21 days. Osteocalcin expression, undetectable at day 6, was observed in 50% of patient samples at day 14 and 67% of patient samples at day 21. Estrogen receptor expression was detected from day 10 in 75% of patient samples and in all samples at day 21. In the presence of dexamethasone and ascorbate, cell proliferation, Type I collagen and alkaline phosphatase were markedly stimulated over 10 to 14 days. Osteocalcin expression was detected in 70% of patient samples as early as day 6. Furthermore, estrogen receptor expression was detected in 57% of patient samples at day 6 and was found to correlate with induction of the osteoblast phenotype. No differences in the patterns of expression were observed between female (n=11) and male (n=8) patients, or, patients over 55 (n=14) or under 55 (n=5). These results demonstrate the detailed development of the mature osteoblast phenotype from early fibroblastic stem cells, which, can be accelerated by treatment with dexamethasone and ascorbate and suggest a role for estrogen in the subsequent quanta differentiation of the committed osteoprogenitors.
P22. Bisphosphonates induce apoptosis in human myeloma cells in vitro
CM Shipman, MJ Rogers, RGG Russell, PJ Croucher
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX

Bisphosphonates are used for the treatment of hypercalcemia and osteolytic bone lesions associated with metastatic bone disease, including multiple myeloma. The effectiveness of bisphosphonates is due to their ability to target to bone mineral, followed by effects on osteoclasts that can lead to osteoclast apoptosis. Bisphosphonates can also cause macrophage apoptosis in vitro, and could therefore affect other cells in the bone microenvironment, including macrophages and osteoblasts. Since myeloma cells localize to sites of active bone resorption they may also be exposed to relatively high doses of bisphosphonates. We therefore examined whether bisphosphonates can affect myeloma cells in vitro and cause apoptosis.

The results show the synthesis of 1,25D in the co-culture system due to interferon-y treatment. The effects of three bisphosphonates, clodronate, pamidronate and incadronate (YM-75) on the proliferation of human myeloma cell lines HS.Sultan, JJN-3 and U266 were assessed by comparing cell numbers using a Coulter Counter. The proportion of cells with a characteristic apoptotic morphology following bisphosphonate treatment was determined by fluorescence microscopy after staining nuclei with DAPI.

Direct effects were seen in HS-Sultan and JJN-3 after exposure to the bisphosphonates for 72 hours. 100nM clodronate and pamidronate (p<0.05) and 500nM incadronate (p<0.001) appeared to inhibit cell proliferation. 100nM incadronate and 500nM pamidronate also inhibited 100% in the proportion of apoptotic cells (p<0.05), while 500nM incadronate caused a 2.38-fold increase (p<0.05). The U266 cell line also responded significantly following bisphosphonate treatment, but was less sensitive than HS-Sultan and JJN-3. The order of potency of the bisphosphonates at inhibiting proliferation and inducing apoptosis correlated with the order of anti-resorptive potency i.e. clodronate < pamidronate < incadronate.

These results suggest that bisphosphonates can directly affect human myeloma cells in vitro by inhibiting proliferation and inducing apoptotic cell death. The clinical significance of these observations remains to be determined.

P23. Synovial fluid fibroblasts regulate the concentration of 1,25-dihydroxyvitamin D made by macrophages in coculture
SJ Fowler, ME Hayes*, EB Mower
University of Manchester Bone Disease Research Centre, Department of Medicine, Manchester Royal Infirmary, Oxford Road, and *Department of Biological Sciences, Manchester Metropolitan University, Manchester

Human synovial fluid macrophages (M) and fibroblasts (Fb) express the 25D-1-hydroxylase (1-OHase) and 25D-24-hydroxylase (24-OHase) enzymes respectively and synthesize 1,25D or 25D,2SD when given 25D as substrate. A co-culture system was designed to investigate whether M0 1,25D could be 25-hydroxylated by Fb to form 1,24,25D. M0 were pre-treated for 24 hours with 5nM interferon-y, a stimulus of M0 1,25D-stimulated enzyme activity and P450,,,, mRNA expression. Following pre-treatment, the cells were incubated together with Fb for 24 hours: ND=non-detectable. The renal 24-OHase enzyme is induced by 1,25D, the hormonal form of vitamin D. This study investigates the regulation of the 24-OHase enzyme, and expression of the cytochrome P450 component of 24-OHase (P450,,,,) by 1,25D and M0 in two cell lines of proximal (LLC- PK1) and human (CL8) origin. Enzymic activity was measured by conversion of 1H-25(OH),D3 to 1H-24,25(OH),D3. P450,,,, mRNA levels were measured by in situ hybridisation (ISH) using a 35S-labelled antisense riboprobe derived from full length human P450,,,, cDNA (supplied by J. Ohmndahl). Induction of 24-OHase activity and P450,,,, mRNA was observed in both cell lines in a dose-dependent manner following 24h treatments with 10-3 to 10-10M 1,25D. A 50% inhibition of 1,25D-stimulated 24-OHase activity was observed following treatment with 10-10 cyclohexamide. Pre-treatment with PMA further increased 1,25D-stimulated enzyme activity and P450,,,, mRNA expression in both cell lines. ISH revealed that PMA (100nM) alone increased the number of positive cells expressing basal levels of P450,,,, mRNA. Further treatment of these cells with 1,25D up-regulated expression more than that observed by treatment with 1,25D alone. We conclude that the action of 1,25D is potentiated by PMA and is inhibited by cyclohexamide. The direct effect of 1,25D alone suggests its actions are mediated through the vitamin D receptor pathway to up-regulate transcription of P450,,,, mRNA followed by increased 24-OHase enzyme activity which can be inhibited by the translation inhibition, cyclohexamide. In addition, PMA enhances the effect of 1,25D by a mechanism which may involve protein kinase C.

Endowed by a Programme Grant from the Medical Research Council.

P24. Regulation of 25-hydroxyvitamin D, 24-hydroxyvitamin (24-OHase) activity and expression in renal cells by 1,25-dihydroxyvitamin D (1,25D) and phloro ester (PMA)
SE Heys, JL Berry, AP Mee, EB Mower
University of Manchester Bone Disease Research Centre, Department of Medicine, Manchester Royal Infirmary, Manchester M13 9WJ

The renal 24-OHase enzyme is induced by 1,25D, the hormonal form of vitamin D. This study investigates the regulation of the 24-OHase enzyme, and expression of the cytochrome P450 component of 24-OHase (P450,,,,) by 1,25D and M0 in two renal cell lines of proximal (LLC-PK1) and human (CL8) origin. Enzymic activity was measured by conversion of 1H-25(OH),D3 to 1H-24,25(OH),D3. P450,,,, mRNA levels were measured by in situ hybridisation (ISH) using a 35S-labelled antisense riboprobe derived from full length human P450,,,, cDNA (supplied by J. Ohmndahl). Induction of 24-OHase activity and P450,,,, mRNA was observed in both cell lines in a dose-dependent manner following 24h treatments with 10-3 to 10-10M 1,25D. A 50% inhibition of 1,25D-stimulated 24-OHase activity was observed following treatment with 10-10 cyclohexamide. Pre-treatment with PMA further increased 1,25D-stimulated enzyme activity and P450,,,, mRNA expression in both cell lines. ISH revealed that PMA (100nM) alone increased the number of positive cells expressing basal levels of P450,,,, mRNA. Further treatment of these cells with 1,25D up-regulated expression more than that observed by treatment with 1,25D alone. We conclude that the action of 1,25D is potentiated by PMA and is inhibited by cyclohexamide. The direct effect of 1,25D alone suggests its actions are mediated through the vitamin D receptor pathway to up-regulate transcription of P450,,,, mRNA followed by increased 24-OHase enzyme activity which can be inhibited by the translation inhibition, cyclohexamide. In addition, PMA enhances the effect of 1,25D by a mechanism which may involve protein kinase C.

Endowed by a Programme Grant from the Medical Research Council.

P25. Pott's disease: a molecular explanation
S Meghji, B Henderson, K Heron, S Nair, P Mascagni*, ARM Coates**
Maxillofacial Surgery Research Unit, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK. *Inlandmark Research Centre, Milan, Italy and **Division of Molecular Microbiology, St George's Hospital Medical School, London, UK

Tuberculosis of the spine (Pott's disease), of which there are up to 1 million new cases yearly, is characterised by massive destruction of the vertebrae. This study asks - is bone destruction due to the direct effect of Mycobacterium tuberculosis? Sonicated M. tuberculosis dose-dependently stimulated the resorption of murine calvaria and the recruitment of calvarial osteoclasts. We had previously shown that the heat shock protein - chaperonin (cpn) 60 of gram-negative bacteria but not of M. tuberculosis is a potent osteolytic agent. We now report that the co-chaperone - cpn 10 of M. tuberculosis stimulates calvarial bone resorption and the recruitment of calvarial osteoclasts at concentrations as low as 1ng/ml (15pM) and that these actions can be completely blocked by a monoclonal antibody SA12 raised to cpn 10. When SA12 was added to calvaria stimulated with M. tuberculosis sonicates it completely blocked bone resorption and osteoclast recruitment. Thus cpn 10 is the only osteolytic component in sonicates of M. tuberculosis and this molecule is a therapeutic target in Pott's disease.
P26. Expression of tenasin-C in bones responding to mechanical load
CMB Webb, G Zaman, JR Mosley, RP Tacker*, LE Lanyon, EF Mackie
Department of Veterinary Basic Sciences, The Royal Veterinary College, London
NW 1 0TU and *Department of Cell Biology and Human Anatomy, University of
California, Davis, California, USA.

A number of early biochemical responses of bone cells to mechanical loading
have been identified, but the full sequence of events leading from strain
to formation of new bone is poorly characterised. The extracellular
matrix protein, tenasin-C, supports differentiation of osteoblast-like cells.
The current study was carried out to investigate whether expression patterns
of tenasin-C in loaded bones are in agreement with a role for this protein
as a mediator of the adaptive response. Tenasin-C expression was investi-
gated in rat ulnae subjected to an established noninvasive loading regimen.
RNA extracted from loaded compared with contralateral control bones six
hours after loading showed a significant increase in tenasin-C transcript
expression. In animals killed at 3 or 5 days after initiation of daily loading,
periosteal surfaces undergoing load-induced reversal showed enhanced im-
munohistochemical staining for tenasin-C. In animals killed at 15 days, the
bone formed in response to loading was demarcated from old bone by
tenasin-C staining of reversal lines. Within this bone, tenasin-C was seen in
the lacunae of older but not more recently embedded osteocytes. These
results indicate that tenasin-C expression by bone cells is enhanced soon
after loading, and suggest that tenasin-C may act as a mediator of the
adaptive response.

P27. Cytokine production by mechanically deformed mouse osteoblasts
and obstruction with Herbimycin
K Arneman, MC Meikle, F McDonald
Department of Orthodontics and Paediatric Dentistry, United Medical
and Dental Schools, London.

We have examined cultured mouse osteoblasts derived by primary culture
and grown on petri dishes capable of deformation. Cells were released by
disodium glycine and grown to confluence in Ham F12 media
calculated on the surface of the dish. A cycle of cell deformation of 10 seconds
every 1.5 minutes was carried out for 48 hours after an initial 24 hours
for the cells to attach. Cytokine production of both stimulated and unstimulated
cells was examined by standard ELISA (enzyme-linked immunoassorbent
assays) of collected media. Media was collected after 8, 16, 24, 32, 40
and 48 hours and stored at -20°C. The mouse cytokines, interleukin 1β, IL-1β, IL-6 and IL-10 were examined in all dishes cells were seen adhering
to the base of the dish at 5 hours and increased to confluence at 48 hours.
Both IL-1β (< 1 pg/ml) and IL-10 (< 1 pg/ml) remained unchanged in production. IL-6 (unstimulated; 87 ± 17.6 pg/ml) was raised when the cells
were grown during mechanical perturbation after 12 hours (178.9 ± 29.7
pg/ml) (n=6 for both stimulated and unstimulated groups). This effect was
reduced over the observation period by the addition of 500 pg/ml of Herbimy-
icin A (0.96 ± 5.8 pg/ml). Levels of IL-6 never declined completely to
unstimulated levels. Mechanical deformation may possibly stimulate osteo-
blast activation by a cytokine based mechanism activated in part by a
tyrosine kinase pathway as a consequence of matrix interaction.
P30. Chondrocytes of the fracture callus directly replace the cartilaginous soft callus with bone matrix
R Clay, HJ Roach, BE Scammell*
Academic Orthopaedic Unit, University of Southampton, SO16 6YD and *Department of Orthopaedic and Accident Surgery, University of Nottingham, NG7 2UH

We have investigated the characteristics of chondrocytes during the transition of the cartilaginous fracture callus to hard (bony) callus in a rabbit fracture model. Prior to vascular invasion, hypertrophic chondrocytes acquired acid phosphatase (TRAP) activity, and also became positive for type I collagen and bone sialoprotein. After vascular invasion, many chondrocytes which had become trapped in the centre of the so-called “mixed spicules” divided. Occasionally, bi-potential cells were present. i.e. cells which simultaneously synthesized cartilage proteoglycans and bone matrix. Initially, this bone matrix was present inside intact chondrocytic lacunae, as confirmed by 3D confocal microscopy. We have termed this “lacunar” bone to distinguish it from the “vascular” bone, i.e. the bone laid down onto the outside of the mixed spicules by osteoblasts derived from marrow stromal cells. With time the lacunar bone spread beyond the confines of the lacunae and gradually replaced all the cartilage matrix that was originally present in the early endochondral spicules. Nevertheless, we could still distinguish the lacunar bone from the vascular bone: Lacunar bone was woven bone, whereas vascular bone was uni-directional lamellar bone. Lacunar bone contained TRAP activity and strong immunoreactivity for bone sialoprotein, whereas vascular bone was not. This mechanism for the relatively rapid activity and strong immunoreactivity for bone sialoprotein, whereas vascular bone did not. This mechanism for the relatively rapid replacement of soft callus by bone has not previously been recognised, but if it did not exist, fracture callus would be considerably less able to withstand external mechanical forces.

P31. Changes in bone turnover and bone mineral density with age
D Fatayergi, AM Cooper, R Eastell
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield

The mechanism of age-related bone loss in men is unclear. Changes in bone turnover with ageing could give insight into this mechanism. Previous results of bone turnover with age are conflicting. We studied 180 healthy Caucasian men, ages 20 to 79 years (30 per decade). Total body bone mineral density (BMD) was measured (Lunar DPX) and fasting morning serum samples and urine collections obtained. Serum osteocalcin and serum bone alkaline phosphatase (bALP), markers of bone formation, were measured by IRMA. Urinary deoxypyridinoline (Dpyd) and N-telopeptides of type I collagen (NTx) markers of bone resorption, were measured by ELISA and expressed as a ratio to urinary creatinine. Serum PTH was measured by IRMA. Bone turnover was high in both young and older men, with a nadir in the fourth and fifth decade - the data was best described by a quadratic function. PTH showed a linear increase with age. Total body BMD showed a linear decrease with age.

|            | Osteocalcin | bALP | Dpyd/Cr | NTx/Cr | PTH | TB BMD |
|------------|-------------|------|---------|--------|-----|-------|
| R²         | 0.33        | 5    | 15      | 15     | 7   | 5     |
| P          | 0.0001      | 0.003| 0.001   | 0.001  | 0.004| 0.004 |

Increased bone turnover in younger men corresponded to the attainment of peak bone mass. During adult life bone turnover slowed, before accelerating after the seventh decade which corresponded to an increase in circulating PTH. These changes may result in loss of bone mineral.

P32. NMDA and AMPA type glutamate receptors in bone
PG Genez, NM Peet, AJ Paton, TM Skerry
Bone and Joint Research Group, Biology Department, York University, Helsingfors, York YO1 5DD

Reports linking the regulation of a synaptic glutamate transporter to mechanical loading of bone suggest a role for excitatory amino acids in cell-cell communication in bone. We investigated expression of different classes of glutamate receptors in rat tissues using commercially available antibodies to different neuronal glutamate receptors. On western blots, an antibody to the GluR-1 AMPA receptor identified identical sized bands in bone and brain and using an antibody to an intracellular loop of the NMDAR-1 receptor, we identified a protein band in extracts of bone, bone marrow and spleen which was approximately 20KDa larger than the receptor in the brain. Immunohistochemistry demonstrated that the cells expressing this receptor were multinucleated/multilobate, and were located predominantly in the bone marrow and stroma of the spleen, although occasionally we saw immunoreactivity in multinucleated cells in contact with bone. A different antibody, to the C-terminus of the NMDAR-1 receptor, which can react with only a subset of CNS splice variants, failed to bind to the bone/marrow/spleen protein, suggesting that the bone receptor sequence may be a known splice variant or could be different from known CNS forms. These findings provide evidence for a role for excitatory amino acids as paracrine osteotropic agents, and could have considerable clinical implications.

P33. Aromatase cytochrome P450 (P450arom) transcripts are detected in fractured bone but not in adjacent normal tissue
C Lee, AM Flanagan
St. Mary’s Hospital Medical School, London

It has been proposed that peripheral conversion of sex steroid precursors to active hormones play a role in the maintenance of the skeleton and that in particular P450arom may be involved in this process. However we propose that this enzyme is not detected in normal bone/marrow but can be induced in pathological conditions such as fracture.

To study this, we obtained bone/marrow from men and women having surgery for fractured necks of femura (n-6); tissue was taken from fracture sites, fatty bone marrow in the femoral shafts, normal cortical bone and subcutaneous buttock adipose tissue (control) of each patient. Bone/marrow and buttck fat were also obtained from elective hip replacements and some of this bone/marrow was also cultured. RNA was extracted from all of these specimens: 2ug RNA was reverse transcribed using a primer specific for P450arom and 0.4ug cDNA underwent PCR (35 cycles), using primers for human P450arom. PCR products (272 bp) were detected in the tissue from all of the fracture sites and buttck fat but no PCR products were amplified from the RNA obtained from the unaffected adjacent bone or fatty bone marrow. Furthermore P450arom transcripts were not detected in bone/marrow obtained from fresh tissues from those individuals undergoing elective surgery but if this tissue were grown in vitro, P450arom transcripts were detected.

To exclude PCR failure in cases when P450arom transcripts were not detected, RNA, from relevant human tissues, was co-amplified with rat RNA known to express P450arom, using primers which detect both rat and human P450arom. These products were analysed by Southern blot using oligonucleotide probes, which label either human or rat P450arom. The blots confirmed the absence of P450arom in non-fractured human bone/marrow and preclude PCR failures.

Our results indicate that P450arom is not detected in normal human bone/marrow but can be induced under pathological conditions. We propose that tissue grown in vitro is analogous to wound healing and this explains the detection of P450arom transcripts in cultured tissue.
P34. Expression of vitamin D receptor polymorphisms in type I diabetes
E Watkins, M Webb, S C Bain, M C Sheppard, M Hewison
Department of Medicine, Queen Elizabeth Medical Centre, Birmingham B15 2TH

The pleiotropic actions of 1,25-dihydroxyvitamin D3 (1,25D3) are dependent on expression of intracellular vitamin D receptors (VDR). Tight regulation of VDR expression appears to be an important feature of differentiating cells particularly in the immune system. Recent studies have suggested that natural variation in VDR expression may occur as a result of VDR gene polymorphisms. In view of the proposed role of 1,25D3/VDR in immunosuppression we have examined the distribution of two VDR polymorphisms (BsmI and TaqI) in a population of patients with the autoimmune disease Type I Diabetes. Using DNA from the first sibling in different families, the results were compared using PCR with specific primers, the resulting samples cut with BsmI and TaqI, and gel electrophoresis patterns analysed. Within the control subjects polymorphic variation was BBtt (17%), BbTt (53%) and bbTT (30%), correlating well with previous studies. Type I diabetic variation was BBtt (21%), BbTt (41.5%) and bbTT (37.5%). Apparent correlation was also found between VDR polymorphisms and HLA types in the diabetics. The data presented here suggest that further investigation of autoimmune diseases may help to clarify the role of VDR polymorphisms in modulating responses to vitamin D.

P35. Production of the 139aa isoform of PTHrP by DU145 prostate cancer cells
JS Carron, N Cross*, NJ Bundrcd**, MJ Diver*, WD Fraser*, JA Gallagher
Human Bone Cell Research Group, Department of Orthopaedics, and University
Department of Clinical Chemistry, The University of Liverpool, Liverpool L69 3BX

The parathyroid hormone-related protein (PTHrP) gene consists of nine exons which give rise to multiple mRNA species by alternative splicing. This diversity is reflected at the protein level by the identification of three distinct PTHrP species, polypeptides of length 139, 141 and 173 amino acids. The regulation of PTHrP expression by osteoblasts can also be regulated by glucocorticoids and growth factors. In this study we have investigated steady state levels of PTHrP mRNA in cells using semi-quantitative low cycle number reverse transcriptase-linked polymerase chain reaction followed by Southern blotting. A decrease in mRNA expression by osteoblasts derived from human bone was detected following 72hr treatment with 10^-7M doses of vitamin D. Furthermore, using specific oligonucleotide primers we investigated the expression of the three PTHrP isoforms in cells. Expression of mRNA encoding the PTHrP isoforms 1-139, 1-141 and 1-173 was detected in osteoblasts, expression of all three isoforms was decreased following treatment with 10^-7M 1,25 dihydroxyvitanin D3. The regulation of PTHrP expression by 1,25 dihydroxyvitamin D3 may be one of the important local mechanisms controlling bone turnover in vivo.

P36. 1,25 dihydroxyvitamin D3 inhibits expression and secretion of PTHrP by human osteoblasts in vitro
CA Walsh, BW Bowler, WD Fraser, P McGrady, JA Gallagher
Human Bone Cell Research Group, Department of Orthopaedics, and University
Department of Clinical Chemistry, The University of Liverpool, Liverpool L69 3BX

Parathyroid hormone related protein (PTHrP) is produced by a wide range of normal and foetal tissues. It has previously been reported that human osteoblasts in vitro express PTHrP mRNA and secrete the protein. Production of PTHrP by osteoblasts in vitro can also be regulated by glucocorticoids and growth factors. In this study we have treated cells derived from human bone with 1,25 dihydroxyvitamin D3, which produced a dose dependent, statistically significant (p<0.05) decrease in the production of immunoreactive PTHrP by the cells. In addition we have investigated steady state levels of PTHrP mRNA in the cells using semi-quantitative low cycle number reverse transcriptase-linked polymerase chain reaction followed by Southern blotting. A decrease in mRNA expression by osteoblasts derived from human bone was detected following 72hr treatment with 10^-7M doses of vitamin D. Furthermore, using specific oligonucleotide primers we investigated the expression of the three PTHrP isoforms in cells. Expression of mRNA encoding the PTHrP isoforms 1-139, 1-141 and 1-173 was detected in osteoblasts, expression of all three isoforms was decreased following treatment with 10^-7M 1,25 dihydroxyvitanin D3. The regulation of PTHrP expression by 1,25 dihydroxyvitamin D3 may be one of the important local mechanisms controlling bone turnover in vivo.

P37. Ca^2+ release from cultured mouse calvaria is very sensitive to ambient pH
S Meghji, B Henderson, MS Morrison, TR Arnett
Maxillofacial Surgery Research Unit, Eastman Dental Institute and Department of
Anatomy and Developmental Biology, University College London, London WC1E 6BT

Resorption pit formation by cultured rat osteoclasts (OC) is activated when extracellular pH is reduced from about 7.25 to 7.05. We studied the effect of 1,25-dihydroxyvitamin D3 (1,25D3) on resorption of cultured mouse calvaria at pH values ranging from 7.17 to 6.95. We measured the release of calcium from cultured 3 d in 1.5 ml BGM medium with 5% serum. Medium final pH and PO2 were measured by blood gas analysers. Data are means ± SEM (n=3).

| Treatment      | pH | Ca^2+ release (mg/d) |
|----------------|----|----------------------|
| Control        | 7.21| 0.62±0.11            |
| 3 mEq/l HCl    | 7.17| 1.73±0.09            |
| 6 mEq/l HCl    | 7.13| 1.95±0.17            |
| 9 mEq/l HCl    | 7.09| 2.03±0.13            |
| 12 mEq/l HCl   | 7.01| 3.14±0.36            |
| 15 mEq/l HCl   | 6.95| 5.12±0.30            |
| 15 mEq/l HCl + 10 ng/ml sCT | 6.88| 2.23±0.06             |
| 10 nM 1,25(OH)2D3 | 7.09| 4.90±0.11             |
| 20 nM 1,25(OH)2D3 | 7.13| 5.88±0.17             |
| 15 mEq/l HCl + 1,25(OH)2D3 | 6.91| 5.45±0.11             |

The results show a near-maximal, 8.2-fold stimulation of Ca^2+ release associated with a pH drop of 0.26 unit, with the greatest changes occurring close to pH 7.0. Increased numbers of large, tartrate resistant acid phosphatase-positive cells were evident in fixed calvaria from cultures with large Ca^2+ effluxes. The inhibitory effect of salmon calcitonin (sCT) indicates that acid-stimulated Ca^2+ release was largely OC-mediated. Interestingly, the osteolytic effects of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) were accompanied by culture medium acidification.
P38. Indomethacin and ibuprofen stimulate pit formation by rat osteoclasts
MS Morrison, TR Arnett
Department of Anatomy and Developmental Biology, University College London, London WC IE 6BT

Prostaglandins (PGs) exert an inhibitory effect on bone resorption in the disaggregated rat osteoclast (OC) pit formation assay. In bone organ culture, however, PGs are osteolytic, and blockers of PG synthesis such as indomethacin (indo) inhibit resorption. We investigated the effects of indo and also ibuprofen (ibup), a more selective cyclo-oxygenase inhibitor, on resorption pit formation by cultured rat OC. Mixed cell populations containing OC obtained from the minced bones of 5 neonatal rats were incubated for 24h at low density on 5mm diameter ivory discs in HICO-,CO2 buffered medium acidified with 10 mM/I HCl. Medium final pH and PO2 were measured by blood gas analyser. Tartrate-resistant acid phosphatase positive OCs, total cell numbers and discrete resorption pits were assessed using transmitted and reflected light microscopy. Data are means ± SEM (n=5); *p<0.01 vs. control.

| pH | No. of OCs | No. of Pits |
|----|------------|------------|
| Expt. 1 Control | 6.887 | 38.8±5.0 | 12.3±1.9 |
| 0.2 µM indo | 6.916 | 34.5±6.8 | 16.0±2.9 |
| 2 µM indo | 6.929 | 42.0±4.7 | 35.3±4.4** |
| 20 µM indo | 6.941 | 31.5±3.0 | 11.0±3.0 |
| Expt. 2 Control | 6.900 | 21.8±6.0 | 3.5±1.5 |
| 0.5 µM ibup | 6.951 | 23.3±3.8 | 15.8±1.2** |
| 5 µM ibup | 6.952 | 17.3±4.1 | 6.3±2.9 |

Our results show that both indo and ibup strongly stimulate pit formation by disaggregated rat OC, suggesting that resorption in these cultures is subject to tonic inhibition via endogenous PG production.

P39. Structure/function relationship of the osteolytic activity of chaperonin 10
S Meghji, K Reddi, S Noir, P Mascagni*, P White, ARM Coates**, B Henderson
Maxillofacial Surgery Research Unit, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK; *Department of Molecular Microbiology, St George’s Hospital Medical School, London, UK

Mycobacterium tuberculosis chaperonin (cpn) 10: (i) stimulates resorption of murine calvaria and recruitment of calvarial osteoclasts; (ii) activates human monocytes to secrete IL-6 and (iii) inhibits the proliferation of MG63 osteoblast-like cells - all activities consistent with an osteolytic protein. Cpn 10 is a heptameric dome-shaped molecule whose subunits have a structural motif consisting of an anti-parallel β-barrel with a β-hairpin appendages extending from the top (cpn 10 roof - residues 45-56) and bottom (mobile loop - residues 16-34) of the protein and a third small putative nucleotide-binding loop around position 71. To determine the structure/function activity of cpn 10, 12 truncated mutant proteins containing these various regions have been synthesised and tested for biological activity. The calvarial bone resorption/osteoclast recruiting activity of cpn 10 is due to the interactions of residues in the mobile loop. In contrast, the monocytes IL-6-inducing activity is produced by a sequence which contains residues running from the roof to the putative nucleotide-binding region. The osteoblast-inhibiting activity could not be induced by any of the 12 mutant proteins and may require multiple sites of attachment which cannot be mimicked by peptides. This data is consistent with the hypothesis that there are a number of different receptors for cpn 10 in cells associated with bone remodelling.

P40. Changes in localisation of oestrogen target cells may be key to skeletal function in laying hens
C Baris, J Hoyland*, DH Carter**, BI Thorp*, AJ Freemont*, IP Braid
University of Manchester Bone Disease Research Centre, Departments of Rheumatology, *Pathological Sciences, **Oral Pathology, and Roslin Institute, Edinburgh

Osteoporosis affects 30% of egg laying hens and is a major welfare problem. By first ovarian follicle maturation, both osteogens and structural bone volume (SBV) peak. Thereafter, osteogen levels fall to a lower plateau and SBV decreases, as medullary osteogenesis, for eggshell calcium production, begins. How bone formation is switched from structural to medullary bone is unclear. Maximisation of peak SBV, however, markedly decreases incidence of osteoporosis. We therefore studied developmental oestrogen receptor (ER) expression in tarsonematomatous from Brown Leghorn hens, by indirect immunofluorescence. In fresh frozen undecalcified sections, and in situ hybridisation with the S18 labelled 1.8 kb cDNA probe (Professor Chambron, Strasbourg) in formalin-fixed paraffin embedded sections. ER was expressed even at 12 weeks, mostly in endosteal osteoblasts. At 20 weeks only (peak oestrogen) ER was expressed in perosteal osteoblasts and osteocytes. Marrow cells were mostly negative. Thereafter, from 23 weeks to 43 weeks, (plateau oestrogen) endosteal and endocortical osteoblasts, some marrow and medullary bone cells expressed ER. We suggest that, since altered location and identity of oestrogen responsive cells is concomitant with peak SBV and osteogen levels, this is key to maximisation of SBV. 1. Thorp et al (1993) Asian Poultry 67: 62

P41. Age-related changes in serum levels of bone markers in immature horses are influenced by the season
B Jackson, CW McIlwraith**, R Easteil, RGG Russell*, LE Lanyon, JS Price
Department of Veterinary Basic Sciences, The Royal Veterinary College, London NW1 OTU; **Department of Human Metabolism and Clinical Biochemistry, Sheffield University Medical School, Beech Hill Road, Sheffield S10 HX and ***Colorado State University, Fort Collins, Colorado, USA 80523

Developmental orthopaedic disease (DOD) is a significant cause of morbidity and mortality in horses. Progress towards reducing its prevalence can only be made if sensitive, non-invasive methods are developed for detecting changes in skeletal metabolism. Biochemical markers fulfill these requirements and the aim of this study was to establish the normal age-related changes in serum bone markers in skeletal immature horses. Serum samples were collected over a 10 month period from 24 thoroughbred foals that were 5-8 months of age at the start of the study (October). We measured bone alkaline phosphatase (BAP), osteocalcin (BGP), the carboxy-terminal propeptide of type I collagen (PICP), and the cross-linked telopeptide domain of type I collagen (ICTP). The assays have been validated for use in horses. a) Age-related changes: levels of each marker fell significantly during the study (p<0.0001), the most significant decrease occurred between 5 and 7 months of age. Levels of BAP and BGP did not change significantly after this, whereas PICP and ICTP concentrations rose between 7-11 months. b) Seasonal effects: when the relationship between the bone formation markers and month of the year was compared, BGP and BAP levels increased between December and April (126% p<0.001, 115% p<0.05). In contrast, there was a very distinct rapid rise in PICP levels in April (141%, p<0.0001). ICTP concentrations increased over three months to peak in March (157%, p<0.001). An increase in daily weight gain is known to occur in yearling thoroughbreds in the spring. These results confirm the importance of measuring a selection of bone markers since each one reflects a different physiological mechanism. The finding that BAP and BGP did not show such a distinct seasonal increase as PICP indicates that during periods of rapid growth type I collagen in tissues other than bone may be a significant source of PICP. If bone markers are to be used clinically to study bone modelling in immature horses, both age and seasonal changes need to be considered.
Biochemical markers of bone resorption have the limitation of day to day variability, probably because most are measured in the urine. We developed a serum assay for one of the markers, serum galactosyl hydroxlysine (S-GH). The aim of the present study was to determine whether S-GH was as sensitive as urine markers using the model of primary hyperparathyroidism (PHPT). S-GH using HPLC approach was measured together with serum bone alkaline phosphatase (BAP), carboxy-terminal propeptide of human type I pro-collagen (PICP), 24 hour urinary GH (U-GH) and cross-linked N-terminal telopeptide of type I collagen (NTx) in 20 postmenopausal women (ages 48 to 80 (63±11, mean±SD)) with PHPT at baseline and over 18 to 30 months (23.9±3.6) after surgery. Thirty three age-matched postmenopausal healthy controls were measured at baseline and at 24 to 27 months (24±4.1). At baseline, the levels of all biochemical markers were increased significantly except serum PICP. There was no significant differences in Z-score between biochemical markers. S-GH was correlated with all other markers measured at baseline (p<0.01 for all measurements). At follow-up, the levels of all biochemical markers of bone turnover returned to reference range. The decrease (5%/year) of bone resorption markers was greater than the decrease of bone formation markers and there was no difference in the decrease between bone resorption markers. It is concluded that serum GH seems promising to monitor the bone resorption during treatment of PHPT in postmenopausal women.

P42. The evaluation of serum galactosyl hydroxysyline in postmenopausal women with primary hyperparathyroidism
C-Y Guo, WEG Thomas*, AW Al-Dehaimi, A Colwell, B Jackson, R Eastell
Department of Human Metabolism and Clinical Biochemistry, Northern General Hospital and Department of Surgery, Royal Hallamshire Hospital, Sheffield

Intravenous clodronate as treatment for Paget's disease of bone
CG Ooi, WD Fraser
Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool
L7 8XP

We evaluated the use of clodronate in the treatment of Paget's disease using an intravenous regime of four 1200 mg doses at 3 monthly intervals. 21 patients (12 male, 9 female) received 1200 mg clodronate (Bonefos, Boehringer Ingelheim) in 500 ml 0.9% saline solution over 4 hours on 4 occasions at 3 monthly intervals. All had serum alkaline phosphatase(ALP) levels above the reference range of 125 U/l (mean 387 U/l, range 163 - 1260). At 3, 6, 9, and 13 months from start of treatment there was a mean percentage reduction in ALP from pre-treatment values of 33%, 40%, 44% and 47% respectively (paired t test, p< 0.01). All but 2 patients had at least 25% reduction in pre-treatment ALP values. ALP values in 10 patients were suppressed to within the reference range(pre-treatment ALP 183-269 U/l). Biochemical relapse has occurred in 12, with a mean relapse time of 18 months (range 13-25 months). Clodronate was well tolerated, with 1 patient complaining of myalgia after the first infusion, which did not recur subsequently.

Intravenous clodronate is effective and well tolerated as treatment for Paget's disease. The use of higher or more frequent doses of intravenous clodronate may be beneficial for patients with high ALP values (>800 U/l).

P43. The expression of cementoblast-specific genes by periodontal ligament cells in vitro
D Tenenbaum, M Shumster, EJ Hughes
Department of Periodontology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Turner Street, London El 2AD

Cementoblasts, the dental cementum-producing cells, appear to express similar features to osteoblasts but their phenotype has not been fully characterised. The periodontal ligament is believed to contain cementoblast precursors as well as osteoblasts and fibroblast cells. In this study we aimed at identifying potential cementoblast-specific genes expressed by periodontal ligament (PDL) cells in vitro. For that differential display RT-PCR was used to visualise differentially expressed gene fragments by PDL as compared with human osteoblast cells (HOB). Total RNA samples were extracted from confluent cultures of PDL and HOB, reverse transcribed and amplified using a set of arbitrary upstream (5'-GCAATCGATG-3') and downstream primers (3'-T12MC-5'). Six PDL differentially expressed cDNA bands were isolated, amplified, cloned and sequenced. A 424bp fragment showed complete homology with a human expressed sequence tag isolated from placenta. This cDNA clone was used as a probe for Northern hybridisation. The results demonstrated that the 424bp fragment is selectively expressed by PDL cells but not by alveolar bone osteoblasts, gingival fibroblasts or the osteoblast cell line MG63. These results suggest the presence of cementoblast-specific genes within the periodontal ligament population and further support the idea of phenotypic diversities between osteoblasts and cementoblasts.

P44. Intravenous clodronate as treatment for Paget's disease of bone
CG Ooi, WD Fraser
Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool
L7 8XP

We evaluated the use of clodronate in the treatment of Paget's disease using an intravenous regime of four 1200 mg doses at 3 monthly intervals. 21 patients (12 male, 9 female) received 1200 mg clodronate (Bonefos, Boehringer Ingelheim) in 500 ml 0.9% saline solution over 4 hours on 4 occasions at 3 monthly intervals. All had serum alkaline phosphatase(ALP) levels above the reference range of 125 U/l (mean 387 U/l, range 163 - 1260). At 3, 6, 9, and 13 months from start of treatment there was a mean percentage reduction in ALP from pre-treatment values of 33%, 40%, 44% and 47% respectively (paired t test, p< 0.01). All but 2 patients had at least 25% reduction in pre-treatment ALP values. ALP values in 10 patients were suppressed to within the reference range(pre-treatment ALP 183-269 U/l). Biochemical relapse has occurred in 12, with a mean relapse time of 18 months (range 13-25 months). Clodronate was well tolerated, with 1 patient complaining of myalgia after the first infusion, which did not recur subsequently.

Intravenous clodronate is effective and well tolerated as treatment for Paget's disease. The use of higher or more frequent doses of intravenous clodronate may be beneficial for patients with high ALP values (>800 U/l).

P45. Application of defined uniform dynamic strain to human bone cells in vitro
B Fermer, M Emerton, J Urban, R Gundle, D Murray
Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Headington, Oxford OX3 7LD and 2 Department of Physiology, Oxford University, Oxford

The mechanical environment plays a major role in the control of bone formation. Suitable in vitro models for studying mechanotransduction will enable mechanisms behind in vivo observations of mechanical loading to be determined. Data from previous in vitro models have been difficult to interpret since the strains applied have been poorly controlled, non-uniform, or non-physiological. This model allows different strains on multiple samples to be investigated.

Human osteoblast-like cells were grown in the presence of 100M dexamethasone and 100uM ascorbate-2-phosphate. Cells were plated onto polycarbonate strips and clamped onto the stretching device. Controlled cyclical strains between 1 and 200 millistrain, with strain rates from 0.1 to 1,000 millistrain/second can be applied.

Cells were subjected to 1, 2 and 4 millistrain for 500 cycles at 4mm/sec. 5 hours later samples were taken. PGE2 was assessed by radio-immunoassay and DNA content using the Hoechst dye method.

Strain gauges showed reliable, reproducible strains were achieved between 1 and 6 millistrain. Photelastic strain analysis showed the strain was evenly distributed. A significant increase in the PGE2/DNA (ng/mg) at 4 millistrain (p<0.05) was detected in 2 out of 3 patients.

This is a reliable model to study mechanotransduction pathways in human cells and their interaction with other modulator of bone remodelling.
P46. 1,25-dihydroxyvitamin D receptor and chromogranin A levels are inversely correlated in abnormal human parathyroid tissue

LK Davесп ort, N Parrott*, EB Mawer
University of Manchester Bone Disease Research Centre, Department of Medicine and *Department of Surgery, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WH.

Chromogranin A (CgA) is an acidic glycoprotein that is co-stored and co-secreted with parathyroid hormone (PTH) in the parathyroid glands. The physiological role of CgA is uncertain, although it is postulated that it is a precursor of a biologically active peptide that inhibits stimulated secretion of PTH and co-stored CgA. 1,25-dihydroxyvitamin D (1,25) inhibits PTH expression and secretion, while stimulating CgA expression and activity in the parathyroids.

We have, therefore, examined distribution of CgA and 1,25 receptors (VDR) within parathyroids from patients with primary hyperparathyroidism (PHPT) and secondary hyperparathyroidism (SHPT) undergoing corrective surgery. Fresh frozen sections were cut (7μm) and immuno-histochemistry performed using antibodies directed against VDR and CgA.

Strong nuclear staining for VDR was observed in parathyroids of PHPT patients whilst immunoreactivity for CgA was weak. Conversely, VDR immunoreactivity was greatly reduced in parathyroids from SHPT patients although there was strong cytoplasmic staining for CgA.

These data suggest that there may be an important physiological role for CgA in the regulation of parathyroid cellular function and secretion.

*Supported by a Programme Grant from the Medical Research Council.

P47. Isolation of LPS-inhibiting proteins from the surface of gram-positive bacteria

SJ Crean, S Nair, K Reddi*, S Meghji, M Harris, B Henderson
Maxillofacial Surgery Research Unit and the *Department of Microbiology, Eastman Dental Institute, University College London, 256 Grays Inn Road, London WC1X 8LD

Bacterial infections of bone are a major cause of bone pathology and lipopolysaccharide (LPS) is believed to be the major osteolytic component of bacteria, presumably acting to induce osteolytic cytokines such as IL-6. However we have shown that components associated with the surface of gram positive bacteria can also stimulate bone resorption in an LPS independent manner. Surprisingly such components are not particularly potent inducers of osteoclastic cytokine production. During studies of these gram-positive components we have discovered that the bacterium Staphylococcus aureus and S epidermidis contain proteins on their outer surfaces which can inhibit the induction of pro-inflammatory cytokines by LPS stimulated human monocytes. Inhibition was dose dependent and evident at concentrations as low as 10μg/ml of the surface protein fraction from these bacteria.

Fractionation of the surface protein fraction by preparative isoelectric focusing has resulted in the partial isolation of the active inhibitor, a protein with an approximate pl of 92.

In conclusion we have isolated an LPS inhibitor from the surface of Gram-positive bacteria which blocks LPS induction of osteolytic cytokines. This may have therapeutic potential and raises the question of whether this capacity to inhibit LPS confers any advantage in mixed bacterial infections.

P48. Differences in the ability of 2 quantitative ultrasound scanners to predict hip fractures

A Stewart, DM Reid
Osteoporosis Research Unit/Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen

Quantitative ultrasound (QUS), and in particular broadband ultrasound attenuation (BUA), has been shown to be discriminative and predictive of osteoporotic fractures, especially hip fractures. There are now 4 commercial machines available to measure QUS - McCue CUBA Clinical, Lunar Arthels, Sahara and the Hologic 575+. We have the McCue CUBA Clinical (McC) and the Walker Sonix 575, a forerunner to the Hologic 575+ (WS) at our unit. The correlations between the BUA measurements taken by the two machines is 0.691 (p < 0.001) in a group of 394 patients with hip fractures. It is clear therefore that the machines are not measuring exactly the same parameters. But does the predictive value differ between the machines? In the group of 394 hip fractures 12 sustained a second hip fracture during a follow-up period. A Receiver operator characteristic (ROC) analysis was performed and the areas under the curves (AUC) compared. BUA WS had an AUC of 0.597, whereas BUA McC had an AUC of 0.652. However this difference was not statistically significant. The relative risks (RR) for a second hip fracture was 1.12 (0.59 to 2.1, 95% confidence limits) for BUA WS and 1.66 (0.88 to 3.15, 95% confidence limits) for BUA McC for 1 SD reduction in measurement. Of those regarded "at risk", (i.e. > 1 SD below the mean) by BUA WS only 76.9% (30/38) are also regarded as risk when BUA McC is used. Although there was no statistically significant differences between the predictive ability of these 2 QUS scanners it does show that these 2 machines do not scan exactly the same parameter. If these 2 machines differ it seems likely that the other 2 QUS machines would also give differing predictive abilities labelling different populations at risk.

P49. Functional analysis of membrane aminopeptidase N (CD13) in osteoclasts

G Down, MA Horton
UCLMS Department of Medicine, Middlesex Hospital, London, W1N 8AA

The CD 13 "leucocyte antigen" is expressed at high level by osteoclasts. CD 13 is identical to membrane aminopeptidase N (EC 3.4.11.2), originally purified from epithelium where it is involved in peptide transport. Its role in haemopoietic cells differs significantly, mediating coronavirus recognition and acting in concert with neutral endopeptidase to activate the activity of peptide mediators. We hypothesise that CD 13 modulates the activity of these 2 @US machines would also give differing predictive abilities labelling different populations at risk.
P50. Culture of a novel cell type from flexor tendons
CE Evans, G Schofield, I Trail
University of Manchester Bone Disease Research Centre, Department of Orthopaedic Surgery, Clinical Sciences Building, Hope Hospital, Salford M6 8HD.
*Upper Limb Unit, Wighton Spring Hospital, Hall Lane, Appleby Bridge, Wigan

 Cultures of cells with fibroblast-like morphology and growth pattern were obtained from explant cultures of flexor tendon. Proteoglycan and collagen secretion by cultures of first passage cells (TNC) was demonstrated by specific histochemical stains from 48h onwards; proteoglycan staining was lost once monolayer is reached 48-72h when cells seeded (≥2.5x10^5/well).

Type I collagen, but not type III was shown to be present in the extracellular matrix by immunohistochemical staining using specific antibodies. TNC had a population doubling time of 31h, a saturation cell density of 5 x 10^6/ml and confluence was attained within 2-5 weeks in primary cultures. Quantitative assay of total collagen showed that synthesis increased rapidly in the first 96 hours after passaging and started to decrease after 96 hours, during the stationary phase. Cultures allowed to become “superconfluent” spontaneously form three-dimensional structures from 4 weeks onward, which in time become macroscopic, resembling tendon-like structures (TLS), which grow longitudinally until the vessel wall is reached. TLS are attached to the culture flask at either end and occasionally along their length. Cells within those structures appear elongated and under cell-generated tension. Sections of tendon, of TLS and TNC monolayers stained with H and E demonstrate a remarkable similarity in morphology, with well-oriented fibrils and scattered cells. Morphology and protein secretion of cultures of skin fibroblasts are currently under comparison with TNC. 

P51. Bone mineral density and turnover in blacks and whites
YM Henry, R Eastell
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield, Sheffield

A number of studies comparing bone mineral density (BMD) between blacks and whites, using dual energy x-ray absorptiometry (DXA) have reported that BMD is 10-15% higher in blacks and rates of bone turnover (BT) are lower. There is no data on BMD and BT between racial groups in young adults. We evaluated bone formation and resorption in both black and white women aged 21-35yrs (mean (SD), 27 (5)) from the local community. The following measurements were made: (a) BMD of the total body, lumbar spine and proximal femur using the Lunar DPA densitometer (b) crosslinked N-telopeptides of Type I collagen (NTx) in urine to evaluate bone resorption (c) bone specific alkaline phosphatase (BAP) in serum by precipitation to evaluate bone formation. Racial differences in the measurements were examined by a two sample t-test. Blacks had higher total body BMD and trochanteric BMD (p=0.003, p=0.04 respectively). Racial differences in bone formation and resorption were not significantly different (NTx output 362 (218) black, 282 (147) nmol BCE/day white, BAP 35 (12.3) black, 34 (7.9) μmol HPLC white [mean (SD)], Therefore our results show that the difference in peak bone mass cannot be explained by bone turnover. We conclude that bone turnover may play an important role in the racial differences in BMD at puberty but not during adulthood.

P52. Renal clearance of free and conjugated pyridinium crosslinks of collagen
A Colwell, R Eastell
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield

Pyridinium crosslinks of collagen are excreted in the urine and their measurement by HPLC reflects the rate of bone resorption. They are present in urine in the free form (40%) and conjugated to peptide fragments (60%). It is not known whether the generation of free crosslinks takes place in bone, kidney, or both. To evaluate the possibility of renal generation of free crosslinks, we developed an HPLC assay for free and total crosslinks in serum using a sensitive fluorimeter. We studied children because they have high turnover. We made timed 2-hour urine collections with blood sampled at 1 hour in 18 children (ages 13 to 18) and the mean fractional excretion (SEM) of free crosslinks was 22 (0.2) and that of conjugated crosslinks was 0.6 (0.1). These ratios both differed significantly from 1 (P<0.001) and this may result from the conversion of conjugated to free crosslinks in the kidney. If this conversion was saturable, free crosslink generation would be related to total crosslink excretion. This may explain the observation that conjugated crosslinks show greater changes than free crosslinks in response to antiresorptive therapy.

P53. Apoptosis in bone regeneration during leg-lengthening
G Li, AS Virdi, JT Triffitt
MRC Bone Research Laboratory, Salford Orthopaedic Centre, University of Oxford, Oxford, OX3 7LD

Apoptosis was studied in an animal model of leg-lengthening in which adult NZ white rabbits with mid-tibial osteotomies were stabilised with external fixation. 7 days later, twice daily distraction was initiated at rates of 0.3 mm, 0.7 mm, 1.3 mm and 2.7 mm/day until 20% lengthening of the tibia was achieved. The distraction zones were processed histologically and embedded in paraffin. Apoptosis was detected by a (TdT) mediated DUTP-digoxigenin nick end labelling (TUNEL) method using a cell death detection kit. In addition, a method of dual labelling of cell proliferation by immunohistochemistry following the TUNEL method was developed. In general, the number of cells undergoing apoptosis is higher than expected. Cell death appeared in the fibrous tissue, but most was seen in the newly formed bone in the central region, in osteocytes mainly and in a few bone-surface cells. The cells of periosteum or newly synthesised woven bone were more proliferative than apoptotic. As the rate of distraction increased, the number of cell undergoing apoptosis in newly formed bone was greatly reduced, but appeared to increase in fibrous tissue. These data suggest that apoptosis may be an important mechanism controlling local turnover during bone regeneration.
**ABSTRACTS**

**P54.** A transgenic animal model for Paget's disease and osteosarcoma

S Forbes-Robertson, EF Wagner, PT Sharpe, A E Grigoriadis
Department of Craniofacial Development, Floor 28, Guy's Tower, London Bridge, London SE1 9RT

Paget's disease is a major degenerative bone disease, characterised by rapid bone turnover and increased activity and numbers of osteoclasts. The cause of the disease is not known but evidence has implicated a complex interaction between paramyxoviruses (e.g. canine distemper virus (CDV)), cytokines (e.g. IL-6) and nuclear oncogenes (e.g. c-fos)(1). The role of c-fos is of particular interest since c-fos transgenic mice develop a 'Paget-like' histology and c-fos knockout mice lack osteoclasts and develop osteopetrosis (2,3).

To address the possible role of c-Fos in osteoclast function we are using a transgenic animal approach in which c-fos is targeted to osteoclasts using the tartrate-resistant acid phosphatase (TRAP) promoter. Two transgenic lines of transgenic mice overexpressing c-fos in osteoclasts should further elucidate the possible role of this gene in osteoclast differentiation and activity, and serve as a possible model for Paget's disease.

1. Mee and Sharpe, 1993, Bioessays 15:783-789.
2. Grigoriadis et al., 1993, J. Cell Biol. 122:685-701.
3. Grigoriadis et al., 1994, Science 266:443-448.

**P55.** Are adults with learning disability at greater risk of fracture compared with community controls?

TJ Aspray, RM Franks, D Rawlings, SJ Quilliam*, SP Tyer**
Musculoskeletal Unit, Freeman Hospital, Newcastle upon Tyne NE7 7DN; *Cantle Surgery, Prudhoe, Northumberland and **Prudhoe Hospital, Northumberland

Little is known about the prevalence of osteoporosis amongst adults with mental handicap. We have therefore studied bone mineral status in 65 subjects with learning disability, using Broad-band Ultra-sound Attenuation (BUA), BMD (Meq) and Speed of Sound (SOS) measurements at the heel (Cuba Clinical, UK). Comparison was made with 30 male and 35 female age- sex matched controls, randomly selected from the local General Practice (BUA;dB MHz?) and Speed of Sound (m/s.) measurements at the heel.

We have used heel ultrasound to examine 65 adults with mental handicap. We have compared BUA and SOS measurements in a group of 65 adults with mental handicap with those in a group of age and sex matched controls from the local General Practice.

We found that BUA and SOS were lower in residents with learning disability compared with controls for both sexes all P<0.005.

These results suggest that this group with learning disability have a low bone mass. Many of them may have modifiable risk factors for osteoporosis, including reduced physical activity and sunlight exposure, hypogonadism and anticonvulsant drug therapy, which warrant further investigation.

**P56.** A prospective study of fracture prediction using heel ultrasound in postmenopausal women

P Thompson, J Taylor, A Fisher
Osteoporosis Dorset, 11 Shelley Road, Bournemouth, Dorset

Heel ultrasound has been shown to predict hip fracture in very elderly individuals but there are few data concerning fracture prediction at younger ages. This abstract reports the early data from a prospective study of a cohort of women aged 45 to 75 years. All women in an urban general practice were invited to attend for heel ultrasound measurement (Lunar Achilles). A fracture register was established and validated. Percentage fractures for each quartile of age adjusted stiffness (using the regression equation age vs stiffness) were compared. 1857 women were scanned (79%). There were 61 fractures with 23 occurring in the lowest quartile of age adjusted stiffness compared with only 10 in the highest quartile. When only fragility fractures were considered there were 14 in the lowest quartile of age adjusted stiffness compared with 5 in the highest quartile.

These data support the concept that heel ultrasound predicts fracture and suggests that this is independent of age.

**P57.** The role of the c-fos / AP-1 transcription factor in osteoclast and macrophage differentiation

J McCluskey, AE Grigoriadis
Department Craniofacial Development, Guy's Tower Floor 28, London Bridge, London SE1 9RT

It has been shown previously that mice lacking functional c-fos develop osteoporosis characterised by defective bone remodelling (1). These mice show a complete absence of functional multinucleated osteoclasts and their immediate precursors but have an increased number of bone marrow macrophages (2). Other genes cannot substitute for c-fos function suggesting an indispensable role for c-fos in determination of the osteoclast / macrophage lineage in vivo.

In vitro co-culture experiments have indicated that infection of Fox knockout spleen cells with not only c-fos but also with a fos B virus, can overcome in part, the block in osteoclast differentiation. These results suggest that other AP-1 genes may be able to compensate for the absence of c-fos if expressed appropriately. Therefore, initial experiments have sought to determine at which point c-fos expression is required during both osteoclast and macrophage ontogeny by investigating the expression of different fos- and jun-related genes, and osteoclast and macrophage markers, during development and in post natal bones of both wild-type and knock-out mice. Preliminary results in wild-type mice have indicated that the time course of osteoclast and macrophage development do not co-incide: Examination of E17-E18 metatarsals shows that whereas TRAP+ve osteoclasts have already invaded into the bone marrow space, F4/80+ve macrophages are present in the dermis and do not seed the bone marrow until birth. Current experiments using metatarsals from Fox knockout mice will determine whether the block in osteoclast differentiation alters the timing of macrophage differentiation. Together, these experiments will provide a detailed temporal and cell type-specific expression profile of c-fos / AP-1 genes to pinpoint when this gene is required during osteoclast and macrophage differentiation.

1. Wang et al, 1992, Nature 360, 741-745.
2. Grigoriadis et al, 1994, Science 266, 443-448.
P58. The cytokines TNF-alpha and beta cause dose-dependent detachment of osteoprogenitor cells
CE Evans, C Ward, M Davies, CSB Galasko
Department of Orthopaedic Surgery, Clinical Sciences Building, Hope Hospital, Salford M6 8HD

We previously showed that human multiple-myeloma cell lines (MC) secrete soluble factors (TNFβ and others) which affect adhesion of human osteoprogenitor cells (HOP) (1). Results are reproducible, but the biological effect is variable. To investigate this variability, conditioned media (CMC) from MC lines were assayed by ELISA for TNFa and TNFβ. Results were correlated with effect on HOP and CM parameters. Some CMC samples contained TNFβ (5-20 pg/ml), all samples were negative for TNFa. MC density and TNFβ concentration were positively correlated (r = 0.58); no correlation was found between TNFβ concentration and biological effect of CM on HOP or MC density and biological effect. As some CMC causing HOP detachment contained no detectable TNFa, TNFa and a concentrations below the detection level of the ELISA were used (5 and 10 pg/ml). HOP detachment was seen (Table 1) (time and dose-dependent). Our results suggest that detachment of HOP incubated with CMC from MC is caused by very low levels of TNFa and TNFa, secreted only at specific stages of MC growth cycle in vitro. We are examining the relationship between TNF secretion, cell growth and cell cycle and the binding of TNF to HOP.

1. Evans CE et al Eur J Musculoskeletal Res 1994 3:137-144

P59. Quantitative ultrasound measurements: short and long term precision
BM Ingle, R Eastell
Department of Human Metabolism and Clinical Biochemistry, University of Sheffield, UK

Coefficient of variation (CV) as a measure of precision is not appropriate for ultrasound measurements because it does not take into account the different ranges of measurement values. Standardised coefficient of variation (SCV) adjusts the CV for the measurement range. We aimed to estimate the SCV in a short term precision study and evaluate the long term (74 weeks) precision of the Lunar Achilles, CUBA Clinical (both calcaneus) and DBM Sonic (finger) ultrasound machines. We measured healthy women (ages 20-37 years) 5 times each using 2 operators. We then measured a further 20 population CV. A low SCV indicates good precision.

| Ultrasound Machine | Short term CV, % | SCV, % | Long term CV, % |
|--------------------|-----------------|--------|-----------------|
| Lunar Achilles BUA | 2.21            | 27.06  | 3.06            |
| Lunar Achilles SOS | 0.93            | 44.01  | 1.66            |
| CUBA Clinical BUA  | 6.08            | 24.61  | 6.15            |
| CUBA Clinical VOS  | 1.16            | 50.28  | 2.88            |
| DBM Sonic          | 1.19            | 50.14  | 1.45            |

Thus, long-term CV tends to be greater than short-term CV, especially for velocity measurements (SOS, VOS). This is partly due to long-term drifts in the instruments. The SCV estimates indicate that BUA measurements are more precise than velocity measurements.

P60. The molecular mechanisms underlying c-fos induced bone tumour formation in transgenic mice
E El-Emir, AE Grigoriadis
Department of Orthopaedic Surgery, Guy’s Tower Floor 28, Guy’s Hospital, London Bridge, SE1 9RT

The mechanisms underlying bone tumour formation are not well understood. Recently, we have reported that transgenic mice overexpressing the c-fos proto-oncogene, a member of the AP1 transcription factor complex including the jun gene family, develop osteosarcomas due to transformation of bone-forming osteoblasts (1). Moreover, co-expression of a c-jun transgene enhanced Fos induced osteosarcoma formation in vitro (2). To further investigate the transformation events triggered by c-fos, we are currently focusing on the earliest stages of tumour development and are investigating the potential role of apoptosis, a process implicated in tumorigenesis. Histological analysis of these tumours showed that the onset of tumour formation occurs at 46 weeks of age. The early lesions arise frequently in the regions of the proximal tibia and fibulae and are characterised by an expanded cortical bone in the metaphyseal region. The tumours contain a large number of osteoblasts which express exogenous c-fos as judged by in situ hybridisation analysis, as well as some chondrocytes and osteoclasts. Upon closer examination of the early lesions, mitotic cells as well as cells undergoing apoptosis could be observed. To investigate further the balance between proliferation and possible cell death, we are currently measuring the mitotic and apoptotic indices at different stages of tumour development.

In a second approach, we are using cell lines derived from Fos single and Fos-Jun double transgenic mice. These cell lines express high levels of the different AP-1 genes as well as many bone cell marker genes and some are tumorigenic when injected into nude mice. To address the potential role of apoptosis in c-fos-induced tumours, we are examining the expression of members of the bel-2 gene family, which are major regulators of apoptosis, in the different cell lines.

Toluena, examining the role of apoptosis using both in vivo and in vitro approaches will yield useful information regarding the mechanisms underlying Fos-induced osteosarcoma formation.

1. Grigoriadis et al., 1993. J. Cell Biol. 122: 685-701.
2. Wang et al., 1995. Cancer Res. 55: 6244-6251

P61. The development of odontomes in the incisors of the obese mouse
C Johnstone, MW Robins
Physics Group, King’s College, London WC2R 2LS

Obese (ob/ob) mice develop odontomes at the base of their incisors by 26 weeks of age (Batt, Int J Obesity 16:29, 1992), however details of earlier stages of odontomes have not been established. The condition as a possible model system for the molecular mechanisms underlying c-fos induced bone tumour formation.

The data indicate that the maxillary incisors of ob/ob mice cannot be distinguished from normal until 150 days, then show progressive increase in all parameters measured. The mandibular incisors show similar, though less distinct series of progressive changes. The results imply that the odontomes arise by progressive deceleration of tooth eruption, coupled with plastic deformation of newly-formed dental tissue, between 150 and 200 days of age.
were treated with LPS (500ng/ml) and hydrocortisone (10W'M). The condi-
tions of 0.022 to 0.046 gicm' were attributable to the following indices of physical
activities.

Significant differences in IL-la (p < 0.05) were found using the two culture systems. We concluded that whole
blood cultures could provide a suitable alternative method for measuring
stimulated secretion of cytokines in whole blood and isolated cell cultures.

blood cultures could provide a suitable alternative method for measuring
activity: walking more than 1/2 hour/day, 'heavy' reported occupational
physical activity, sporting activity have been shown to be associated with the risk of vertebral
deformities despite compensatory increases in bone density.

ABSTRACTS

P62. Approaches to the measurement of cytokines in peripheral blood in
patients with rheumatoid arthritis
A Rogers, S Rahman, RGG Russell, R Eastell
Department of Human Metabolism and Clinical Biochemistry, Clinical Sciences
Centre, Northern General Hospital, Herries Road, Sheffield S5 7AU

Measurement of circulating levels of cytokines may provide insights into the
pathogenesis of bone and joint diseases. It is unclear as to whether measurement
of serum levels of cytokines or whole blood cultures could replace the
more cumbersome method of peripheral blood mononuclear cell (PBMC)
cultures. This study assesses the possibility of using whole blood cultures by
testing this method against the use of peripheral blood mononuclear cell
cultures and the measurement of circulating cytokines. We studied ten
patients (6 women, 4 men) with seropositive rheumatoid arthritis and ten
healthy age and sex matched controls. Samples of blood were taken and used for
mononuclear cell cultures, whole blood cultures and serum. The cultures were
treated with LPS (500ng/ml) and hydrocortisone (10W'M). The condi-
tioned medium from cultures, and the serum were then assayed for IL-6,
IL-1a, IL-1B, IL-1ra, TNFa and GM-CSF by ELISA. We found that LPS
stimulated secretion of cytokines in whole blood and isolated cell cultures,
and that this was attenuated by hydrocortisone.

Significant differences in IL-1a (p = 0.03) and IL-1ra (p = 0.02) response to
LPS were found using the two culture systems. We concluded that whole
blood cultures could provide a suitable alternative method for measuring
cytokines in peripheral blood.

P63. Are risk factors for vertebral deformities associated with reduced
bone density? The EVOS study
P Masaryk, M Lunt, K Weber, C Scheidt-Nave, D Reid, H Pols, T O'Neill,
D Felsenberg, C Dodenhof, J Dequeker, J Cannata, L Benevolenskaya, G
Poor, J Falch, A Silman, J Reeve
European Prospective Osteoporosis Study (EPoS), Institute of Public Health,
Cambridge CB2 2SR

In the EVOS cohort, smoking, alcohol consumption and various measures of
physical activity have been shown to be associated with the risk of vertebral
deformity. The aim of the present study was to determine the association
between these risk factors and bone density in 13 EVOS centres that
performed bone densitometry. Each of the variables was fitted into a regres-
sion model adjusted for age, height, weight and centre.

BMD differences in the spine, femoral neck and/or trochanter in the range
0.022 to 0.046 g/cm' were attributable to the following indices of physical
activity: walking more than 1/2 hour/day, 'heavy' reported occupational
physical activity, sporting activity > 2 hours per week. Smoking was associ-
ated with lower bone density at all three sites in men (p < 0.02) and
Milk intake was significantly associated with bone density in women.

With the notable exception of heavy physical activity in men, these results
of the present study are consistent with previous findings in EVOS between
the same risk factors and vertebral deformity. They may be best explained by
an association of vertebral deformity with reduced bone density which might be
over-ridden in some men by the effects of heavy physical activity leading to
deformities despite compensatory increases in bone density.

P64. Induction of osteoblastic commitment in mesenchymal stem cells by
bone morphogenetic proteins (BMPs)
W Turner, FJ Hughes
Department of Periodontology, St. Bartholomew's and the Royal London
School of Medicine and Dentistry, Turner Street London E1 2AD

BMPs induce commitment of mesenchymal stem cells to osteoblastic and
chondroblastic phenotypes, although their mechanisms of action are un-
known. In this qualitative pilot study we stimulated confluent C3HOT112
cells, a mouse mesenchymal cell line known to be responsive to BMP, with
BMP 2, 4 and 6 over a time course of 2-72 hours. The cells were first kept in
serum free medium for 24 hours prior to stimulation and maintained for a
further 72 hours post stimulation in medium with 10% FCS, prior to histo-
chemical staining for alkaline phosphatase (ALP). Stimulations were carried
out in both serum-free medium and medium containing 10% FCS. C3HOT112
cells responded to BMP 2, 4 and 6 in a dose-dependent manner
(80ng/ml - 500ng/ml) by expressing ALP, whereas unstimulated cells did not
express ALP. ALP induction was seen even with cells treated with BMPs in
serum free medium for only 2 hours.

The results confirm that BMPs can induce osteoblastic commitment of
mesenchymal stem cells. Furthermore the finding of activity after only 2
hours stimulation in serum-free medium suggests that BMP-induced cell
commitment is a single, irreversible event which does not require the pres-
ence of other co-factors.

BMPs were a generous gift of Dr J Wozney, Genetics Institute, Cambridge MA
USA. Supported by an MRC Clinical Training Fellowship.

P65. Assessment of bone mass in multiple myeloma by quantitative ultra-
sound
J Dillon, A Stewart, D M Reid, R Souta*
Departments of Medicine and *Haematology, University of Aberdeen, Forester-
hill, Aberdeen, AB25 2ZD

Multiple myeloma (MM) is a clonal plasma cell neoplasm characterised by
bone marrow plasmacytosis, the presence of a monoclonal immunoglobulin
and osteolytic skeletal lesions. Current methods of assessing bone disease in
myeloma are unsatisfactory. Skeletal imaging is insensitive but often not specific and X-ray densitometry is useful but
often not accessible. We have assessed bone mass in patients with myeloma
by using quantitative ultrasound. Broad-band Ultrasound Attenuation (BUA),
was measured in the calcaneus of 12 patients with MM (6 males, 6 females,
mean age 68.8 years) using a Walkersonix UBA 575 scanner (short-term
precision in our hands 2.6%) and the results compared with those from 48
age, sex and weight matched controls. In the group as a whole BUA was
found to be significantly reduced by 24.4% (P=0.022) in the patients with
myeloma. Male patients had the greatest reduction (30.5%, P=0.009) but
there was also a trend to reduced values in the females (17.5%).

Although the reduction in BUA is not specific for myeloma the system is
cheap and highly portable and, if these results are confirmed in a larger
series, it could be well suited for use in the outpatient haematology clinic.
The role of quantitative ultrasound in targeting and possibly monitoring
bisphosphonate therapy should be examined.
P66. Cellular characteristics of osteoblasts derived from patients with craniosynostosis
RD Evans, K Arnerman, MC Meikle, F McDonald
Department of Orthodontics and Paediatric Dentistry, United Medical and Dental Schools, and Institute for Child Health, London

In this report we have examined cultured human osteoblasts from patients diagnosed with Crouzon's syndrome. We have examined the cellular production of IL-1α, IL-6, IL-8, IL-11, M-CSF and osteocalcin. Standard ELISA assays were used for assessments. Cells were prepared either by explant culture or by enzyme digest as previously reported and were grown to confluence in Ham's F12/DMEM media which contained 10% foetal calf serum, 50μg/ml streptomycin, 50μg/ml penicillin and 100μg/ml amphotericin. Once confluent the cells were trypsinised and pooled. 10^6 cells were added to each well of a 24 well plate. The cells were stimulated with IGF-I (10 nM), TNFα (10 nM), TGF-β (10 nM) and one series remained as a control. The cells were grown for 7 days and 300 ml of media was removed at 12, 24, 48 and 96 hours and frozen at -20°C until ready for analysis. In all wells cells were seen adhering to the base of the 24 hour and increasing to confluence at 48 hours. Both IL-1α and IL-11 showed negligible production (< pg/ml). IL-6 was always present irrespective of the stimulus (34.9 ± 12.3 pg/ml). This is low in comparison to other reported levels. Conversely osteocalcin was always high (60.9 ± 16.8 ng/ml) except when stimulated with IGF-I. In this case osteocalcin production was 21.3 ± 14.3 ng/ml. M-CSF was at a similar level to previously reported osteoblasts at 96 hours (3,196.8 ± 187.9 pg/ml). IL-8 was high in all samples starting at low levels (< pg/ml) increasing to similar high levels to those previously reported after 96 hours (IGF-1 5,712.0 ± 165.4; TNFα 6,123.0 ± 196.3; TGFβ 8,122.9 ± 296.0). In conclusion, cells derived from patients with a Crouzon's syndrome appear to produce increased quantities of osteocalcin and decreased quantities of IL-6.

P67. An education package that increases calcium intake in schoolgirls
C Jones, L Nixom, P Thompson
Osteoporosis Dorset, 11 Shelley Road, Bournemouth, BH1 4QG, Dorset

This study was designed to prospectively test the ability of the package to influence calcium intake in a cohort of schoolgirls. 44 pupils in an independent girls school were sent a validated dietary diary at the beginning of year 7. An education package ("Pays to look after Your Bones") was integrated into the curriculum. Dietary diaries were circulated at the end of year 7. At baseline 77% of the girls had calcium intakes <800mg/day and 9% <400mg/day. There was a significant increase in mean (SD) calcium intake from 730(288)mg/day to 783(310)mg/day during the study (p<0.001). This difference could in part be explained by protein suggesting an increased intake of dairy products. At the end of the study 60% were taking less than 800mg/day and 7% less than 400mg/day.

We conclude that the education package integrated into year 7 can modestly increase calcium intake. However a significant minority remain well below recommended calcium requirements and this deficiency is associated with low protein/caloric intakes.

P68. Osteoporosis in postmenopausal women with impaired mobility
D Wright, MB Barnes, D Williams, TJ Daymond
Dept of Rheumatology, Derriford Hospital, Plymouth, and Hunters Moor Regional Rehabilitation Centre, Newcastle Upon Tyne

Twenty women with impaired mobility were randomly selected from a population attending a regional rehabilitation centre, to determine their risk of osteoporotic fracture. Potential risk factors and mobility were assessed, and seven day dietary calcium intake was measured. Bone mineral density was measured using the Lunar Expert DEXA scanner, and biochemical markers of bone turnover were analysed. Of the 20 women (mean age 61 yrs), 13 met the WHO criteria for osteoporosis at the lumbar spine or hip (ie, T score <2.5), including all 7 women who reported previous fragility fractures, and 8 who reported loss of height. Mean BMD for the whole group was 0.759 g/cm² (0.547-1.64) left hip, 0.743 (0.134-1.214) right hip, and 1.025 (0.601-1.816) at the lumbar spine. Mean decamperoxidine/creatinine ratio was 8.2 nmol/mmol (range 5.1-12.2) suggesting increased bone resorption, mean ISAP was 28 ug/l (14-51). No significant relationship between level of mobility (median OPCS score 1.6, range 1.1-1.2) and BMD was detected.

Fourteen women reported falls within the previous 12 months. Most of these women are at risk of fractures due to low bone density and falls. A larger study is now required to determine which patients are most at risk, and possible management options.

P69. Early proliferative responses following tibial osteotomy
G Li, AH RW Simpson, J Kenwright, J Triffitt
MRC Bone Research Laboratory, Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Oxford, OX3 7LD

The exact nature and locations of the osteogenic progenitors which are activated following skeletal fracture are unknown. To address these issues the present study has examined cell proliferation in the early stages of tibial osteotomy in an animal model. In vivo uptake of bromodeoxyuridine (BrdU) and its detection by an anti-BrdU antibody (Bua20A) was used to identify proliferating cells and the locations and temporal sequences of the cellular responses were determined.

Mid-tibial osteotomies were performed on adult male rabbits. The osteotomies were stabilised with unilateral external fixators applied remote from the osteotomy site so that cellular responses at the fracture site could be studied without additional interference. After 7 and 14 days the animals were killed and the regenerated tissue was excised and fixed in paraformaldehyde before decalcification. Paraffin sections were cut and immunostained with Bua20A monoclonal antibody. The results have defined the sites of cellular regeneration and indicate the importance of the periosteal and medullary regions of bone in the early stages of fracture healing.
Secondary fracture repair is associated with extensive intracartilaginous and intramembranous ossification. Since bone displays immunoreactivity to several neuropeptides and research suggests that calcitonin gene related peptide (CGRP) influences bone cell activity; CGRP expression was examined in the repairing rodent femur.

CS1 mice (mid-third femoral fractured and non-fractured groups, n=30) had tissues removed at 3, 4 and 5 weeks post-fracture; before processing for histology, indirect immunofluorescence labelling of CGRP and radioimmunoassay. Confocal microscopy showed CGRP labelling of osteocondral osteoclasts, nerve-like fibres in bone and marrow elements. CGRP levels at 3 weeks were 2.8 ng/g (SE=0.24) in fractured limbs vs 7.3 ng/g (SE=0.19) in non fractured limbs; at 4 weeks 5.1 ng/g (SE=1.05) vs 7.83 ng/g (SE=0.22) and at 5 weeks 5.45 ng/g (SE=0.32) vs 7.05 ng/g (SE=0.26). Reductions in CGRP levels post-fracture of 62% (3 wks), 35% (4wks) and 23% (5wks) compared to non-fractured levels suggests that bone cells contribute to enhancement of CGRP with time post-fracture and that the deficit in expression reflects incomplete restoration of sensory innervation.

For research grant support we wish to thank the DHSS, N. Ireland.