Abstracts from the Bone and Tooth Society Annual Meeting 2003

9-11 July 2003

Hallam University,
Sheffield, UK

Organisers

Dr Aubrey Blumsohn, Dr Nicky Peel
University of Sheffield

Bone and Tooth Society

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

Annual Meeting 2003

Venue

The meeting will take place at Hallam University, Sheffield, UK.

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Meeting Organiser

For further information please contact our Meeting Organiser:
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Outline programme

	Wednesday 9th July	Thursday 10th July	Friday 11th July
Morning	11:00 Registration and coffee **11:30 Official Opening** **11:45 Oral Communications** 12:45 Lunch	**08:45** RANKL: **Larry Riggs** (Rochester MN, USA) **09:30 Oral Communications** **10:30** Bone microstructure in archaeology: **Andrew Chamberlain** (Sheffield, UK) 10:50 Coffee **11:20 Symposium: New hormonal actions** • Phosphate metabolism: **Gordon Strewler** (Boston MA, USA) • Osteoblast sensitivity to corticosteroids: **Mark Cooper** (Birmingham, UK) • Thyroid hormone actions in bone and cartilage: **Graham Williams** (London, UK) 12:50 Lunch	**08:45** Genetic control of bone remodeling: insights from rare diseases: **Michael Whyte** (St Louis MO, USA) **09:30 Oral Communications** 10:30 Coffee **11:00 Poster Discussion Session** **12:00 Bone and Tooth Society AGM/ Lunch**
Afternoon	**13:45 Symposium: New thoughts about old hormones** • Vitamin D: **Mike Holick** (Boston, MA) • Parathyroid hormone: **Edward Nemeth** (Toronto, Canada) **14:45 Tea and Posters** **16:00 Symposium: Hearts and bones** • Statins and bone: **Greg Mundy** (San Antonio TX, USA) • Atherosclerosis: an inflammatory disease with calcification: **David Crossman** (Sheffield, UK) • The Barker Hypothesis: Early life influences on bone and cardiovascular diseases: **Cyrus Cooper** (Southampton, UK)	**14:00 Clinical Case Presentations** **15:00 Oral Communications** **16:00 Tea and Posters** **17:00 Debate:** Clinical trials without placebo controls are unethical: **Juliet Compston** (Cambridge, UK) **John Kanis** (Sheffield, UK)	**13:00 Oral Communications** **14:00 Symposium: Insights from rare diseases** • Molecular pathology of renal chloride channels and calcium metabolism: **Raj Thakker** (Oxford, UK) • "Juvenile Pagets", OPG mutations: **Tim Cundy** (Auckland, New Zealand) **15:00 Presentation of Awards** **15:15 Close of meeting**
Evening	**19:30 Reception**	**19:30 Dinner**	

Programme

Wednesday 9 July

11:00 Registration and coffee

11:30 Official Opening
Bob Boucher, Vice-Chancellor, University of Sheffield

11:45 Oral Communications

Chairs: Tim Chambers (London, UK)/Greg Mundy (San Antonio TX, USA)

11:45 OC1 LIMITIN, A TYPE-I INTERFERON-LIKE CYTOKINE, INHIBITS OSTEOCLASTOGENESIS WITHOUT AFFECTING MACROPHAGE PROLIFERATION AND FUNCTION
T Sato, Y Hakeda. Department of Oral Anatomy, Meikai University, School of Dentistry, Saitama, Japan

11:57 OC2 MATRIX METALLOPROTEINASE INHIBITION DOES NOT RECAPITULATE THE ACTION OF CHEMICALLY MODIFIED TETRACYCLINES ON OSTEOCLAST LINEAGE CELLS
S G Holmes[1], D J Buttle[2], N J Bishop[1], P S Grabowski[1]. [1]Academic Unit of Child Health; [2]Division of Genomic Medicine, University of Sheffield, UK

12:09 OC3 ZOLEDRONIC ACID INHIBITS ADHESION OF BREAST AND PROSTATE CANCER CELLS VIA INHIBITION OF PROTEIN PRENYLATION
J P Coxon, L M Pickering, K W Colston. Department of Cellular & Molecular Medicine, St George's Hospital Medical School, London, UK

12:21 OC4 VISUALISATION OF THE UPTAKE OF A NOVEL FLUORESECENT BISPHOSPHONATE INTO INTRACELLULAR VESICLES IN RESORBING OSTEOCLASTS AND J774 CELLS
F P Coxon, J Langton, M J Rogers. Bone Research Group, University of Aberdeen, UK

12:33 OC5 DISTINCT REGULATION OF BONE AND MUSCLE MAINTENANCE DURING HINDLIMB SUSPENSION BY A CONCENTRIC RESISTANCE EXERCISE REGIMEN
D S Perrien[1], N S Akel[1], D C Montague[1], M Knox[2], J D Fluckey[2], E E Dupont-Versteegden[2], C A Peterson[2], L Suva[1], D Gaddy[1]. [1]Physiology and Biophysics, and Center for Orthopaedic Research, Orthopaedic Surgery; [2]Geriatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

12:45 Lunch

13:45 Symposium: New thoughts about old hormones

Chairs: Cyrus Cooper (Southampton, UK)/Gordon Strewler (Boston MA, USA)

13:45 IS1 Vitamin D: **Mike Holick** (Boston MA, USA)

14:15 IS2 Parathyroid hormone: **Ed Nemeth** (Toronto, Canada)

14:45 Tea and Posters

16:00 Symposium: Hearts and bones

Chairs: Richard Eastell (Sheffield, UK)/Mike Rogers (Aberdeen, UK)

16:00 IS3 Statins and bone: **Greg Mundy** (San Antonio TX, USA)

16:30 IS4 Atherosclerosis: an inflammatory disease with calcification: **David Crossman** (Sheffield, UK)

17:00 IS5 The Barker Hypothesis: Early life influences on bone and cardiovascular diseases: **Cyrus Cooper** (Southampton, UK)

17:30 Close

19:30 Reception
Level 2, Atrium, Owen Building
Generously supported by Eli Lilly & Co

Thursday 10 July

08:45 IS6 Paracrine regulation of postmenopausal increases in bone resorption: role of RANK Ligand (RANKL): **Larry Riggs** (Rochester MN, USA)

Chair: Richard Eastell (Sheffield, UK)

09:30 Oral Communications

Chairs: Dana Gaddy (Little Rock AR, USA)/Nigel Loveridge (Cambridge, UK)

09:30 OC6 MEGAKARYOCYTE SYNTHESIS OF OSTEOPROTEGERIN IS STIMULATED BY ESTROGEN WHILST RANKL EXPRESSION IS SUPPRESSED
S Bord[1], E Frith[1,2], D C Ireland[1], M A Scott[2], J I O Craig[2], J E Compston[1]. [1]Cambridge University School of Clinical Medicine; [2]Department of Haematology, Addenbrooke's Hospital, Cambridge, UK

09:42 OC7 MEGAKARYOCYTES STIMULATE OSTEOBLAST SYNTHESIS OF COLLAGEN AND OSTEOPROTEGERIN PROTEIN AND mRNA
S Bord[1], E Frith[1,2], D C Ireland[1], M A Scott[2], J I O Craig[2], J E Compston[1]. [1]Cambridge University School of Clinical Medicine; [2]Department of Haematology, Addenbrooke's Hospital, Cambridge, UK

09:54 OC8 REGULATION OF BONE RESORPTION AND FORMATION THROUGH THE CELL-SURFACE PROCESSING OF RANKL AND JAGGED-1
D J Dallas-Skerry[1], G Murphy[2], T M Skerry[1]. [1]Royal Veterinary College, London, UK; [2]Cambridge Institute for Medical Research, UK

10:06 OC9 INNOVATIVE BIOMINERALISED POLYSACCHARIDE TEMPLATES FOR HUMAN BONE CELL AND GENE DELIVERY
D W Green[1], I Leveque[2], D Walsh[2], K A Partridge[1], S Mann[2], R O C Oreffo[2]. [1]University Orthopaedics, General Hospital, Tremona Road, Southampton, UK; [2]Department of Chemistry, University of Bristol, UK

10:18 OC10 RETARDATION OF LONGITUDINAL BONE GROWTH BY GLUCOCORTICOIDS IS REVERSED BY IGF-I IN FOETAL MOUSE METATARSAL ORGAN CULTURES
T Mushtaq, S F Ahmed, C Farquharson. Bone Biology Group, Division of Integrative Biology, Roslin Institute, Roslin, UK

10:30 IS7 Bone microstructure in archaeology: **Andrew Chamberlain** (Sheffield, UK)

Chair: Aubrey Blumsohn (Sheffield, UK)

10:50 Coffee

11:20 Symposium: New hormonal actions

Chairs: Jonathan Reeve (Cambridge, UK)/David Reid (Aberdeen, UK)

11:20 IS8 Phosphate metabolism: **Gordon Strewler** (Boston MA, USA)

11:50 IS9 Osteoblast sensitivity to corticosteroiods: **Mark Cooper** (Birmingham, UK)

12:20 IS10 Thyroid hormone actions in bone and cartilage: **Graham Williams** (London, UK)

12:50 Lunch

14:00 Clinical Case Presentations

Moderators: Tim Cundy (Auckland, NZ)/Michael Whyte (St Louis MO, USA)

14:00 C1 RIB FRACTURES IN A YOUNG MAN
M W J Davie. Robert Jones and Agnes Hunt Hospital, Oswestry, Shropshire, UK

14:15 C2 CHONDROSARCOMA ASSOCIATED WITH HYPERPARATHYROIDISM: A REPORT OF 2 CASES
A Bhatia[1], S Cannon[1], T Briggs[1], R W Keen[1,2]. [1]Royal National Orthopaedic Hospital, Metabolic Bone Unit, Brockley Hill, Stanmore, Middlesex, UK; [2]University College London, Rheumatology Department, Arthur Stanley House, 40-50 Tottenham Street, London, UK

14:30 C3 MOLECULAR DIAGNOSIS OF HYPOPARATHYROIDISM : 2 ILLUSTRATIVE CASES
G Hampson[1], M R Bowl[2], M A Nesbit[2], M Giannoulis[3], R Jones[3], R V Thakker[2]. [1]Department of Chemical Pathology, St Thomas' Hospital, London, UK; [2]Nuffield Department of Clinical Medicine, University of Oxford, UK; [3]Diabetes and Endocrine, St Thomas' Hospital, London, UK

14:45 C4 SEVERE OSTEOPOROSIS IN TWO YOUNG IMMOBILISED MEN
D O'Gradaigh, S Bord, J E Compston. Bone Research Group, Dept. of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, UK

15:00 Oral Communications

Chairs: Larry Riggs (Rochester MN, USA)/Peter Selby (Manchester, UK)

15:00 OC11 THE EFFECTS OF DIETARY IMPROVEMENT ON BONE METABOLISM IN ELDERLY UNDERWEIGHT WOMEN WITH OSTEOPOROSIS : A RANDOMISED CONTROLLED TRIAL
G Hampson[1], F Martin[2], K Moffat[3], S Vaja[1], S Sankaralingam[1], J Cheung[1], G M Blake[4], I Fogelman[4]. [1]Department of Chemical Pathology; [2]Elderly Care Unit; [3]Dietetics, St Thomas' Hospital, London, UK; [4]Osteoporosis Screening Unit, Guy's Hospital, London, UK

15:12 OC12 CHANGES IN BONE MASS FOLLOWING TIBIAL SHAFT FRACTURE
S W Veitch[1,2], S C Findlay[1], A J Hamer[2], R Eastell[1], B M Ingle[1]. [1]Bone Metabolism Group, Clinical Sciences (North), University of Sheffield, UK; [2]Orthopaedic Department, Northern General Hospital, Sheffield, UK

15:24 OC13 DOES LOW MAGNITUDE, HIGH FREQUENCY MECHANICAL LOADING IMPROVE DIAPHYSEAL STRENGTH OF LONG BONES?
K A Ward[1], C W Alsop[1], J M Caulton[2], C T Rubin[3], M Lunt[1], J E Adams[1], M Z Mughal[2]. [1]University of Manchester, UK; [2]Central Manchester and Manchester Children's University Hospitals NHS Trust, UK; [3]State University of New York, New York, USA.

15:36 OC14 BIOCHEMICAL MARKERS OF BONE TURNOVER ARE ASSOCIATED WITH RISK OF BONE METASTASES IN WOMEN WITH PRIMARY OPERABLE BREAST CANCER
S K Bal[1], R Tahtela[2], A Paterson[3], T Powles[4], S Atula[2], J Kanis[1], S Vasireddy[1], J Nevalainen[2], E McCloskey[1]. [1]University of Sheffield, UK; [2]Leiras Oy, Clinical Research, Helsinki, Finland; [3]Tom Baker Cancer Center, Calgary, Canada; [4]Royal Marsden Hospital, Sutton, UK

15:48 OC15 THE RELATIONSHIP BETWEEN BONE DENSITY BONE SIZE AND FRACTURE SYNDROMES IN POSTMENOPAUSAL WOMEN
C M Smith, J A Clowes, R Eastell. Bone Metabolism Group, University of Sheffield, UK

16:00 Tea and Posters

17:00 Debate:
Clinical trials without placebo controls are unethical.

For: **John Kanis** (Sheffield, UK)

Against: **Juliet Compston** (Cambridge, UK)

Chair: David Hosking (Nottingham, UK)

18:00 Close

19:30 Dinner
Millennium Galleries and Winter Garden

Friday 11 July

08:45 IS11 Genetic control of bone remodeling: insights from rare diseases: **Michael Whyte** (St Louis MO, USA)

Chair: Raj Thakker (Oxford, UK)

09:30 Oral Communications

Chairs: Tim Skerry (London, UK)/Graham Williams (London, UK)

09:30 OC16 PLEIOTROPHIN - A NEW INHIBITOR OF BMP-MEDIATED OSTEOINDUCTION: POSSIBLE RELEVANCE TO FIBRODYSPLASIA OSSIFICANS PROGRESSIVA?
H I Roach[1], R S Tare[1], R O C Oreffo[1], E M Shore[2], F S Kaplan[2]. [1]University Orthopaedics, University of Southampton, CF86, General Hospital, Southampton, UK; [2]Dept. of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, USA

09:42 OC17 VITAMIN D RECEPTOR GENE POLYMORPHISMS: ASSOCIATION WITH BONE DENSITY AND OSTEOPOROTIC FRACTURES: A CASE CONTROL STUDY
A,Rogers, F Gossiel, J A Clowes, R Eastell. Bone Metabolism Group, Section of Human Metabolism, Division of Clinical Sciences (North), University of Sheffield, UK

09:54 OC18 C-FOS BLOCKS CARTILAGE DIFFERENTIATION IN VITRO BY INHIBITING THE EFFECTS OF BONE MORPHOGENETIC PROTEIN (BMP)-2 AND -4
I Anagnostopoulos, D P Thomas, A E Grigoriadis. Department of Craniofacial Development, King's College London, Guy's Hospital, Guy's Tower, London, UK

10:06 OC19 POLYMORPHISMS IN THE P450 C17 (17-HYDROXYLASE/17,20-LYASE) AND P450 C19 (AROMATASE) GENES : ASSOSIATION WITH SERUM SEX STEROIDS CONCENTRATIONS AND BONE MINERAL DENSITY IN POST-MENOPAUSAL WOMEN
J Somner[1],S Mclellan[1],J Cheung[1],Y T Mak[1], M L Frost[2], K M Knapp[2], A S Wiercbicki[1], M Wheeler[1], I Fogelman[2], S H Ralston[3], G Hampson[1]. [1]Department of Chemical Pathology, St Thomas' Hospital, London, UK; [2]Osteoporosis Screening Unit, Guy's Hospital, London UK; [3]Bone Research Group, Department of Medicine and Therapeutics, University of Aberdeen Medical School, UK

10:18 OC20 EPIDEMIOLOGY OF FRACTURES IN BRITISH RACEHORSES IN TRAINING
K L P Verheyen[1], J S Price[2], J L N Wood[1]. [1]Animal Health Trust, Newmarket, UK; [2]Royal Veterinary College, London, UK

10:30 Coffee

11:00 Poster Discussion Session

Chairs: Sharyn Bord (Cambridge, UK)/Juliet Compston (Cambridge, UK)

11:00 P1 GENDER VARIATION IN PTH SENSITIVITY FOLLOWING GROWTH HORMONE REPLACEMENT IN ADULT GROWTH HORMONE DEFICIENT PATIENTS
H D White[1], A M Ahmad[1], A A Syed[1], R Peter[1], B H Durham[2], J P Vora[1], W D Fraser[2]. [1]Department of Diabetes and Endocrinology; 2]Department of Clinical Chemistry, Duncan Building, Royal Liverpool University Hospital, UK

11:05 P2 OSTEOPONTIN EXPRESSION AT SITES OF OSTEOCLAST EROSION IN RHEUMATOID ARTHRITIS
D O'Gradaigh, S Bord, J E Compston. Bone Research Group, Dept. of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, UK

11:10 P3 CHANGES IN BODY COMPOSITION DURING TREATMENT FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA WITH A CONTEMPORARY PROTOCOL
J H Davies[1], B Evans[1], M Jenney[2], W Evans[3], E Jones[3],J Gregory[3]. [1]Department of Child Health, UWCM, Cardiff, UK;[2]Department of Paediatric Oncology, Llandough Hospital, UK; [3]Department of Medical Physics, UHW, Cardiff, UK

11:15 P4 ESTROGEN AND THE FORMATION OF REMODELLING CLUSTERS
N Loveridge, S Vedi, K L Bell, J E Compston, J Reeve. Department of Medicine, University of Cambridge Clinical School, UK

11:20 P5 IN VIVO HUMAN BONE AND CARTILAGE FORMATION USING POROUS POLYMER SCAFFOLDS ENCAPSULATED WITH BONE MORPHOGENETIC PROTEIN-2
X B Yang[1], M J Whitaker[2], N M P Clarke[1], W Sebald[3], S M Howdle[2], K M Shakesheff[4], R O C Oreffo[1]. [1]University Orthopaedics, University of Southampton, Southampton General Hospital, UK; [2]School of Chemistry, University of Nottingham, UK; [3]Department of Physiological Chemistry, University of Wurzburg, Germany; [4]School of Pharmaceutical Sciences, University of Nottingham, UK

11:25 P6 PASTEURELLA MULTOCIDA TOXIN HAS DIFFERENTIAL EFFECTS ON MURINE AND HUMAN OSTEOCLAST DIFFERENTIATION AND ACTIVITY
N W A McGowan[1], D Harmey[1], G Stenbeck[2], A E Grigoriadis[1]. [1]Craniofacial Development, King's College London, UK; [2]Bone and Mineral Centre, University College London, UK

11:30 P7 TGF-BETA-INDUCED SOCS3 EXPRESSION AUGMENTS TNF-ALPHA-INDUCED OSTEOCLAST FORMATION
S W Fox[1], A C Lovibond[1], S J Haque[2], T J Chambers[1]. [1]Department of Cellular Pathology, St George's Hospital Medical School, London, UK; [2]Department of Cancer Biology & Department of Pulmonary Critical Care Medicine, Cleveland Clinic Foundation, Cleveland, USA

11:35 P8 A ROLE FOR RETINOIC ACID IN REGULATING OSTEOCLAST FUNCTION IN REGENERATING DEER ANTLERS
S P Allen[1], M Maden[2], J S Price[1]. [1]Veterinary Basic Sciences, Royal Veterinary College, London, UK; [2]Developmental Neurobiology, Kings College, London, UK

11:40 P9 OLDER PEOPLE IN CHINA AND UK DIFFER IN THE RELATIONSHIPS BETWEEN PARATHYROID HORMONE, VITAMIN D AND BONE MINERAL STATUS
L Yan[1,2], A Prentice[1], S D'Ath[1], A Laidlaw[1], M A Laskey[1], B Zhou[2]. [1]MRC Human Nutrition Research, Cambridge, UK; [2]Shenyang Medical College, Shenyang, PR China

11:45 P10 CORTICAL POROSITY DURING FAST GROWTH IN THE IMMATURE SKELETON: THE ROLE OF PERIOSTEAL OSTEOBLAST PROLIFERATION
D H Murray[1], N Loveridge[2], C Farquharson[1]. [1]Bone Biology Group, Roslin Institute, Edinburgh, UK; [2]Bone Research Group, Addenbrooke's Hospital, Hills Road, Cambridge, UK

11:50 P11 ADHESION OF BREAST AND PROSTATE CANCER CELLS TO EXTRACELLULAR MATRIX PROTEINS IS DEPENDENT ON GROWTH FACTOR RECEPTOR EXPRESSION AND INHIBITED BY ZOLEDRONIC ACID
L M Pickering, J P Coxon, K W Colston. Department of Cellular and Molecular Medicine, St. George's Hospital Medical School, London, UK

11:55 P12 A NOVEL APPROACH TO THE GENERATION OF ANTIBODIES AGAINST ADULT HUMAN MESENCHYMAL STEM CELLS
J Letchford[1], A Cardwell[1], K Stewart[1], M J Perry[2], J N Beresford[1]. [1]University of Bath, UK; [2]University of Bristol, UK

12:00 Bone and Tooth Society AGM, Lunch

13:00 Oral Communications

Chairs: Agi Grigoriadis (London, UK)/Richard Oreffo (Southampton, UK)

13:00 OC21 ER ALPHA ACTIVATES THE BMP-6 PROMOTER IN BONE AND BREAST CELLS VIA DISTINCT MECHANISMS
D B Ong[1], S M Colley[1], M R Norman[1], R Kitazawa[2], S Kitazawa[2], J H Tobias[1]. [1]Academic Rheumatology and University Research Centre for Neuroendocrinology, University of Bristol, UK; [2]2nd Department of Pathology, Kobe University, Kobe, Japan

13:12 OC22 NEURONAL NOS IN ADULT HUMAN CORTICAL OSTEOCYTES
A M Caballero-Alías[1], N Loveridge[1], A Lyon[1], V Das-Gupta[2], A Pitsillides[2], J Reeve[1]. [1]Bone Research Group (MRC), University of Cambridge, UK; [2]Royal Veterinary College, London, UK

13:24 OC23 BIOMIMETIC MICROENVIRONMENTS - STIMULATION OF HUMAN OSTEOPROGENITOR DIFFERENTIATION BY A SYNTHETIC PEPTIDE COLLAGEN BINDING DOMAIN: P-15
X B Yang[1], H I Roach[1], N M P Clarke[1], R S Bhatnagar[2], S Li[3], R O C. Oreffo[1]. [1]University Orthopaedics, University of Southampton, General Hospital, Southampton, UK; [2]Laboratory of Connective Tissue Biochemistry, University of California San Francisco, USA; [3]Department of Bioengineering, University of California Berkeley, USA

13:36 OC24 DEFINING THE SECRETOME OF BONE CELLS: NOVEL BONE BIOACTIVE PROTEINS
G P Thomas, P Moffatt, P Salois, M-C Bessette, K Sellin, M-H Gaumond, C Lanctot. Phenogene Therapeutiques, 416 de Maisonneuve Ouest, Suite 1020, Montreal, Quebec H3A 1L2, Canada.

13:48 OC25 ACTIVATORS OF PPAR ISOFORMS ALPHA AND/OR DELTA CAUSE AN INCREASE IN BONE FORMATION IN VITRO AND IN VIVO
J Clarke[1], D Hughes[2], M Perry[3], A Scutt[1], K Still[1]. [1]Child Health, University of Sheffield, UK

14:00 Symposium: Insights from rare diseases

Chair: Roger Smith (Oxford, UK)

14:00 IS12 Molecular pathology of renal chloride channels and calcium metabolism: **Raj Thakker** (Oxford, UK)

14:30 IS13 Juvenile Pagets, OPG mutations: **Tim Cundy** (Auckland, New Zealand)

15:00 Presentation of Awards

15:15 Close of meeting

IS1

VITAMIN D: IMPORTANT FOR PREVENTION OF CANCER, DIABETES, HEART DISEASE, AND OSTEOPOROSIS

M F Holick. Boston University School of Medicine, Boston, MA, USA

Most of our vitamin D requirement comes from exposure to sunlight. This is because very few foods naturally contain vitamin D. In Europe very few foods are fortified with vitamin D. The result is that most children and adults are in a chronically vitamin D insufficient state which has important implications for their overall health and well-being. Once made in the skin, vitamin D is metabolized sequentially in the liver and kidney to 1,25-dihydroxyvitamin D [1,25(OH)2D]. Receptors for 1,25(OH)2D are present in most cells and tissues in the body. 1,25(OH)2D not only is critically important for regulating calcium homeostasis, but appears to be very important in the regulation of cell growth, immune function, insulin secretion and renin production. It is also now realized that many tissues, including colon, breast, prostate and skin can express the 25-hydroxyvitamin D-1-hydroxylase (1-OHase). Thus, WHEN vitamin D is metabolized in the kidney it acts not only to regulate calcium homeostasis, but also insulin production and regulates renin secretion. The local production of 1,25(OH)2D may be important as a sentinel for regulating cellular growth and could be the explanation for why vitamin D deficiency (decreased exposure to sunlight) is associated with an increased risk of developing and dying of colon, prostate, ovarian and breast cancer. The general public, physicians and health care regulatory agencies need to recognize the importance of vitamin D nutrition and sun exposure, not only for bone health but for overall health and well-being of children and adults. Monitoring of 25-hydroxyvitamin, the only measure of vitamin D status, should be as routine a test as is a serum lipid profile. The correction of vitamin D insufficiency by increasing fortification programs of various foods, including juice products, is worthy of consideration worldwide.

IS2

PARATHYROID HORMONE

E F Nemeth. NPS Pharmaceuticals, Toronto, Ontario, Canada

Two recent developments prompt new thoughts about parathyroid hormone (PTH). The first is the compelling evidence suggesting that circulating fragments of PTH act on a novel receptor to alter skeletal physiology. Peptide fragments are secreted by parathyroid cells and additionally result from metabolism of circulating PTH. These peptide fragments neither bind to nor activate the PTH/PTHrP receptor (PTHR1) yet they alter the activity of bone cells in vitro and affect systemic calcium homeostasis in vivo. Moreover, these peptide fragments affect the responses of bone cells derived from mice lacking the PTHR1. It is not unreasonable to suppose that we are on the brink of discovering an aspect of PTH physiology involving new peptide ligands and their cognate receptor.

The second development prompting new thoughts about PTH is the discovery of pharmacological tools to control secretion of PTH. Calcimimetic and calcilytic compounds act as activators and antagonists, respectively, of the calcium receptor on parathyroid cells. These compounds permit the direct control of PTH secretion without altering blood levels of calcium, phosphate, or vitamin D. Calcilytic compounds rapidly increase, whereas calcimimetic compounds decrease, circulating levels of PTH. Depending on the pharmacokinetics of the particular compound, the changes in circulating levels of PTH can be either sustained or intermittent. Calcilytic compounds can transiently increase circulating levels of PTH and stimulate new bone formation in rat models of osteopenia. Calcimimetic compounds decrease circulating levels of PTH in patients with primary or secondary hyperparathyroidism and improve bone quality in patients with renal osteodystrophy. In animal models of secondary hyperparathyroidism, calcimimetics can be used to show that intermittent, but not sustained decreases in circulating levels of PTH increased bone mineral density. These findings suggest that transient decreases in circulating levels of PTH might also have anabolic effects on bone.

IS3

STATINS AND RELATED ANABOLIC AGENTS FOR BONE

G R Mundy. University of Texas Health Science Center at San Antonio, Texas, USA

The Bone Morphogenetic Protein-2 (BMP-2) gene is regulated by a complex promoter. This promoter in cells in the osteoblast lineage is regulated by nitric oxide, whose expression in osteoblast lineage bone cells is enhanced by increased expression of eNOS mRNA. The statins stimulate BMP-2 transcription, which ultimately leads to osteoblast proliferation and differentiation by enhancing eNOS mRNA stability, which in turn leads to enhanced NO generation and BMP-2 transcription. The effects of statins to increase mRNA stability is mediated by their capacity to inhibit HMG CoA reductase, which in turn leads to impaired generation of small GTPases that require prenylation for activity. This latter step is responsible for increasing expression of eNOS mRNA. A similar process occurs in endothelial cells and is responsible for NO generation and beneficial effects on cerebral blood flow and protection against ischemic cerebral infarcts. These observations suggest novel ways in which effects of statins unrelated to their capacity to lower serum cholesterol may have important effects in target cells which in turn may lead to therapeutic benefit. These mechanisms will be discussed during this presentation.

IS4

Abstract not received

IS5

THE BARKER HYPOTHESIS: EARLY LIFE INFLUENCES ON BONE AND CARDIOVASCULAR DISEASES

C Cooper[1], E M Dennison[1], M K Javaid[1], H E Syddall[1], S Shore[1], K M Godfrey[1], R Oreffo[2], H I Roach[2], N K Arden[1]. [1]MRC Environmental Epidemiology Unit; [2]University Department of Orthopaedics, University of Southampton, UK

There are several similarities in the epidemiological patterns of osteoporosis and coronary heart disease: (a) the two disorders share similar secular trends and geography; (b) BMD has been shown to predict both death from cardiovascular disease and a number of risk factors for the disorder. It has now been demonstrated that environmental influences during early life interact with the genome in establishing the functional level of a variety of metabolic processes which are involved both in cardiovascular and osteoporosis risk. This review will address the role played by such environmental influences during intrauterine or early postnatal life. The evidence that osteoporosis risk might be programmed in this way stems from four groups of studies: (1) Epidemiological studies which confirm that subjects who are born light and whose growth falters in the first year of postnatal life, have significantly lower bone size and mineral content, at age 60 to 75 years; (2) Epidemiological cohort studies have demonstrated that subsequent lower trajectories of childhood growth are associated with an increased risk of hip fracture among such men and women; (3) Detailed physiological studies of candidate endocrine systems which might be programmed have shown that birthweight and growth in infancy alter the functional settings of the GH/IGF-1, and hypothalamic pituitary adrenal axes; (4) Studies characterising the nutrition, body build and lifestyle of pregnant women which relate these to the bone mass of their newborn offspring, have identified a number of important determinants of reduced fetal mineral accrual (maternal smoking, low maternal fat stores and maternal undernutrition, intense levels of weight-bearing physical activity in late pregnancy).

These data suggest that undernutrition and other adverse influences arising in fetal life or immediately after birth have a permanent effect on body structure, physiology and metabolism, which might independently influence the later risk of cardiovascular disease and osteoporotic fracture.

IS6

PARACRINE REGULATION OF POSTMENOPAUSAL INCREASES IN BONE RESORPTION: ROLE OF RANK LIGAND (RANKL)

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Over the last decade, studies on the paracrine mediation of increased bone resorption due to estrogen deficiency have focused on upregulation of the pro-inflammatory cytokines (IL-1b, TNFa, M-CSF, IL-6, and PGE2), which act mainly by increasing the pool of pre-osteoclasts in the bone marrow microenvironment. However, the recently discovered cytokine, RANKL, is the final and most important effector of osteoclastogenesis. RANKL is expressed on the surfaces of bone marrow cells, acts by cell-to-cell contact through its receptor, RANK, on osteoclast lineage cells, and is neutralized by binding to its soluble decoy receptor, osteoprotegerin (OPG), secreted by osteoblast lineage cells. Although OPG production is upregulated by estrogen in vitro, thus far, RANKL has not been shown to be regulated. Thus, we used flow cytometry and fluorescent-labeled OPG as a probe to isolate bone marrow cells expressing RANKL on their surfaces.

Bone marrow aspirates are passaged through a Ficoll-gradient and 2-color flow cytometry was employed to classify cells expressing RANKL as marrow stromal cells (MSC), T-cells or B-cells using antibodies against alkaline phosphatase, CD3, and CD19, respectively. Isolated MSC were shown to express bone-related genes and to form mineralized nodules when cultured in a differentiating medium. We studied 12 premenopausal women (Premps), 11 early (<10 yrs after menopause) postmenopausal women (Postmps), and 13 postmenopausal women (matched for years after menopause) receiving long-term estrogen therapy (Postmps + E). Based on fluorescence intensity, RANKL surface concentration per cell in the Postmps women was increased over that in the Premps women in MSC by 304% ($P<0.001$), in T-cells by 248% ($P<0.01$), in B-cells by 238% ($P<0.01$), and in total RANKL positive cells by 254% ($P<0.01$). Moreover, the bone resorption marker, serum CTx, was correlated ($P=0.005$) with RANKL expression per cell in MSC ($r=0.52$), T-cells ($r=0.46$), B-cells ($r=0.57$), and total RANKL positive cells ($r = 0.46$) and inversely ($r=-0.31$, $P=0.01$) with serum estradiol in total RANKL positive cells. Although these findings do not exclude contributions by other cytokines, they indicate that upregulation of RANKL on MSC, T-cells and B-cells in bone marrow cells is a major determinant of increased bone resorption during E-deficiency.

IS7

BONE MICROSTRUCTURE IN ARCHAEOLOGY

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Mineralised tissues are durable after death, and bones and teeth are therefore an important category of material studied by archaeologists and forensic scientists. Archaeologists use macroscopic, microscopic, chemical and biomolecular methods to investigate ancient skeletal remains with the aim of identifying species, estimating demographic parameters, diagnosing ancient disease, investigating metabolic processes and examining post-mortem diagenetic change. Forensic scientists use similar investigative methods but with different aims, for example to establish the personal identity of missing persons and to ascertain time, place and cause of death. This paper reviews some of the evidence obtained from microstructural studies of bones and teeth from archaeological sites and suggests some directions for future research.
Bone is exposed to varying degrees of degradation after death, with both the mineral and the organic phases subject to decay depending on the temperature, acidity and presence of microorganisms in the burial environment. Characteristic patterns of decay can be observed in microscopic thin sections of bone, and the changes observed correlate well with physical and chemical measures of bone protein content, porosity and mineral crystallinity. The extent of microscopic degradation in bone depends on the species, with different vertebrate species varying in the structural, cellular and vascular organisation of their skeletal tissues.
Well-preserved bone can be recovered from a range of environments including alkaline soils, anaerobic sediments and cave deposits. In specimens with intact collagen, the disposition of lamellar bone and secondary osteons can be recorded from undecalcified thin sections observed with transmitted plain and polarised light. A preliminary study of bone microstructure in modern cattle has indicated that populations of small diameter osteons may develop in the cortical bone of animals that are exploited intensively for milk production. These patterns have also been identified in the bones of prehistoric cattle, thereby providing information about past animal husbandry practices

IS8

RICKETS, PHOSPHATONINS, AND THE CONTROL OF PHOSPHATE METABOLISM

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The existence of a phosphaturic hormone, a phosphatonin, was long ago invoked to explain the pathogenesis of phosphate wasting syndromes. The identification of FGF23 as a phosphaturic substance has linked together all three of the most important phosphaturic syndromes. FGF23 is produced in large amounts by tumors that cause osteomalacia, and serum levels of FGF23 are high in patients with tumor-induced osteomalacia. Autosomal dominant hypophosphatemic rickets (ADHR)is caused by mutations that prevent the degradation of FGF23 by proteolytic cleavage. The most common of inherited phosphate wasting disorders, X-linked hypophosphatemia (XLH), is characterized by elevated serum levels of FGF23 and is caused by loss of a protease, PHEX, that is a putative FGF23 cleavage enzyme. (It has been difficult, however, to show that PHEX cleaves FGF23.) Deletion of both copies of the FGF23 gene in mice produces a syndrome of hyperphosphatemia, increased 1,25-dihydroxyvitamin D levels and hypercalcemia. This result indicates that FGF23 has a significant role in the physiologic regulation of phosphate, calcium and vitamin D metabolism and raises a number of questions. Is there a phosphate sensor and where is it located? What is the physiologic site of production of FGF23 and how is it linked to phosphate sensing? What is the physiological site of FGF23 degradation, and is degradation a physiologically important regulatory mechanism for FGF23? Does FGF23 have a unique receptor, and what are the cellular mechanisms that link the FGF23 receptor to renal phosphate reabsorption? What tissues other than the proximal renal tubule are targets for FGF23 action? What is the physiological significance of the tight relationship between phosphate excretion and vitamin D activation that is disclosed by the phosphaturic syndromes as well as the knockout mouse? There is much work to be done on phosphate homeostasis.

IS9

OSTEOBLAST SENSITIVITY TO CORTICOSTEROIDS

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Corticosteroids are essential for differentiation of osteoblasts in vitro but when used clinically can decrease bone formation and lead to osteoporosis. In recent years our understanding of corticosteroid action has increased and it has become clear that distinctions between glucocorticoids and mineralocorticoids depend not only on receptor affinity but on metabolism by intracellular enzymes. Osteoblasts express both glucocorticoid receptors and mineralocorticoid receptors but these interactions are regulated by expression of glucocorticoid modifying enzymes. 11beta-hydroxysteroid dehydrogenases (11b-HSDs) interconvert hormonally active cortisol and inactive cortisone. 11b-HSD1 is primarily a glucocorticoid activator and 11b-HSD2 an inactivator. 11b-HSD2 is expressed in human foetal osteoblasts and osteosarcoma cell-lines whereas 11b-HSD1 is expressed in human osteoblasts in vivo and in primary osteoblast cultures. In human osteoblasts 11b-HSD1 can generate cortisol from cortisone and also prednisolone from prednisone. Expression of 11b-HSD1 in osteosarcoma cells induces differentiation and slows cellular proliferation by sensitising cells to cortisone. Circulating levels of cortisone in vivo (and prednisone during prednisolone treatment) are within the active range of the enzyme. In normal volunteers the effect of prednisolone treatment on bone formation markers is dependent on pre-treatment 11b-HSD1 activity suggesting that 11b-HSD1 is a major regulator of osteoblastic glucocorticoid sensitivity in vivo. Recent work suggests that 11b-HSD1 is dynamically regulated during osteoblast differentiation such that osteoblasts are able to regulate their sensitivity to corticosteroids. In addition to enzymatic regulation of corticosteroid sensitivity similar mechanisms are likely to regulate sensitivity to other steroid hormones.

IS10

THYROID HORMONE ACTIONS IN BONE AND CARTILAGE

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Thyroid hormone (T3) is required for skeletal development during childhood and T3 regulates bone turnover and mineralisation in adults. Thyrotoxicosis is an established risk factor for osteoporosis. We and others have shown that T3 receptors (TRs) are expressed in osteoblasts and growth plate chondrocytes, which represent primary T3-target cells in the skeleton. T3 effects on osteoclast-mediated bone resorption are thought to be mediated by osteoblasts via paracrine pathways. Nevertheless, the mechanism of T3-action in bone is poorly understood. We analysed skeletal development in TRalpha0/0 mice (which lack all products of the TRalpha gene), TRbetaPV mice (which harbour a mutation in TRbeta that causes severe resistance to thyroid hormone) and TRalphaPV mice (which have the same mutation in TRalpha but exhibit only very mild thyroid gland failure). TRalpha0/0 mice are biochemically euthyroid and have normal levels of growth hormone production. They exhibit a phenotype of delayed endochondral ossification and reduced bone mineralisation. TRbeta-null mice display no skeletal phenotype. Homozygous TRbetaPV mutant mice have severe disruption of the pituitary-thyroid axis with circulating T4 and T3 levels increased 9-15 fold and TSH levels elevated greater than 400-fold. Surprisingly, they exhibit advanced endochondral and intramembranous ossification with increased bone mineralisation. TRalphaPV heterozygous mice are severely growth retarded with grossly impaired ossification despite normal circulating T4 and T3 levels. In further studies, we identified that fibroblast growth factor receptor-1 (FGFR1) expression and activity are stimulated by T3 in bone. FGFR1 expression and T3-stimulated functional activity was reduced in osteoblasts from TRalpha0/0 mice whereas its expression was increased in bone from TRbetaPV mice. These data suggest that TRalpha0/0 mice exhibit a hypothyroid phenotype in bone, whereas in TRbetaPV mice the phenotype reflects a thyrotoxic skeleton. These data are supported by findings in TRalphaPV mice and indicate that TRalpha is the major functional TR in bone in vivo and suggest that important T3-effects on bone are mediated via a novel pathway involving activation of FGFR1. Genetically modified mice are a powerful resource that will allow further elucidation of the mechanism of T3 action in bone.

IS11

GENETIC CONTROL OF BONE REMODELING: INSIGHTS FROM RARE DISEASES

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Although individually rare, a considerable number and variety of heritable disorders have recently revealed the importance of specific molecules and biochemical pathways in the genetic control of bone remodeling.

Osteoclast biology is profoundly disrupted in the *osteopetroses*. Diminished osteoclast action disturbs skeletal growth, modeling, and remodeling. Persistence of unresorbed primary spongiosa, synthesized during endochondral bone formation, establishes this pathogenetic hallmark. Although animal models show that knockout of many genes could cause human osteopetrosis, deactivating mutations in only 3 genes (*CA II*, *TCIRG1*, *CLCN7*) have been identified thus far in humans and explain most cases. Impaired carbonic anhydrase II, α_3 subunit of a H^+ pump, and chloride channel 7 cause *osteopetrosis/renal tubular acidosis/cerebral calcification, malignant osteopetrosis*, and *Albers-Schönberg disease*, respectively. Notably, all 3 genes function in acid secretion by osteoclasts. Now, excesses of potent bisphosphonates can cause drug-induced osteopetrosis in children. Cure for heritable osteopetrosis following allogeneic marrow cell transplantation from matched donors supports a hematopoietic stem cell origin for osteoclasts. *Pycnodysostosis* is caused by cathepsin K deficiency.

Osteoclast formation and activity are critically regulated by the OPG/RANKL/RANK/NF-κB signaling system. *Familial expansile osteolysis* as well as *expansile skeletal hyperphosphatasia* and *juvenile Paget's disease* feature accelerated skeletal remodeling due to activating and deactivating mutations in *RANK* and *OPG*, respectively. Homozygous deletion of *OPG* in Navajos with JPD proves lethal by young adult life from progressive skeletal insufficiency due to rapid bone turnover.

Osteoblast function is compromised in *hypophosphatasia* and *osteogenesis imperfecta* (OI). In hypophosphatasia, deactivating mutations in *TNSALP* diminish bone alkaline phosphatase activity. Increasingly, inorganic pyrophosphate is incriminated — acting as the key pathogenetic inhibitor of mineralization that must be hydrolyzed extracellularly by TNSALP to enable hydroxyapatite crystal growth. In OI, there are quantitative and often qualitative defects in type I collagen biosynthesis. Marrow cell transplantation has been reported in a few cases to have benefited both osteoblast disorders, perhaps by improving healthy osteoblast numbers after introducing mesenchymal stem cell precursors.

Osteoblast regulation is compromised by deactivating mutations in the *CBFA1* transcription factor causing *cleidocranial dysplasia*. Deactivation of *LRP5* involving wnt signaling causes *osteoporosis/pseudoglioma syndrome*; activation causes a *high bone mass phenotype*.

IS12

MOLECULAR PATHOLOGY OF RENAL CHLORIDE CHANNELS AND CALCIUM METABOLISM

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Disturbances in the renal homeostasis of calcium metabolism may result in hypercalciuria, which is the commonest cause of renal stones (nephrolithiasis), that affect 12% of men and 5% of women by the age of 70 years. The molecular mechanisms and renal transport proteins that regulate urinary calcium excretion remain to be elucidated. The occurrence of hypercalciuric nephrolithiasis as a familial disorder in 45% of patients indicates a genetic basis, and hypercalciuria may occur as a monogenic disorder, or as a polygenic trait involving 3 to 6 susceptibility loci in man and rat, respectively. Studies of some monogenic forms of hypercalciuric nephrolithiasis in man eg. Bartter's syndrome, Dent's disease, autosomal dominant hypocalcaemic hypercalciuria (ADHH) and hypercalciuric nephrolithiasis with hypophosphataemia, have helped to identify a number of transporters, channels and receptors that are involved in regulating the renal tubular reabsorption of calcium. Thus, Bartter's syndrome, an autosomal recessive disease, may be caused by mutations of the bumetamide sensitive Na+-K+Cl- (NKCC2) co-transporter, or the outwardly rectifying renal potassium channel (ROMK), or the voltage-gated chloride channel, CLC-Kb; Dent's disease, which is an X-linked disorder characterised by low molecular weight proteinuria, hypercalciuria and nephrolithiasis, is due to mutations of the voltage-gated chloride channel, CLC-5; ADHH is associated with activating mutations of the calcium-sensing receptor, which is a G-protein coupled receptor; and hypophosphataemic hypercalciuric nephrolithiasis associated with osteoporosis is due to mutations in the type 2a sodium-phosphate co-transporter (NPT2a). These molecular genetic studies have provided valuable insights into the renal tubular pathways that regulate calcium reabsorption and predispose to kidney stones.

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IS13

IDIOPATHIC HYPERPHOSPHATASIA - GENOTYPE & PHENOTYPE

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Idiopathic Hyperphosphatasia (IH) is a rare autosomal recessive bone disease (MIM 239000). Affected children are normal at birth, but develop progressive long bone deformities, fractures, vertebral collapse, short stature, skull enlargement and deafness, with striking radiological changes. However, considerable phenotypic variation exists: some patients present in infancy, others not until later childhood. The earlier the diagnosis is made, the more severe the phenotype. Deformity can progress rapidly during the pubertal growth spurt, so even 'mild' phenotypes can end up with severe deformity, shortening life expectancy. Both plasma alkaline phosphatase activity and the excretion of type I collagen breakdown products are very high, indicating that IH is a disorder of dysregulated bone turnover. Two recent publications have implicated deletion or mutation of the gene TNFRSF11B in the aetiology of IH. TNFRSF11B encodes osteoprotegerin (OPG) an osteoblast-derived peptide that binds to RANK-L, inhibiting the RANK-L/RANK interaction that stimulates osteoclastogenesis. Deficient, or inactive OPG would allow unfettered osteoclastogenesis, and very high bone turnover would result.

We have identified 11 subjects with a clinical diagnosis of IH, from 9 families. In 8 subjects from 6 families we have identified homozygous mutations in TNFRSF11B, and been able to relate the mutations to the putative effect on OPG, and to phenotype. The TNFRSF11B gene has five exons: exon1 encodes a signal peptide, exons 2-3 the ligand (RANK-L) binding region, and exons 4-5 heparin-binding and dimerization domains. The ligand-binding region, crucial for OPG activity, consists of 4 cysteine-rich domains, each with 4 (or 6) cysteine residues forming 2 (or 3) disulphide bonds. Major deletions in the gene result in a severe phenotype, with presentation before the age of 18 months, delayed walking and short stature (<3rd centile). Mutations of the cysteine residues in the ligand-binding domain produce a similarly severe phenotype, but non-cysteine mutations in this region result in a milder phenotype, with later diagnosis (~5 yrs), no delay in walking, and characteristic x-ray appearances. The mildest phenotype seen, with normal stature and minimal deformity, resulted from a deletion/insertion mutation in exon 5.

C1

RIB FRACTURES IN A YOUNG MAN

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A 36yr old man was referred for bone densitometry because of rib fractures. He had pain in the chest, leg and foot for 1 year and been treated for costochondritis and arthritis. He had no children. There was a family history of osteoporosis, but none of rickets. Examination suggested that he was in pain; proximal weakness was not evident. He was tall (height 192cm:Span 202cm). Testes were small, but secondary sex characteristics were unremarkable. No masses were palpable.

Investigations showed Ser Ca 2.3mmol/l, PO4 0.5mmol/l, Alkaline Phosphatase 156 iu/l (1.3xUL normal) and Tubular Reabsorption of Phosphate 55%. Investigations for Fanconi syndrome negative. PTH was 4.7pmol/l (normal), antiendomysial antibodies negative and serum testosterone 7.1nmol/l, LH 2.2 IU/L, FSH 7.1 IU/L. Myeloma screen was negative. Levels of 1, 25(OH)2D3 (27pmol/l; nr 20-120pmol/l) were low, whilst FGF23 was elevated {209 RU/L (nr less than 100)} (courtesy Dr W Fraser, Liverpool). Bone density at L2-4 was 0.665g/cm2 (z score -3.6) and femoral neck 0.485g/cm2 (z score -3.2). 99TcMDP scanning revealed multiple areas of skeletal uptake. 111Indium Octreotide was taken up at the lower end of the left femur. MRI scanning showed a tumour at the lower end of the left femur, and an insufficiency fracture in the medial tibial condyle. Iliac crest bone biopsy showed thick osteoid seams. Lower left femur biopsy showed a tumour, probably mesenchymal, but not neoplastic. Treatment with Calcitriol 500ng/day and Phosphate Sandoz ii/day (limited by diarrhoea) has not affected symptomatology or altered Ser PO4 or Alkaline Phosphatase towards normality. Wide resection of the tumour is planned. Low serum phosphate and elevated alkaline phosphatase values were important pointers to the diagnosis and octreotide scanning was valuable in locating the tumour.

The presence of significant hypophosphataemia and of elevated values of FGF23 associated with a tumour and in the absence of a family history of rickets indicates that this patient probably has oncogenic osteomalacia. Further studies on the resected tumour and follow up biochemical studies should clarify the diagnosis. Recovery following resection would be expected.

C2

CHONDROSARCOMA ASSOCIATED WITH HYPERPARATHYROIDISM: A REPORT OF 2 CASES

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The association between primary hyperparathyroidism and primary malignant tumours of bone is uncommon. There is, however, current interest in this area as intermittent, daily dosing of parathyroid hormone (PTH) in rats has been found to result in osteosarcoma. We report two cases of female patients presenting with chondrosarcoma, where primary hyperparathyroidism was diagnosed preoperatively on the basis of a raised serum calcium. Details of the cases are shown below:

	Case 1	Case 2
Age (yrs)	66	36
Site of chondrosarcoma	Scapula	Tibia
Serum calcium (mmol/l) [normal range: 2.10-2.70]	3.51	2.96
Serum phophate (mmol/l) [normal range 0.70-1.50]	0.57	0.64
PTH (pmol/l) [normal range 0.9-7.3]	28.5	14.0

Both patients underwent surgical neck exploration, and a single enlarged parathyroid gland was identified and removed in each case. This resulted in return of their biochemistry to normal. Their orthopaedic operations were uncomplicated.

The rarity of case-reports in the literature with both hyperparathyroidism and bone tumours may suggest that these two patients represent only a chance association identified in a specialist centre. Alternative explanations include the fact that prolonged exposure to PTH may result in malignant transformation in predisposed individuals. The PTH receptor is expressed in cartilage and PTH-related protein regulates chondrogenesis. The exact onset of hyperparathyroidism in these patients is unclear, although on the basis of symptoms we believe it was some time prior to their diagnosis. There may also be a genetic linkage between the two diseases, as loss of heterozygosity of the proximal long arm of chromosome 10 has been reported in patients with chondrosarcoma. This genomic region also contains the RET oncogene, and mutations of this gene have been identified in the hereditary cancer syndromes of multiple endocrine neoplasia type II (MEN 2). Further genetic analysis is now being undertaken in these two patients to determine if they have an underlying genetic basis to their condition. Screening of first-degree family members is also being conducted.

C3

MOLECULAR DIAGNOSIS OF HYPOPARATHYROIDISM : 2 ILLUSTRATIVE CASES

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We report 2 cases. The first case is a 31 year old male who presented at the age of 10 with cramps and leg spasms. At age 3, he was noted to have nail changes with mucocutaneous candidiasis and patchy hair loss. Relevant history included a family history of scalp and skin problems and Finnish ancestry on the maternal side. On examination, he was of short stature, had brittle nails, partial alopecia and a positive Chvostek's sign. Biochemical investigations revealed a low serum calcium with undetectable PTH. A short synacthen test showed a flat response. A diagnosis of auto-immune polyglandular syndrome (APS) type 1 was made. He was started on 1-alphacalcidol 1.0 mcg/day and replacement with hydrocortisone and fludrocortisone. Genetic analysis revealed a homozygous nonsense mutation in exon 6 (R257X) of the auto-immune regulator gene (AIRE) which changes an arginine codon to a stop codon at amino acid position 257.

The second case is an 18 year old male who failed his hearing screening test at the age of 8 months. This was later confirmed to be sensori-neural in type. At the age of 13, he presented as an emergency with muscular spasms and convulsions. Laboratory tests showed a low serum calcium (1.03 mmol/l), phosphate (1.7 mmol/l) and PTH (6 ng/l, normal range 10-64). A diagnosis of hypoparathyroidism was made. He is now on maintenance treatment with sandocal 1g/day and 1-alphacalcidol 1.0 mcg b.d. Renal investigations revealed no abnormalities. There is no known relevant family history. The findings were suggestive of 'partial' HDR syndrome which consists of a triad of clinical features including hypoparathyroidism, deafness and renal abnormalities. Genetic analysis in our patient revealed a nonsense mutation in exon 6 of the GATA3 gene, a 'C' to 'T' transition at codon 367 which alters the DNA-binding abilities of GATA 3. GATA 3 is a transcription factor which is thought to play an essential role during vertebrate development.

Identification of genes implicated in the pathogenesis of hypoparathyroidism will help in the understanding of the development and function of the parathyroid gland.

C4

SEVERE OSTEOPOROSIS IN TWO YOUNG IMMOBILISED MEN

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Peak bone density is influenced by hormonal, genetic and nutritional factors and is an important determinant of the risk of osteoporosis and fracture. Here, we describe two young men who have established osteoporosis at a young age as a consequence of immobility since infancy, and we discuss some of the practical clinical issues that arise.

RB, now 17 years of age, was referred for bone densitometry and fracture risk assessment following a supracondylar left femoral shaft fracture sustained in an epileptic fit. He had previously fractured the left femur aged 8 years. This young man had loss of movement in all limbs, profound learning difficulties and epilepsy, which was well controlled with sodium valproate and clobazam but had previously required phenytoin. He was wheelchair-dependent since 1996. Investigations confirmed normal bone biochemistry (serum calcium, phosphate, alkaline phosphatase, parathyroid hormone and 25-hydroxyvitamin D), FSH, LH and sex-hormone binding globulin. As serum testosterone was at lower limit of normal (9.6nmol/L), replacement therapy was commenced. Bone densitometry was obtained (only of the lumbar spine with considerable practical difficulty), with a T-score of -4.9. Intravenous pamidronate was recommended because of the difficulties with maintaining an upright posture.

TF is 20 years of age, and was referred following a femoral shaft fracture which occurred during a transfer to a (semi-)upright position. Diagnosed with cerebral palsy in infancy, he could stand for short periods using a frame until surgery for progressive kyphoscoliosis in 1997 was followed by loss of all lower limb function. Recurrent respiratory tract infections necessitated a tracheostomy tube and regular becotide inhaler. Bone biochemistry (as listed above) was normal. However, testosterone was low (3.9 nmol/L). T-score on total hip DXA was -3.9 (surgical implants precluded spine scans). He has been treated with testosterone replacement patches, and with cyclical etidronate and calcium as he is unable to maintain the upright posture recommended for both alendronate and risedronate.

These cases illustrate the importance of weight bearing in attaining peak bone strength, and highlight factors that complicate the management of osteoporosis in young chronically immobilised people.

OC1

LIMITIN, A TYPE-I INTERFERON-LIKE CYTOKINE, INHIBITS OSTEOCLASTOGENESIS WITHOUT AFFECTING MACROPHAGE PROLIFERATION AND FUNCTION

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Recently, limitin, a type-I interferon (IFN)-like cytokine, has been identified. A unique feature of limitin is to selectively suppress B lymphopoiesis via IFN-alpha/beta receptors without myelosuppression. Since ovariectomy of mice increases the numbers of osteoclast precursors and B220-positive pre-B lymphocytes, but not other myeloid cells including macrophages, we hypothesized that if limitin could inhibit osteoclastogenesis, it is a useful agent for inhibition of osteoclastogenesis caused by estrogen deficiency with no adverse effects on myelopoiesis. In this study, we explored this possibility and an involvement of limitin in bone metabolism in vivo.

In cultures of unfractionated bone cells, limitin inhibited osteoclastogenesis induced by 1,25(OH)2D3. We previously reported that purified B220-positive cells differentiate into osteoclasts by coculturing with stromal ST2 cells in the presence of 1,25(OH)2D3. In this culture, limitin suppressed B220-positive cell proliferation, resulting in a decreased osteoclastogenesis. Since IFN-beta has been shown to inhibit osteoclastogenesis from macrophages via IFN-alpha/beta receptors, we next examined the effects of limitin on macrophages. Limitin inhibited osteoclastogenesis induced by soluble RANKL from bone marrow (BM)-derived macrophages and clonal macrophages, RAW264.7 cells. In contrast to IFN-beta, limitin showed no effect on LPS-induced NO production by macrophages as well as their proliferation. The frequency analysis of osteoclast precursors indicated that limitin decreases osteoclast precursors generated by M-CSF from BM cells, while limitin had no effect on macrophage-colony formation induced by M-CSF. Finally, we examined limitin mRNA expressions in vivo. The results showed that limitin mRNA was highly expressed in spleen, thymus, lung, as well as femur, compared to kidney, brain, intestine and liver. Among different parts of femur, limitin mRNA was predominantly expressed in epiphysis, a main site of osteoclastogenesis, compared to flushed-out BM and diaphysis. Interestingly, ovariectomy stimulated limitin mRNA expression in femur, suggesting a possible pathophysiological involvement in bone metabolism.

In conclusion, limitin not only inhibited osteoclastogenesis from B220-positive cells but also inhibited macrophage differentiation into osteoclasts as well as M-CSF-induced generation of osteoclast precursors without suppression of macrophage proliferation and function. Thus, limitin might be useful for clinical usage due to lack of adverse effects as seen for IFN-alpha and -beta.

OC2

MATRIX METALLOPROTEINASE INHIBITION DOES NOT RECAPITULATE THE ACTION OF CHEMICALLY MODIFIED TETRACYCLINES ON OSTEOCLAST LINEAGE CELLS

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Chemically modified tetracyclines (CMTs) are non-antibiotic tetracycline derivatives that inhibit bone resorption in vitro and in vivo. CMT-3 and CMT-8 inhibit enzymes of the matrix metalloproteinase (MMP) sub-family, with IC50's in the range of 0.2 - 50 micromol/litre and the action of these tetracycline compounds on bone has been attributed by others to their MMP-inhibitory activity. We have previously shown that CMT-3 and CMT-8 are potent inducers of apoptosis in mature and culture-derived osteoclasts in vitro, at concentrations that inhibit bone resorption and that would be expected to significantly inhibit MMPs. We are unaware of any literature that implicates MMP inhibition with induction of apoptosis in osteoclast lineage cells. While some reports suggest that TIMP-3 may promote apoptosis through a mechanism independent of MMP inhibition, many reports indicate that TIMP-1, TIMP-2 and other MMP inhibitors are protective against apoptosis and promote proliferation in a variety of cells. We therefore studied whether inhibition of MMPs with BB94, a broad spectrum MMP inhibitor, could recapitulate the apoptosis induced by CMTs in osteoclast lineage cells. We grew osteoclast-like cells from human peripheral blood mononuclear cells stimulated with RANKL and MCSF over 21 days (hOC), and from RAW264.7 cells stimulated with RANKL over 7 days (mOC). Treatment with CMT-3 (20 micromol/litre) for 24 hours led to apoptosis in 81% and 85% of multinucleated cells in hOC and mOC respectively. At this concentration, we observed 25% of control MMP-13 activity in a fluorimetric kinetic assay. In contrast, treatment with BB94 (1 micromol/litre) for 24 hours led to apoptosis in less than 5% of multinucleated cells in hOC or mOC, not significantly different from control cultures. At this concentration, MMP-13 activity was completely inhibited in a fluorimetric kinetic assay. We conclude that MMP-inhibition is not sufficient for the pro-apoptotic action of CMT-3 on osteoclasts.

OC3

ZOLEDRONIC ACID INHIBITS ADHESION OF BREAST AND PROSTATE CANCER CELLS VIA INHIBITION OF PROTEIN PRENYLATION

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Breast and prostate cancer are known to preferentially metastasise to bone, where both are thought to initially induce bone resorption by osteoclasts. Bisphosphonates are potent inhibitors of osteoclasts and have therefore been proposed for use in metastatic bony lesions. Recently these drugs have been shown to have direct effects on cancer cells themselves. The more potent amino-bisphosphonates, such as zoledronic acid (ZA), have been shown to inhibit the mevalonate pathway, which normally allows prenylation of proteins, an important form of post-translational modification.

In this study, we examined effects of ZA on adhesion of human breast and prostate cancer cells. We also investigated how these effects were altered by adding analogues of intermediates in the mevalonate pathway, mixed isomers farnesol (FOH), or all-trans geranylgeraniol (GGOH). We also compared effects with those induced by C3-exoenzyme (C3X), an inhibitor of Rho, which is known to play an important role in cell adhesion. Two breast cancer cell lines, MCF-7 and MDA-MB-231, and two prostate cancer cell lines, DU-145 and PC-3, were used. To test for effects on adhesion, cells were exposed for 24 hours to varying concentrations of ZA before being seeded onto dentine slices and left for a further 24 hours. Cells were then fixed, stained and counted. We then repeated this protocol but also exposed cells for 3 hours to 40microM of either FOH or GGOH prior to addition of 50microM ZA. Effects of ZA were compared to adding 5microg/ml of C3X.

ZA induced inhibition of adhesion in all cell lines. Higher concentrations were required for a significant effect on breast cancer cells (50-100microM) than prostate cancer cells (1microM). In MCF-7 cells, this inhibitory effect on adhesion was rescued by adding FOH, but not GGOH. In DU145 and PC-3 cells, inhibition of adhesion was rescued by GGOH, not FOH. Effects of C3X were examined in MCF-7, DU145 and PC-3 cells. In each, C3X caused a significant inhibition of adhesion.

These results support a role for ZA inhibiting adhesion of breast and prostate cancer cells, and suggest the underlying mechanism involves inhibiting prenylation of proteins, such as Rho.

OC4

VISUALISATION OF THE UPTAKE OF A NOVEL FLUORESECENT BISPHOSPHONATE INTO INTRACELLULAR VESICLES IN RESORBING OSTEOCLASTS AND J774 CELLS

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Bisphosphonates (BPs) are potent inhibitors of bone resorption that act by inhibiting the intracellular enzyme farnesyl diphosphate (FPP) synthase in osteoclasts, resulting in the inhibition of prenylation of small GTP-binding proteins that are essential for osteoclast function. BPs do not readily cross the cell membrane, and the exact mechanism by which they are internalised by osteoclasts remains unclear. To investigate this, we synthesised a fluorescently-labelled bisphosphonate (alendronate; ALN) by conjugation with alexa-fluor 488 succinimidyl ester. Labelled ALN was separated from unreacted fluorochrome by calcium precipitation. The resulting compound (ALN-AF488) retained high affinity for bone mineral, and bound avidly to the surface of dentine discs.

Confocal microscopy was used to examine the uptake of ALN-AF488 by osteoclasts cultured for 24 hours on dentine slices precoated with ALN-AF488. The ALN-AF488 enabled visualisation of the dentine surface, while anti-vitronectin receptor and TRITC-phalloidin staining were used to identify active osteoclasts. ALN-AF488 was internalised by resorbing osteoclasts into distinct, highly fluorescent intracellular vesicles which became dispersed throughout the cell. The presence of labelled vesicles at the basolateral surface suggests that BPs may enter a transcytotic pathway. By contrast, osteoclasts that had not resorbed the dentine surface showed no evidence of uptake of ALN-AF488.

Although J774 macrophages could not internalise bone-bound ALN-AF488, when cultured on coverslips these cells did internalise ALN-AF488 in solution into discrete vesicles throughout the cytoplasm. When cultured in the presence of TRITC-dextran, a marker of fluid-phase endocytosis, most of the ALN-AF488 and dextran co-localised to the same vesicles and were taken up at similar rates. By contrast, Alexa-fluor633-transferrin, a marker of receptor-mediated endocytosis, localised to intracellular vesicles distinct from those containing dextran and ALN-AF488. This indicates that ALN-AF488 is internalised by fluid phase endocytosis. However, the uptake of ALN-AF488, but not that of TRITC-dextran, was almost completely prevented by the presence of a ten-fold excess of clodronate. This suggests the involvement of an additional stage of recognition of BP at the cell surface, prior to internalisation by endocytosis. In summary, these studies provide novel visual insights into the mechanism by which BP drugs are internalised by osteoclasts and other cells in culture.

OC5

DISTINCT REGULATION OF BONE AND MUSCLE MAINTENANCE DURING HINDLIMB SUSPENSION BY A CONCENTRIC RESISTANCE EXERCISE REGIMEN

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Exposure to microgravity and/or spaceflight causes dramatic loss of muscle and bone mass. In normal gravity, resistance exercise has been used to increase muscle and bone mass. We tested a novel form of resistance exercise training (RT) using only concentric force production to offset the loss of musculoskeletal mass during 2 weeks of hindlimb suspension (HS), an unloading model of disuse osteopenia. Male, Sprague-Dawley rats (6-months old; 4 per group) were operantly conditioned to perform RT, and then randomly assigned to groups of sedentary control (CON) or HS, +/- RT (CONRT and HSRT, respectively). Resistance exercise consisted of 2 sets of ~21 repetitions with 100-300 Newtons of concentric force, 3 days/week for 2 weeks (during suspension).

Bone density (BMD) and architecture were analyzed by pQCT and microCT. Using pQCT, HS significantly ($p<0.05$) reduced BMD of trabecular bone in the tibia, which was restored to CON levels with HSRT. Similarly, microCT analysis demonstrated that 2 weeks of HS significantly ($p<0.05$) increased trabecular spacing, and decreased trabecular number, thickness and bone volume (BV/TV) (0.114 +/-0.010) in HS rats compared to CON (0.166+/-0.019). HSRT values for trabecular spacing, number, thickness, and BV/TV (0.145 +/- 0.0124) were indistinguishable from CON rats ($p<0.05$). Further, the trabecular thickness of HS was decreased compared to HSRT.

Surprisingly, this concentric exercise training regimen did not prevent loss of muscle mass during the 2 week HS period. Both soleus muscle weight and muscle weight to body weight ratio in control rats (180.9 +/- 11.3 and 0.366+/-0.011, respectively) were significantly greater ($p <0.05$) than both HS (96.0 +/-5.8 and 0.249 +/-.009, respectively) and HSRT (109.1 +/-15.0 and 0.262 +/- 0.016) Gastrocnemius weights and weight ratios were also not maintained by HSRT.

Together, these data demonstrate that this concentric force production exercise regimen was able to maintain bone mass, but not muscle mass. These data suggest that distinct thresholds of training force exist, and/or the type of exercise regimen (concentric versus eccentric) utilized may distinctly regulate bone and muscle maintenance.

OC6

MEGAKARYOCYTE SYNTHESIS OF OSTEOPROTEGERIN IS STIMULATED BY ESTROGEN WHILST RANKL EXPRESSION IS SUPPRESSED

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High-dose estrogen (E) has been shown to produce anabolic skeletal effects in postmenopausal women and is associated with increased megakaryocyte (MK) population in the bone marrow.

To investigate further mechanisms by which MKs may influence bone remodelling, CD34 positive cells were isolated from cord blood by magnetic bead technology (MACS) and cultured for 6, 9 and 12 days in a collagen-based system plus or minus 100nM 17beta estradiol. Collagen films were dried and cells immunolocalised for osteoprotegerin (OPG), RANKL and CD61, a marker of early megakaryocyte maturation, using an indirect immunoperoxidase technique. Specific protein expression was measured quantitatively by image analysis. Fluorescence-based immunocytochemistry was used to co-localise OPG and RANKL with CD61.

At 6 days, when only very immature MKs were evident OPG expression was suppressed 3-fold ($p<0.01$) in the E-treated cultures whilst RANKL remained at basal levels compared to untreated cells. However by 9 days in the E-treated cultures the maturing MKs, demonstrated by a 2-fold induction of CD61 ($p<0.001$), showed a 2.5-fold ($p<0.01$) increase in OPG expression. RANKL levels were reduced 0.3-fold ($p<0.02$) in cells cultured in the presence of E at this time point. Maximal OPG expression was seen at 12 days with a 3-fold induction of expression ($p<0.001$), whilst RANKL levels were further suppressed by 0.5-fold compared to controls ($p<0.01$). Co-localisation of CD61 with OPG and RANKL at 12 days confirmed expression of these proteins by MKs. OPG staining in the MKs was markedly more intense in the E-treated cultures, whilst there was no difference in RANKL staining between the E-treated and untreated cultures.

We have demonstrated that in vitro, E stimulates the colony forming potential of CD 34 positive cells to a more megakaryocytic phenotype. These maturing MKs show increased OPG and suppressed RANKL expression with E treatment. Our results provide further evidence that megakaryocytes may play a role in bone remodelling and in E-induced changes in osteoclastogenesis and bone resorption.

OC7

MEGAKARYOCYTES STIMULATE OSTEOBLAST SYNTHESIS OF COLLAGEN AND OSTEOPROTEGERIN PROTEIN AND mRNA

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Increasing evidence suggests that megakaryocytes (MKs) may play a role in bone remodelling, possibly by their interactions with cells at the bone surface. To investigate direct effects of MKs on osteoblasts, CD34 positive cells were isolated from cord blood and cultured in liquid medium supplemented with serum, thrombopoietin, IL-6 and IL-3. After 7 days, maturing MKs (CD61 positive cells) were isolated and added to cultures of human osteoblasts from 2 different donors aged 4 months and 6 years. Osteoblasts alone and osteoblasts treated with CD61 negative cells were used as control cultures. After 48 hours in culture, cells were fixed and protein expression for procollagen and osteoprotegerin (OPG) determined by immunocytochemistry using an indirect immunoperoxidase method. Specific protein expression was quantitatively measured by image analysis. Similar cultures were used for RNA extraction and mRNA for Col 1A1 and OPG measured by RT-PCR.

Osteoblasts cultured alone showed high levels of expression of procollagen with 74% (± 7%) of cells staining positively. When cultured with MKs the number of positively staining cells remained similar but the intensity of expression was significantly increased 1.54-fold ($p< 0.02$). OPG was expressed by 32% (± 6.3) of osteoblasts. This increased to 51% (± 5.5) when cultured in the presence of MKs ($p<0.01$) with a 1.63-fold increase in intensity of expression ($p<0.01$). Osteoblasts treated with CD61 negative cells showed no differences in procollagen or OPG expression levels to osteoblasts cultured alone. mRNA data supported the protein findings with a 3.1-fold increase in Col 1A1 expression in the MK treated cultures compared to controls ($p<0.02$). Low level OPG mRNA expression was detected in the osteoblasts which was increased 8.14-fold in osteoblasts cultured in the presence of MKs ($p<0.01$). There were no significant differences between osteoblasts from the two different donors.

These results demonstrate that in vitro MKs have direct effects on osteoblasts. The stimulation of collagen synthesis suggests a direct role in bone formation, whilst the up-regulation of OPG suggests an indirect effect on bone resorption. These data provide further evidence that MKs may play an imortant role in bone remodelling.

OC8

REGULATION OF BONE RESORPTION AND FORMATION THROUGH THE CELL-SURFACE PROCESSING OF RANKL AND JAGGED-1

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The ADAMs (A Disintegrin And Metalloproteinases) have been implicated in cell-surface processing of numerous membrane-anchored proteins. Here we show that the ADAMs, TNF alpha converting enzyme (TACE) and ADAM10, may play central roles in co-ordination of bone remodelling through cell-surface processing of i) RANK ligand during osteoblast-mediated osteoclastogenesis and ii) Notch receptor ligand Jagged-1 during osteoprogenitor differentiation. Specifically, we show that normal human osteoblasts and human osteosarcoma cells (MG63) constitutively express and synthesise several ADAM family members including Meltrin gamma, ADAM10, TACE and Metargidin. Using immunofluorescent techniques we show that Meltrin gamma, Metargidin and TACE are localised on the plasma membrane and within vesicles dispersed throughout the cytoplasm. Over-expression of TACE and ADAM10 by MG63 cells, but not Meltrin gamma or Metargidin caused marked changes in cell morphology and differentiation status of cells. Flow cytometry and pulse chase analysis confirmed that several cell surface proteins were released from the surface of TACE or ADAM10 over-expressing cells including TNF alpha, TGF alpha, Jagged-1 and RANKL. Cell-surface processing of RANKL and Jagged-1 involves cleavage close to the plasma membrane, which releases the extracellular domains of the ligands. Treatment of MG63 cells and primary rat calvaria-derived cell cultures with media conditioned by TACE or ADAM10 over-expressing cells increased alkaline phosphatase activity and the formation mineralised nodules in vitro in the same way as a synthetic ligand homologous to the ligand binding domain of Jagged-1. Furthermore, osteoblasts in co-cultures infected with adenoviral constructs of TACE or ADAM10 promoted a significant decrease in osteoclastic bone resorption, consistent with the reduced activity of soluble RANKL compared with cell-associated RANKL previously demonstrated.

The similar effects of ADAM-10 and TACE suggest that either they function in a physiological regulatory capacity under control of some specific spatial/temporal cues, or that specific physiological regulation of processing of Jagged-1 and RANKL involves related but as yet unidentified ADAMs. These findings show that cell-surface processing of ligands by ADAMs can alter the location, efficacy and mode of action of osteotropic ligands. Through this mechanism, ADAMs may play a pivotal role in the co-ordination of the bone remodeling process.

OC9

INNOVATIVE BIOMINERALISED POLYSACCHARIDE TEMPLATES FOR HUMAN BONE CELL AND GENE DELIVERY

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The clinical need for biodegradable materials, with broad applicability, is evidenced by the fact that tissue loss as a result of injury or disease provides reduced quality of life for many at significant socio-economic cost. We describe the development of innovative microcapsule scaffolds based on chitosan and alginate tailored to musculoskeletal regeneration with potential application to a number of human cell types for a variety of tissues.

Semi-permeable polysaccharide microcapsules were produced by a one-step method, in which the deposition of a semi-permeable alginate/chitosan membrane around droplets of sodium alginate was coupled with in-situ precipitation of amorphous calcium phosphate as described by Leveque et al (2002)#. Nucleation of calcium phosphate could be controlled by the phosphate concentration in the alginate droplets to produce capsules of varying mechanical strength and permeability. Hybrid spheres (750-10,000mm) were generated encapsulating primary human bone marrow cells; STRO-1 selected osteoprogenitors (using magnetic activated cell sorting) and isolated articular chondrocyte populations. The potential for gene delivery and growth factor delivery was assessed using adenoviral green fluorescent protein AdGFP transfected osteoprogenitors and rhBMP-2. Encapsulated cells remained viable within polysaccharide microcapsules for over 2 weeks as shown by positive alkaline phosphatase staining of encapsulated cells. Thin-walled capsules split and degraded in vitro within 4 days releasing viable osteoprogenitor cells as assessed by alkaline phosphatase activity. Cells expressing GFP, within microspheres, were observed using photomicroscopy indicating the ability to deliver cells, factors and selected genes. Encapsulation and delivery of active BMP-2 was confirmed using the promyoblast cell line C2C12 known to be exquisitely sensitive to BMP-2. Finally aggregation of the polysaccharide microspheres into extended frameworks was achieved using a designed droplet/vapour aerosol system resulting in foams of aggregated beads.

These composite scaffolds offer stable mechanical and chemical biomimetic environments conducive to bone cell function. These natural polysaccharides are also highly amenable to complexation with a range of bioactive molecules to allow molecular level biomimicry and consequently offer tremendous potential in tissue engineering and regeneration of not only hard tissues but soft tissues as well.

ref:- #Leveque, I., Rhodes, R., Mann, S. Biomineral-inspired fabrication of semi-permeable calcium phosphate-polysaccharide microcapsules. J. Mater. Chem.,2002; 12; 2178-2180.

OC10

RETARDATION OF LONGITUDINAL BONE GROWTH BY GLUCOCORTICOIDS IS REVERSED BY IGF-I IN FOETAL MOUSE METATARSAL ORGAN CULTURES

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Glucocorticoids (GC) are used extensively in children and may cause growth retardation. This may be a consequence of direct interactions between GC and growth plate chondrocytes and involve disruption of the chondrocyte IGF-I signaling pathway. In this present study we have used embryonic mouse metatarsals to study the effects of dexamethasone (Dex) on bone growth and to determine if any adverse effects can be ameliorated by IGF-I . 18-day-old fetal metatarsals were cultured in triplicate for 12 days in serum-free medium supplemented with either Dex 10-4M, IGF-I (100ng/ml) or both. Total metatarsal growth was recorded every second day as a percent change from their initial length at harvesting (day 0). Dry weight and [3H]-thymidine uptake (dpm/microgram dry weight) were determined on day 12.

Dex caused a decrease in metatarsal length of 22% and IGF-I an increase of 23% compared to control bones ($p<0.05$), which increased by 84% over the 12-day period. In addition, IGF-I treatment showed a significant acceleration in linear growth from day 2 (40% compared to 24% in controls)($p<0.05$), whereas Dex treatment caused a reduction in longitudinal growth from day 10 ($p<0.05$). No significant differences in bone width were detected between the treatment groups. Dex had no effect on dry weight (82 ± 5.3 micrograms); whereas IGF-I and IGF-I/Dex treated bones were significantly heavier (152 ± 5 micrograms and 148 ± 2.3 micrograms, respectively) than controls (84 ± 2.0 micrograms)($p<0.05$). [3H]-thymidine uptake was significantly reduced with Dex ($p<0.05$), which may explain the reduced linear growth. However, IGF-I also caused a decrease in [3H]-thymidine uptake ($p<0.05$), which possibly reflected the slowing of growth at day 12 compared to the controls. The combined effects of Dex and IGF-I were intermediate on all parameters suggesting that IGF-I partly ameliorated the effects of Dex.

The results indicate that Dex and IGF-I had opposite effects on longitudinal bone growth. Further, IGF-I rapidly promoted bone growth whereas the adverse Dex effects were dependent on duration of exposure. The amelioration of Dex induced growth retardation by IGF-I may offer potential as a therapeutic approach to counter GC induced growth retardation in children.

OC11

THE EFFECTS OF DIETARY IMPROVEMENT ON BONE METABOLISM IN ELDERLY UNDERWEIGHT WOMEN WITH OSTEOPOROSIS : A RANDOMISED CONTROLLED TRIAL

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Malnutrition in elderly people contributes to osteoporosis and fracture. The aim of the study was to investigate the effects of nutritional improvement on bone metabolism in elderly community-dwelling women. A 12-month randomised controlled trial of 71 ambulant women aged >70 years with BMI < 21 kg/m2 and osteoporosis at the hip was undertaken. They received either calcium (1 g) and vitamin D (800 units of cholecalciferol) only (Group 1 :n =35) or calcium/vitamin D and nutritional supplements (Group 2 : n = 36). Body composition and bone mineral density (BMD) were assessed at baseline and 12 months. Biochemical markers of bone turnover were measured at 1, 3, 6, 9 and 12 months. Group 2 gained significantly more weight (mean [SD]) Group 1 : 0.15 [2.45], Group 2 :2.66 [2.8] kg $p < 0.001$) and fat mass (Group 1 : - 0.26 [1.8], Group 2 : 1.9 [1.7] kg $p < 0.001$). BMD at the spine, femoral neck and total hip did not change significantly, although there was a positive trend at the total hip in Group 2 (Group 1 : -0.5 [5.2], Group 2 : 1.25 [3.3] %, p =0.13). A significant reduction in serum CTX, a marker of bone resorption, was seen in Group 2 (% decrease at 3 month, Group 1 : 1 [8.7], Group 2 : 32 [5.8], $p < 0.01$). Serum osteoprotegerin (OPG) increased significantly in Group 2 with a maximal increase (27%) observed at 6 ($p < 0.01$) and 9 months ($p < 0.05$). Bone-specific alkaline phosphatase did not change significantly, although a small increase was seen at 12 month (% increase Group 1 : 5 [5], Group 2 : 17 [6], p=0.05). Serum osteocalcin increased at 12 month in Group 2 (p = 01). Dietary improvement in elderly women with low BMI is associated with a reduction in bone resorption with a small but 'net' positive effect on bone formation.

OC12

CHANGES IN BONE MASS FOLLOWING TIBIAL SHAFT FRACTURE

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Regional bone loss is a recognised occurrence following a fracture, but the exact pattern of these changes following tibial shaft fractures is unclear. Early weight bearing may have a protective effect on this bone loss. We aimed to 1) investigate the changes in bone mineral density (BMD) and quantitative ultrasound (QUS) following tibial shaft fracture 2) determine the effect of weight bearing and 3) investigate impaired fracture healing on bone loss.

Eighteen subjects (16 men, 2 postmenopausal women; mean age 34, range 18 to 78), treated with either cast or intramedullary nail, were recruited following tibial shaft fracture. BMD measurements of the tibia and hip were made using dual-energy X-ray absorptiometry (DXA) and of the tibia using peripheral quantitative computed tomography (pQCT). QUS was measured at the calcaneus. Measurements were made in both fractured and non fractured limbs in the first 2 weeks and at weeks 8, 12 and 24 following fracture.

Six subjects (33%), treated with a nail had radiologically determined delayed union. Eight subjects bore weight early (within 6 weeks) and seven subjects bore weight late (after 12 weeks).There was a significant decrease in BMD at the tibia and hip in the fractured limb with the biggest losses occurring at week 24 in the trabecular rich metaphyseal regions of the tibia (26 to 32%, $p<0.001$), the trochanter region of the hip (10%, $p<0.001$) and in bone ultrasound attenuation of the calcaneus (13%, $p=0.01$) measured using QUS. There was no difference in proximal femur or calcaneal BMD between those 1) with delayed and normal union or 2) who bore weight early and late.

An increased amount of bone loss was seen at the distal tibia in subjects treated with a cast (32%), compared to those treated with a nail (18%). The increased freedom of movement and mobility in patients treated with a nail appears to limit regional bone loss, when compared to those treated with a plaster cast, despite the fracture requiring longer to heal.

OC13

DOES LOW MAGNITUDE, HIGH FREQUENCY MECHANICAL LOADING IMPROVE DIAPHYSEAL STRENGTH OF LONG BONES?

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We have previously reported that low magnitude high frequency loading treatment resulted in 20.5% net increase in the proximal tibia volumetric trabecular bone mineral density in children with disabling conditions (1). In this pilot randomised controlled trial (RCT), 20 pre-or post pubertal disabled but ambulant children were randomised to standing on active (n=10; 0.3G, 90Hz) or placebo (n=10) devices (Exogen Optimass Device, Smith & Nephew) for 10 minutes/day, 5 days/week for 6 months. We now report the results of tibial cortical changes in these children.

Quantitative computer tomography (Philips SR-4000) was used to acquire a 3D scan of the tibia (90mm block); BonAlyse (v1.3) was used for image analysis. In each subject, measurements were made at the same cortical site approximately 25-50% from the proximal epiphysis at baseline and 6 months. The primary outcome was bone circumference.

Data were analysed using the same strategy as previously, adjusting for weight, height, disability category, pubertal status, calcium intake, baseline vTBMD and number of days in the study.

The changes in whole bone circumference showed a trend towards increases in the treatment group compared to controls, however after controlling for covariates this difference was not significant ($p=0.16$). This result may partly be due to the large imbalance of pubertal stage between the treated and untreated groups (mean difference in bone circumference = 6.24mm, SE 1.78mm, $p<0.01$). ANCOVA analysis in the untreated group was used to investigate whether all of this difference was due to pubertal stage or whether some of the positive effect was due to treatment. The analysis suggests that the difference in pubertal stage between the groups does not completely account for the difference in outcome (mean diff = 2.7mm, SE 1.31mm, $p=0.074$), suggesting a treatment effect.

The results of this pilot RCT suggest that low magnitude high frequency loading treatment might have the potential of improving bending strength of the diaphysis of long bones by increasing the bone diameter through periosteal expansion. Further larger RCTs of this novel physical treatment are required in a more homogenous group of children.

(1) Ward, K. et al. Osteoporos Int 12, S9 (2001).

OC14

BIOCHEMICAL MARKERS OF BONE TURNOVER ARE ASSOCIATED WITH RISK OF BONE METASTASES IN WOMEN WITH PRIMARY OPERABLE BREAST CANCER

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It is hypothesised that increased bone turnover may be associated with a higher risk of bone metastases in breast cancer. We have examined the relationship between bone biochemical markers and metastatic risk in women with primary operable breast cancer.

We studied 560 women within a double-blind, randomized, placebo-controlled study of adjuvant clodronate treatment to prevent bone metastases in patients receiving local and/or systemic therapy. The women received two years of treatment with clodronate 1600mg daily (n=277) or placebo (n=283) and were followed for a median of 5.5 years. Samples were collected at annual intervals for the assessment of serum bone specific alkaline phosphatase (BAP, mg/ml), the amino-terminal propeptide of type I collagen (PINP, mg/l) and carboxyterminal telopeptide of type I collagen (ICTP, mg/l).

The treatment groups were well matched at entry with respect to menopausal status, hormone receptor status and cancer management. None of the biochemical markers measured at baseline predicted future metastatic risk. Furthermore, in the placebo group, values for each of the markers were similar at 12 and 24 months in the women who subsequently did or did not develop bone metastases. In contrast, within clodronate treated women, serum PINP was significantly elevated at 12 and 24 months in women who developed bone metastases (median, interquartile range) (30.0, 23.0-56.0 vs. 24.0, 15.0-38.25, $p=0.041$ and 35.5, 23.25-65.0 vs. 25.0, 16.0-36.0, $p<0.001$ respectively). Similar results were obtained at 24 months for BAP (10.5, 8.00-14.75 vs. 8.0, 7.0-11.0, $p<0.001$) and ICTP (3.65, 2.83-4.5 vs. 3.10, 2.7-3.8, $p=0.022$). These differences persisted even when women developing clinically evident bone metastases during the first two years were excluded from the analysis. The decreases in biochemical markers and metastatic risk were greater in women who were postmenopausal at diagnosis compared to those who were premenopausal.

These data suggest that a decrease in bone turnover during therapy is associated with a lower risk of metastasis. Biochemical markers of bone turnover may play a role in determining a satisfactory response to treatment and may prove of value in monitoring women with primary breast cancer during anti-resorptive therapy.

OC15

THE RELATIONSHIP BETWEEN BONE DENSITY BONE SIZE AND FRACTURE SYNDROMES IN POSTMENOPAUSAL WOMEN

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A previous study demonstrated that women with vertebral fractures have smaller vertebrae with less bone within the bone envelope. The relationship between bone size, bone density and non-vertebral fractures remains unclear.

We recruited women (aged 55 - 80 years) who had sustained hip (48) or vertebral (66) fractures. These were compared to a population-based sample of 490 women (aged 55 - 80 years) from the Sheffield cohort of the Osteoporosis and Ultrasound Study (OPUS). We measured PA lumbar spine L1 - L4 bone area (BA, cm2) and bone mineral content (BMC, g) using DXA (Hologic QDR 4500A). BMC was corrected for bone size using BMAD (g/cm3), calculated as BMC/bone volume (BV, cm3), where BV = BA1.5. Z-scores were calculated to correct BMC, BV and BMAD for age or height. The fracture groups were compared to the postmenopausal OPUS group using one-way ANOVA with a Bonferroni correction.

Subjects with hip fracture had reduced BMC for age and height (mean (SD) ZSc -0.5 (0.99) and -0.64 (0.99) respectively, $p<0.05$). This reduced BMC was due to a reduced BMAD for age and height (mean (SD) -0.79 (0.92) and -0.88 (0.89) respectively, $p<0.05$), but not a reduced BV, either for age or height (mean (SD) ZSc 0.00 (1.44) and 0.20 (2.24) respectively).

Subjects with vertebral fracture were both short and light for their age compared to postmenopausal controls (mean (SD) ZSc -0.33 (1.0) and -0.41 (1.06) respectively, $p<0.05$). They had reduced BMC for age and height (mean (SD) ZSc -1.15 (0.88) and -1.14 (0.84) respectively, $p<0.05$). This was the result of both a reduced BV for age and height (mean (SD) ZSc -0.59 (1.21) and -0.54 (1.33) respectively, $p<0.05$), and BMAD for age and height (mean (SD) ZSc -1.01 (1.0) and -1.03 (0.99) respectively, $p<0.05$).

In conclusion both fracture types were associated with reduced BMC and BMAD after adjusting for age or height. However, subjects with vertebral fractures also had reduced bone volume. This may suggest bone size has a greater contribution to the risk of fracture at sites that are predominately trabecular bone.

OC16

PLEIOTROPHIN - A NEW INHIBITOR OF BMP-MEDIATED OSTEOINDUCTION: POSSIBLE RELEVANCE TO FIBRODYSPLASIA OSSIFICANS PROGRESSIVA?

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Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease, in which major striated muscles are progressively replaced by bone, frequently following trauma. The causes relate to a dysregulation of the BMP pathways of osteoinduction, but no defects have been identified in the genes for BMPs, BMP-receptors or noggin, a BMP-inhibitor. Hence the disease remains a clinical and biological mystery.

We have previously investigated the roles of pleiotrophin (PTN), a putative osteotrophic factor, during bone development. PTN was produced by osteoblasts, stored in bone matrix and was chemotactic for osteogenic cells, suggesting a role in bone remodelling(1,2,3). PTN also enhanced osteogenic differentiation of marrow-derived stromal cells at pg/ml concentrations(1,3). The aims of this study were to determine whether PTN was osteoinductive and/or whether it influenced BMP-mediated osteoinduction. To test for osteoinduction, we used pro-myoblast C2C12 cells, which are exquisitely sensitive to BMPs. In the presence of 100 ng/ml BMP-2, ~50% of C2C12 cells became positive for alkaline phosphatase (ALP) after two days, indicative of osteoinduction. By contrast, PTN failed to induce ALP activity, demonstrating that PTN was not osteoinductive. When C2C12 cells were co-treated with PTN+BMP-2, PTN inhibited the BMP-mediated osteoinduction by ~80%, an effect that was apparent at concentrations of PTN as low as 0.05 pg/ml. However, when PTN was added to C2C12 cells after the osteoinduction by BMP-2 had taken place, PTN stimulated further osteogenic differentiation, suggesting that the window of PTN inhibition of osteoinduction was critical. Examination of PTN expression in skeletal muscle demonstrated high levels of PTN protein in muscle, consistent with a possible negative regulation by PTN of osteogenesis in muscle.

These serendipidous findings identified PTN as a new inhibitor of BMPs, which might be of considerable importance in FOP. PTN binds to cell-surface heparan-sulfate proteoglycans (HSPGs) and may act as accessory protein or co-factor for primary signalling factors(1,2). BMPs and BMP-antagonists also bind to HSPGs, hence the interactions between BMPs and their inhibitors with cell-surface HSPGs prior to receptor binding warrant further investigations, which may lead to finding the causes of FOP.

1.J Bone Miner Res(2002)17:2009-2020. 2.Biochem Biophys Res Comm(2002)298:324-332. 3.J Bone Miner Res(2003)18:47-57

OC17

VITAMIN D RECEPTOR GENE POLYMORPHISMS: ASSOCIATION WITH BONE DENSITY AND OSTEOPOROTIC FRACTURES: A CASE CONTROL STUDY

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Osteoporosis has a strong genetic component and vitamin D is essential for normal bone metabolism. Polymorphisms of the vitamin D receptor (VDR) gene have thus been studied extensively in relation to bone mass. However there have been fewer studies powered to detect associations between VDR genotype and osteoporotic fracture. The aim of this study was to examine the relationship between BsmI and FokI VDR polymorphisms and osteoporotic fracture.

We studied 719 postmenopausal women ages 55 to 80 (mean 68) years. Of these, 265 women had sustained a distal forearm (79), humeral (69), vertebral (66) or hip (51) fracture. The remainder was a population-based sample of 454 postmenopausal women from the Sheffield centre of the osteoporosis and ultrasound study (OPUS) study. Bone mineral density (BMD) was measured by DXA (Hologic QDR 4500A) at the lumbar spine (LSBMD) and total hip (THBMD). BsmI and FokI polymorphisms were assayed using an ABI Prism 7200 Sequence Detection System (Taqman). After correction for multiple comparisons, $P < 0.008$ was significant at the 95% confidence interval.

LSBMD was significantly lower ($P<0.0001$ ANOVA) in the fracture cohort (Mean 0.794; (SEM 0.157) g/cm2) compared to the population-based cohort (1.054 (0.187) g/cm2). THBMD was significantly lower ($P<0.0001$) in the fracture cohort (0.752 (0.139) g/cm2) compared to the population-based cohort (0.906 (0.145) g/cm2). There was no difference in the frequency of BsmI and FokI genotypes between fracture and control groups ($P > 0.05$, ChiSq analysis). Fracture, age and weight, but not genotypes were significant factors in relation to LSBMD and THBMD in the whole group by multiple regression analysis. Haplotype analysis demonstrated that the bbFF haplotype had significantly higher LSBMD when fracture subgroups (upper limb, vertebral and hip) were analysed (All $P < 0.004$). There was a similar trend for THBMD, but this did not reach significance after correction for multiple comparisons (All $P<0.05$). BsmI and FokI polymorphisms, individually or in combination, did not predict fracture independent of BMD by logistic regression.

In conclusion VDR polymorphisms are not related to osteoporotic fracture in this cohort of elderly women. However our results suggest that individuals with the bbFF haplotype may have a higher bone density in the region of the lumbar spine.

OC18

C-FOS BLOCKS CARTILAGE DIFFERENTIATION IN VITRO BY INHIBITING THE EFFECTS OF BONE MORPHOGENETIC PROTEIN (BMP)-2 AND -4

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The c-fos proto-oncogene, a member of the AP-1 transcription factor, plays an important role in bone and cartilage differentiation as shown in gain/loss-of-function studies in mice. To study the role of c-Fos in greater detail, we have generated an in vitro inducible c-Fos system in ATDC5 chondrocytes (e.g. clone DT12.4), and have shown that exogenous c-Fos inhibits chondrocyte differentiation, although the mechanisms are not known. We have previously hypothesised that c-Fos may act downstream of growth factor signalling to regulate chondrogenesis. To this end, we have now investigated the effects of BMPs on cartilage differentiation and gene expression in DT12.4 cells as well as in primary chondrocytes isolated from c-Fos mutant mice.

We first investigated the effects of exogenous BMP-2 and BMP-4 on in vitro differentiation of DT12.4 cells in the absence or presence of exogenous c-Fos. BMP-2 and -4 caused a dose-dependent increase in cartilage nodule formation as assessed by Alcian blue staining. In addition, BMP-2 and -4 rescued the c-Fos-dependent inhibition of differentiation, although higher doses of BMPs were required to restore cartilage differentiation in the presence of c-Fos. RT-PCR analysis for collagen types II and X expression confirmed that higher BMP concentrations were required to stimulate these differentiation markers in the presence of c-Fos. These results suggest that overexpression of c-Fos in chondrocytes results in a decreased responsiveness to BMP-2 and BMP-4 stimulation. We further investigated this by examining BMP responsiveness in primary chondrocytes from c-Fos knockout mice. BMP-2 treatment of c-Fos null chondrocytes resulted in a higher number of Alcian blue-positive cartilage nodules compared to wild-type controls, confirming that c-Fos may negatively regulate BMP signalling in chondrocytes. To identify a possible mechanism, we performed Northern blot analysis on DT12.4 cells and showed that induction of c-Fos inhibited the expression of endogenous BMP-4 mRNA during the early phases of DT12.4 cell differentiation.

Taken together, these experiments suggest that c-Fos interferes with cartilage differentiation by disrupting the autocrine regulation of chondrogenesis by BMP signalling. We are currently testing this hypothesis by analysing the effects of BMP-2 and -4 on ex vivo limb cultures of c-Fos mutant mice.

OC19

POLYMORPHISMS IN THE P450 C17 (17-HYDROXYLASE/17,20-LYASE)AND P450 C19 (AROMATASE) GENES : ASSOSIATION WITH SERUM SEX STEROIDS CONCENTRATIONS AND BONE MINERAL DENSITY IN POST-MENOPAUSAL WOMEN

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Genes involved in the synthesis of androgens and estrogens are potential candidate genes for osteoporosis. The CYP 17 and CYP 19 genes encode 2 cytochrome P450 enzymes ; 17- hydroxylase/17,20 lyase and aromatase respectively, involved in the sex hormone biosynthetic pathway. We investigated the association between 2 common polymorphisms in (1) the promoter region ('T' to 'C' substitution) of CYP 17 and (2) exon 3 ('G' to 'A')of CYP 19, bone mineral density (BMD) and serum androgen / estradiol concentrations in 252 post-menopausal women aged (mean [SD] 64.5 [9.2] years). Genotypes frequencies did not deviate from Hardy-Weinberg equilibrium. In a multiple linear regression analysis model, a significant association was seen between BMD values at the femoral neck and total hip with CYP 17 but not CYP 19 genotype in the whole study population. Subjects with the 'CC' genotype had significantly lower BMD (mean [SD} 'TT': 0.7 [0.16], 'CC' : 0.6 [0.08] g/cm2 , $p = 0.006$). The CYP 17 genotype accounted for approximately 1.2 % of the variance in femoral neck BMD. In women > 10 years since the menopause, those with the CYP 19 'GA' and 'GG' genotypes had lower BMD at the hip and an increased prevalence of fractures than those with the 'AA' genotype. This effect was dependent on estradiol concentrations as CYP 19 genotype was significantly associated with serum estradiol ($p =0.002$). Women with the 'AA' genotype had significantly higher serum estradiol concentrations compared to the 'GG' genotype ($p= 0.03$). We found no significant association between CYP 17 genotype and serum androgens and estradiol concentrations, suggesting that a different mechanism may be implicated in its effect on BMD, possible through the glucocorticoid synthetic pathway. In conclusion, both CYP 17 and CYP 19 are candidate genes for osteoporosis in post-menopausal women.

OC20

EPIDEMIOLOGY OF FRACTURES IN BRITISH RACEHORSES IN TRAINING

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Musculoskeletal injuries are a major cause of morbidity, mortality and wastage in racehorses. The aim of this observational study was to estimate the incidence of fractures sustained by UK racehorses in training and to determine associated risk factors, relating to the horse and its training regime.

Thirteen racehorse trainers participated in the study and horses were studied for a two-year period. Horse and daily training data were collected, including type of exercise, distance and track surface. A case was defined as any animal with a fracture, confirmed by appropriate diagnostic methods (excluding small osteochondral fragments). Training intensity was quantified as the total cumulative distances exercised at canter (10-13 m/s) and gallop (13-17m/s) in 30- and 60-days periods prior to fracture in a nested case-control study, with cases and controls matched on date of fracture of the case. Multivariable conditional logistic regression methods were used to estimate the effect of training intensity on the risk of injury.

1178 Horses provided 386,803 days at risk and 148 fractures were included for analyses. 78% of fractures occurred in training, 22% while racing. 58% of injuries were stress fractures. The overall fracture incidence was 1.15 per 100 horse months (95% CI: 0.97, 1.35), with a higher incidence rate for racing fractures (18.74 per 100 horse months, 95% CI: 12.91, 26.28) compared to fractures occurring during training (0.90 per 100 horse months, 95% CI: 0.75, 1.09). A strong interaction existed between the cumulative distances cantered and galloped in both the 30- and 60-day periods, with horses both cantering and galloping larger distances being at particularly increased risk of fracture. The risk of injury varied between trainers and according to type of fracture.

This is the first large-scale study to describe fracture occurrence and associated risk factors in UK racehorses in training. The high proportion of stress fractures and substantially higher incidence of racing-related fractures suggest that training regimes often do not engender appropriate adaptive changes in bone mass and architecture to prevent injury during competitive performance. The results of this study provide a scientific basis for developing racehorse training regimes that may reduce fracture incidence.

OC21

ER ALPHA ACTIVATES THE BMP-6 PROMOTER IN BONE AND BREAST CELLS VIA DISTINCT MECHANISMS

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Although anti-estrogens are known to stimulate target genes in certain tissues such as bone, the molecular basis for this tissue selective action is unknown. To investigate this further, we examined whether estrogens or anti-estrogens regulate the bone morphogenetic protein 6 (BMP-6) promoter differently in cells derived from distinct tissues. Upon sequence analysis of the BMP-6 promoter, no consensus oestrogen response elements were identified, but a number of putative AP-1 regulatory domains were present. We generated a 4.3Kb fragment of the 5' flanking region of the BMP-6 gene (encompassing multiple putative AP-1 binding sites) and created a reporter construct in which this fragment of BMP-6 promoter controlled expression of the luciferase gene. Cells were transiently transfected with the reporter construct and an expression plasmid for human oestrogen receptor (ER) alpha before subsequent to exposure to ligand and assessment of luciferase activity. Data was corrected for transfection efficiency following analysis of beta-galactosidase control activity within each sample. In osteoblast-like cells (ROS 17/2.8, MG63 and SaOS-2), significant stimulation of luciferase activity was observed relative to vehicle-treated cells in response to ICI 182,780 (ICI) ($P<0.0001$, as assessed by One-Way ANOVA) but not 17beta-oestradiol (E2). In contrast, human MCF-7 and T47D breast cancer cells and HepG2 hepatoma cells showed no response to ICI but significant stimulation following E2 treatment ($P<0.0001$, E2 vs. vehicle treated cells as assessed by One-Way ANOVA). We then compared the pathways involved in ERalpha-dependent stimulation of BMP-6 reporter activity in response to ICI and E2 in MG63 and MCF-7 cells respectively, by repeating these studies using different ER mutants. We found that both pathways are dependent on ERa as opposed to ERb, and require the presence of the ERalpha AF-1 activation domain. However, in contrast to the stimulatory response of MCF-7 cells to E2, the response of MG63 cells to ICI was AF-2 independent. In conclusion, we have found that although ERalpha stimulates the BMP-6 promoter in a variety of cell types, the ligand-specificity and AF-2-dependence of this response differs according to tissue of origin, which may provide a molecular basis for the tissue selective action of anti-estrogens.

OC22

NEURONAL NOS IN ADULT HUMAN CORTICAL OSTEOCYTES

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Until now, eNOS has been considered to be the predominant osteocytic NOS isoform in bone. We previously studied the distribution of eNOS protein expression in the human femoral neck because of its possible involvement in the response to load. Studies in rat and human fracture callus have shown that nNOS mRNA is expressed sometime after fracture but no study has yet immunolocalised the nNOS isoform in mature adult human bone. In this study, we have analysed the density and distribution of eNOS and nNOS isoforms in iliac and femoral neck human osteocytes.

Ten micron frozen sections were cut from 8 transiliac biopsies from osteoporitic patients and from 7 post-mortem femoral neck biopsies (age range=56-80 y). Sections were incubated overnight in antiserum for eNOS and nNOS followed by peroxidase/VIP substrate detection. We used eNOS antisera directed against the C-terminus. For nNOS three different antisera were used, two binding to different C-terminal epitopes and one binding to a N-terminal epitope. Sections were then incubated in propidium iodide or methyl green to detect all osteocytes. eNOS antibody was able to detect eNOS epitopes in osteocytes. All three nNOS antibodies detected nNOS epitopes in osteocytes but those directed against the C-terminus had higher detection rates. The percentage of osteocytes positive for nNOS was higher than that for eNOS in both sites (iliac crest: nNOS 84.04%, eNOS 61.78% $p<0.05$, femoral neck: nNOS 60.98%, eNOS 40.41% $p<0.05$). Given that nNOS isoform is expressed in over 50% of osteocytes, some osteocytes must express both NOS isoforms. In other tissues, both NOS isoforms have been shown to be presence in different intracellular compartments. Further investigation of the location of NOS isoforms in osteocytes is needed to identify its targets. Studies of NOS isoforms expression in human osteocytes and their relation to bone remodelling are currently being undertaken.

In conclusion, both eNOS and nNOS are present in osteocytes but the nNOS isoform is more prevalent.

OC23

BIOMIMETIC MICROENVIRONMENTS - STIMULATION OF HUMAN OSTEOPROGENITOR DIFFERENTIATION BY A SYNTHETIC PEPTIDE COLLAGEN BINDING DOMAIN: P-15

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The formation of biomimetic microenvironments that exploit extracellular matrix cues for mesenchymal cell differentiation offers tremendous potential for skeletal regeneration. Type-1 collagen provides a structural framework for connective tissues and plays a central role in the temporal cascade of events leading to the formation of new bone from progenitors. This study has examined the ability of a synthetic 15-residue peptide, P-15, related biologically to the active cell binding domain of type I collagen, to promote human osteoprogenitor attachment, proliferation and differentiation on 3-D scaffolds.

Human osteoprogenitors were cultured on particulate anorganic bone mineral (ABM) and polyglactin vicryl mesh coupled with or without P-15 in basal (aMEM/10% FCS) or osteogenic (aMEM/10% FCS / 10nM dexamethasone and 100uM ascorbate-2-phosphate) conditions. Immobilized and soluble P-15 increased alkaline phosphatase activity and bone morphogenetic protein-2 (BMP-2) gene expression after 1 and 5 days as determined using real time PCR. Soluble P-15-induced BMP2 expression was significantly lower than that induced by immobilized P-15. After 10 days, no significant differences in gene expression between soluble and immobilized P-15 constructs were observed. P-15 coupled ABM and polyglactin mesh promoted human osteoprogenitor cell attachment, spreading and patterning over 5-24 hours compared to culture on ABM & vicryl mesh alone as observed by light, SEM and confocal photomicroscopy using fluorescent labels.

P-15coupled ABM increased alkaline phosphatase specific activity, cell proliferation as assessed by thymidine incorporation and DNA synthesis in basal and osteogenic cultures in the presence of P-15. The presence of mineralised bone matrix and extensive cell ingrowth and cellular bridging between 3-D ABM matrices and polyglactin vicryl mesh adsorbed with P-15 was observed by alizarin red and von Kossa staining and SEM. In contrast, negligible cell growth was observed on ABM or mesh alone. In vivo diffusion studies using MF1nu/nu mice showed bone matrix formation and organised collagen formation as assessed by Sirius red/ alcian blue staining and collagen birefringence after 6 weeks.

The collagen peptide, P-15 provides a permissive biomimetic microenvironment for osteoprogenitor function and demonstrates the potential for the exploitation of extracellular matrix cues for osteogenesis and their application to bone tissue engineering.

OC24

DEFINING THE SECRETOME OF BONE CELLS: NOVEL BONE BIOACTIVE PROTEINS

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Osteoblasts are highly 'secretory' cells, producing large amounts of protein that play key structural and regulatory roles in bone. To further characterise the bone cell 'secretome', we have used a virus based signal-trap screening approach to identify bone associated secreted proteins. This approach has identified three novel bone-specific genes, osteocrin, PGTI041 and PGTI011, with bone bioactive properties.

Osteocrin is a 130aa secreted protein, sharing no homology with known proteins. However, there are two evolutionarily conserved motifs, reminiscent of dibasic cleavage sites suggesting possible pro-hormone-like processing. Expressed specifically in active matrix producing osteoblasts, maximal expression occurs during growth with a marked decrease in aged bones. Treatment of primary osteoblasts with 1,25(OH)2D3 decreases osteocrin expression. Primary osteoblasts, treated with conditioned media containing recombinant osteocrin exhibited reduced mineralisation and downregulation of osteocalcin and alkaline phosphatase suggesting a role in modulating the mature osteoblastic phenotype.

PGTI041, is a 108aa secreted protein expressed predominantly in bone. The protein has a coiled-coil motif, possibly indicating protein-protein interactions. Peak expression of PGTI041 in vivo is seen in embryonic and neonatal bones with very low expression in adult bones, and the gene is also expressed in primary osteoblast cultures. Overexpression of PGTI041 in UMR106 osteosarcoma cells using an adenovirus vector results in elevated proliferation relative to cells infected with GFP expressing adenovirus. Currently, we tentatively propose a role for PGTI041 in the early stages of osteoblast development.

PGTI011, is a 131aa membrane protein with two transmembrane domains and shares high homology to members of an interferon-inducible gene family. PGTI011 is expressed and is downregulated by 1,25(OH)2D3 treatment in primary osteoblasts and UMR106 cells but is not expressed in MC3T3.E1, SaOS-2 or MG-63 osteoblast cell lines. Overexpression of PGTI011 by adenovirus in both primary osteoblasts and UMR cells resulted in an increase in mineralisation suggesting a role in enhancing late stage osteoblast differentiation.

In conclusion, we have further defined the 'secretome' of bone cells and have identified 3 novel bone proteins with potential roles at different stages of osteoblast development. Further elucidation of the functions of these proteins may provide important avenues for bone disease therapy.

OC25

ACTIVATORS OF PPAR ISOFORMS ALPHA AND/OR DELTA CAUSE AN INCREASE IN BONE FORMATION IN VITRO AND IN VIVO

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A number of substances, in particular prostaglandin E2 (PGE2), are known which stimulate bone formation when administered to adult animals. In vivo, PGE2 is rapidly degraded, to PGA2, and we have previously reported that some of the bone anabolic effects of PGE2 may be caused by PGA2 (Still and Scutt, Prostaglandins and other lipid mediators, 65:21-31, 2001). PGA2 is known to bind to the family of PPAR nuclear receptors. Therefore, we investigated the effects of a number of PPAR agonists on colony formation using the CFU-f assay.

In these studies, PPARalpha/delta agonists (Bezafibrate, Fenofibrate and linoleic acid) caused a dose-dependent increase in osteoblastic colony number, achieving numbers similar to, or greater than that of PGE2. PPARgamma agonists (ciglitazone, 15-d PGJ2) had no effect on osteoblastic colony number.

The effect of these drugs was examined in vivo. Briefly, Wistar rats were injected daily with linoleic acid, Bezafibrate or Fenofibrate for 12 weeks. Metaphyseal bone mineral density was increased in all groups compared to the vehicle. Linoleic acid caused an increase of approximately 3% and 8% at doses of 1 mg/kg/d and 0.3 mg/kg/d. Bezafibrate caused an increase of approximately 6% and 8% at doses of 10 mg/kg/d and 1 mg/kg/d. Fenofibrate caused an increase of approximately 3% and 11% at doses of 10 mg/kg/d and 1 mg/kg/d.

Histomorphometric analysis at the proximal metaphysis suggests that this may be due to an increase in trabecular number although the differences seen are small. However, macroscopic examination of the bones, showed a persistence of trabecular elements along the diaphysis.

In conclusion, PPARalpha/delta agonists increase osteogenesis in vitro and increase bone mineral density in vivo suggesting that PPARs play a role in bone formation.

P1 (OP1)

GENDER VARIATION IN PTH SENSITIVITY FOLLOWING GROWTH HORMONE REPLACEMENT IN ADULT GROWTH HORMONE DEFICIENT PATIENTS

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Adult Growth Hormone Deficiency (AGHD) is associated with reduced bone turnover and osteoporosis. PTH plays an important role in bone metabolism and reports suggest that bone and renal insensitivity to the effects of PTH may contribute to changes in bone turnover observed in AGHD. Growth Hormone Replacement (GHR) results in an increase in bone turnover and bone mineral density (BMD), with greater changes seen in men. Therefore, the aim of this study was to determine the presence of gender differences in PTH sensitivity, phosphocalcium metabolism and bone turnover in patients with AGHD, before and after GHR.

20 patients (10 men) with AGHD were recruited. Half-hourly blood and 3-hourly urine samples were collected before and 1, 3, 6 and 12 months following GHR, for PTH, calcium, phosphate, 1,25(OH)2D3, nephrogenous cyclic AMP (NcAMP, marker of renal PTH activity), type-1 collagen C-telopeptide (CTx, bone resorption marker) and procollagen type-1 amino-terminal propetide (PINP, bone formation marker). Serum calcium was adjusted for albumin concentration (ACa).

24-hour mean PTH was higher at all visits in women compared with men ($p<0.001$). Following GHR, PTH decreased in both genders ($p<0.001$), with the maximum change completed at 6 months in men but 12 months in women. There was no significant difference in maximum PTH percentage decrease between the genders. Increases in NcAMP ($p<0.05$), serum phosphate ($p<0.001$) and 1,25(OH)2D3 ($p<0.001$) were seen in both genders, with greater percentage changes observed in men ($p<0.03$). The maximal increase in NcAMP occurred at 1 month in men, but 3 months in women. ACa increased in both genders ($p<0.001$), with a greater percentage change in women ($p<0.001$). Urinary calcium and phosphate decreased in men only ($p<0.05$). Bone markers increased simultaneously after 1 month GHR in men ($p<0.001$), whereas in women, CTx increased at 3 months ($p<0,001$), while PINP increased at 12 months ($p<0.001$).

Following GHR, women with AGHD had a reduced and delayed response in improvement of PTH sensitivity, delayed and non-simultaneous increase in bone turnover and higher PTH concentrations compared with men; factors which may contribute to the development of reduced BMD response to GHR previously observed in women.

P2 (OP2)

OSTEOPONTIN EXPRESSION AT SITES OF OSTEOCLAST EROSION IN RHEUMATOID ARTHRITIS

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Osteopontin (OPN), a non-collagenous bone matrix protein, influences pathological bone remodelling. OPN deficient mice are resistant to osteoporosis following tail-suspension or ovariectomy, ectopically implanted bone from an OPN -/- mouse is resorbed less efficiently than wild-type bone, and osteocyte expression of OPN is increased by the inflammatory cytokine interleukin-1 or by nitric oxide (NO). OPN binding to alpha-v beta-3 integrin on osteoclasts is required for location of these cells on the bone surface and for their resorptive activity. As osteoclasts are responsible for bone erosion in the joints of patients with rheumatoid arthritis (RA), we postulated that expression of OPN would differ between sites of joint erosion compared with non-eroded sites.

Sections of formalin-fixed, decalcified and paraffin embedded bone/synovium from the metacarpophalangeal joints of RA patients undergoing joint replacement were stained for osteoclasts by TRAP-staining and for OPN by indirect immunoperoxidase immunohistochemistry. TRAP-stained sections were examined to identify areas of osteoclast-associated erosion, and areas of bone-synovium interface without erosion. Some areas that were eroded without visible osteoclasts were also noted. The corresponding areas were then identified on the OPN-stained serial sections and the area of positive staining measured (Lucia G image analysis software - threshold intensity set using negative control-stained sections).

OPN staining was significantly greater at sites of erosion compared with non-erosive sites of bone-synovium interface (osteoclastic erosion, 20.6% [SD 10.2]; non-erosive sites 5.6%[4.7], $p=0.0002$). There was no significant difference in OPN staining of erosive sites with (above) and without (15.8% [9.5]) active osteoclasts.

These data indicate that OPN is a relevant signal in the pathogenesis of erosion in RA. We propose that OPN expression is increased by high IL-1 in the adjacent synovial tissue, with resulting location and sustained activation of osteoclasts at those sites. Alternatively, OPN expression may be increased by altered osteocytic NO expression in response to mechanical or inflammatory effects of the synovial mass. In areas where OPN expression is not induced, erosion does not occur. The absence of osteoclasts at some sites of high OPN expression is likely to be an artefact of serial sectioning of a three-dimensional structure.

P3 (OP3)

CHANGES IN BODY COMPOSITION DURING TREATMENT FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA WITH A CONTEMPORARY PROTOCOL

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Disturbances in body composition, specifically osteopenia and obesity, are complications that may occur in survivors of childhood acute lymphoblastic leukaemia (ALL). Prior treatment with cranial irradiation was thought to be the main aetiological factor. However, recent evidence suggests that these changes were seen even in survivors treated with chemotherapy alone. Therefore, we prospectively evaluated the longitudinal changes in body composition in children with ALL during treatment with chemotherapy but without cranial irradiation.

Whole body DEXA scanning was undertaken at diagnosis and during treatment in 14 children (7 male), mean (SD) age 8.6 (4.4) years. Treatment was with chemotherapy and steroids, 11 with prednisolone and 3 with dexamethasone. Measurements of bone mineral content (BMC) at the hip and lumbar spine were expressed as a percentage of the predicted value and as a standard deviation score (SDS). The ratio of fat mass (FM) to lean mass (LM) was expressed as a percentage of the predicted value, and body mass index (BMI) as an SDS.

The mean hip %BMC SDS was reduced by 6 months (-1.50 SDS;$p<0.01$) and remained significantly low by 24 months of therapy. The mean lumbar spine %BMC SDS was reduced by 12 months (-1.31 SDS;$p<0.01$) which persisted until 24 months. The BMI SDS increased by 24 months (+0.78 SDS;$p<0.01$) whereas the corrected %FM/LM increased from 6 months of treatment ($p<0.01$).

In conclusion, treatment with a contemporary protocol predisposes individuals to the development of osteopenia and obesity during treatment even in the absence of cranial irradiation. We speculate these findings are a direct result of the administration of chemotherapy, including steroids.

P4 (OP4)

ESTROGEN AND THE FORMATION OF REMODELLING CLUSTERS

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Recent evidence has suggested that, in long bones, cortical remodelling is spatially clustered. The cause of such clustering is unknown but it is independent of age or gender. This study was designed to determine whether clustering occurred in the ilium and whether it was modulated by estrogen. Trans-iliac biopsies were taken from young women with endometriosis before or after 6 months treatment with GnRH (GnRH, n=10). Similar biopsies were taken from older women before and after treatment with HRT for an average of 2 years (HRT, n=10).

After sectioning and staining with Toluidine Blue, the number and location of osteoid bearing (%forming) and crenellated (%resorbing) canals were noted in each cortex. Clustering was analysed with 0.32mm (2x mean inter-osteonal distance for all biopsies) as the cluster radius. The results were analysed using regression models in which, subject nested within treatment group (GnRH or HRT), treatment group and estrogen status were the independent variables with %forming, %resorbing and %clustering (the proportion of active canals contained within clusters) as the dependent variables. Forming canals were independent of treatment ($p=0.38$) but significantly higher in the GnRH group (GnRH 18.4±2%, HRT 12.6±1.8%, $p=0.044$). They were more clustered than predicted by chance (mean difference +23.5±3.5%, $p<0.0001$, paired t-test) but this was unaffected by treatment group ($p=0.48$) or estrogen status ($p=0.86$). Resorbing canals were marginally higher in the GnRH group (GnRH: 17.5±2.4%, HRT: 11.2±2.3%, $p=0.11$) and in the absence of estrogen (without: 16.5±1.5%, with:- 13±1.5%, $p=0.10$). Such canals were more clustered than would be predicted by chance (mean difference +16.9±3.7%, $p<0.0001$, paired t-test) but this was unaffected by treatment group ($p=0.13$) or estrogen status($p=0.75$).

In conclusion, this study has shown that cortical remodelling in the ilium is spatially clustered. However, neither the addition (in HRT treatment) nor removal (GnRH treatment) of estrogen affected clustering suggesting, that estrogen status plays little role in the clustering process. However, given that the merging of osteonal canals and the subsequent formation of a large pore is one of the key features of femoral neck fracture, determining the cause of clustering is fundamental to understanding the aetiology of fragility fractures.

P5 (OP5)

IN VIVO HUMAN BONE AND CARTILAGE FORMATION USING POROUS POLYMER SCAFFOLDS ENCAPSULATED WITH BONE MORPHOGENETIC PROTEIN-2

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The ability to deliver, over time, biologically active osteogenic growth factors using designed scaffolds to sites of tissue regeneration offers tremendous therapeutic opportunities in a variety of musculo-skeletal diseases. The aims of this study were to generate porous biodegradable scaffolds encapsulating an osteogenic protein, bone morphogenetic protein-2 (BMP-2) and to examine the ability of BMP-2 released from encapsulated constructs to promote human osteoprogenitor adhesion, migration, expansion and differentiation on 3-D scaffolds in vitro and within an innovative ex vivo chick chorioallantoic membrane bone formation model as well as by in vivo bone formation assays.

BMP-2 encapsulated Poly(DL-lactic acid) (PLA) scaffolds (100ng rhBMP-2/mg PLA) were generated using an innovative supercritical fluid process developed for solvent sensitive and thermolabile growth factors. The bioactivity of rhBMP-2 encapsulated PLA scaffolds were confirmed by induction of the C2C12 promyoblast cell line into the osteogenic lineage as detected by alkaline phosphatase expression. No induction of alkaline phosphatase-positive cells was observed using blank scaffolds. BMP-2 released from encapsulated constructs promoted adhesion, migration, expansion and differentiation of human osteoprogenitor cells on 3-D scaffolds. Angiogenesis and enhanced matrix synthesis (evidenced by alcian blue, sirius red and collagen birefringence) on growth factor encapsulated scaffolds was observed following culture of human osteoprogenitors on explants of chick femoral bone wedge defects in an ex vivo model of bone formation developed using the chick chorioallantoic membrane model. In vivo studies using diffusion chamber implantation (10 weeks) and subcutaneous implantation (6 weeks) of human osteoprogenitors on rhBMP-2 encapsulated scaffolds showed morphologic evidence of new bone matrix and cartilage formation in athymic mice as assessed by x-ray analysis, immunocytochemistry and birefringence. These studies provide evidence of controlled release of BMP-2 from biodegradable polymer scaffolds initiating new bone formation in vivo. The successful generation of 3-D biomimetic structures incorporating slow release osteoinductive factors indicates the potential for de novo bone formation and the potential for more complex tissue regeneration strategies that will incorporate temporal and sequential release strategies for the augmentation of tissue regeneration.

P6 (OP6)

PASTEURELLA MULTOCIDA TOXIN HAS DIFFERENTIAL EFFECTS ON MURINE AND HUMAN OSTEOCLAST DIFFERENTIATION AND ACTIVITY

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Pasteurella multocida toxin (PMT) is a bacterial protein toxin that causes the porcine bone resorbing disease, atrophic rhinitis. PMT is a potent mitogen and induces cytoskeletal rearrangements through the small GTPase Rho. It also stimulates phospholipase C, leading to activation of protein kinase C, increases in inositol phosphates and intracellular calcium, and signals indirectly through the Ras/MAP kinase pathway. Our previous work has established that PMT is a potent inhibitor of osteoblast differentiation in vitro, in part via activation of Rho and Rho kinase. Furthermore, PMT inhibits osteoblast-mediated osteoclastogenesis, partly through a reduction in the ratio of RANKL to OPG. Despite the marked effects on osteoblasts, the potential direct effects of PMT on osteoclast precursor differentiation and bone resorption are less clear.

In this study, the effects of PMT on osteoclasts were assessed in two well-established systems, sRANKL and MCSF-based cultures of murine bone marrow cells and human peripheral blood mononuclear cells (PBMCs). Osteoclast differentiation was assessed by TRAP staining (murine osteoclasts), and by vitronectin receptor (VnR) staining and F-actin rings (human osteoclasts). In all cultures, osteoclast activity was measured by quantifying the area resorbed on dentine slices. Continuous exposure to PMT completely inhibited osteoclast formation and resorption in a dose-dependent manner in both culture systems. Pulse experiments in murine cultures revealed that addition of PMT for the early proliferation (0-3d) or later differentiation (3-6d) stages of culture was sufficient to inhibit osteoclast formation and resorption, whereas addition after osteoclasts were formed (6-9d) had a lesser effect. PMT also markedly inhibited osteoclast differentiation and resorption when present for initial stages in human PBMC cultures (0-7d), although no inhibitory effects were observed when PMT was added after osteoclasts had formed. In contrast, preliminary evidence suggested that PMT may have an activating effect in late cultures, as evidenced by an increase in the proportion of F-actin ring-containing VnR-positive osteoclasts, and a concomitant increase in resorption pit formation. Taken together, these findings indicate that PMT has possible divergent effects on osteoclast differentiation and activity. Further studies are in progress to investigate the signalling mechanisms activated by PMT in mouse and human osteoclasts.

P7 (OP7)

TGF-BETA-INDUCED SOCS3 EXPRESSION AUGMENTS TNF-ALPHA-INDUCED OSTEOCLAST FORMATION

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The loss of bone in mice following ovariectomy has in part been attributed to elevated TNF-alpha levels. TNF-alpha, like RANKL, directly induces osteoclast formation from mononuclear precursors. However, not all precursors form osteoclasts in the presence of TNF-alpha, suggesting that other factors are required to ensure all precursors become osteoclasts at sites of resorption. TGF-beta augments the proportion of precursors that form osteoclast in the presence of TNF-alpha. Furthermore, TNF-alpha-induced osteoclast formation is abolished by anti-TGF-beta antibodies, suggesting that osteoclasts that form without the addition of exogenous TGF-beta are dependent on TGF-beta present in the medium or produced by precursors themselves.

The mechanism by which TGF-beta facilitates TNF-alpha-induced osteoclast formation is unknown. One possibility is that the environment in-vitro is essentially pro-inflammatory, due to the presence of agents such as interferon-beta, and TGF-beta opposes this. Interferons signal via the JAK/STAT pathway, and TGF-beta might therefore block these signals. Interestingly, we have recently shown that TGF-beta-induces the expression of SOCS3, an inhibitor of the JAK/STAT pathway, in osteoclast precursors and SOCS3 expression is enhanced by TGF-beta in RANKL-induced osteoclasts. However, the role SOCS3 in TNF-alpha-induced osteoclast differentiation is not known. Therefore, we examined the effect of TGF-beta on SOCS3 mRNA expression in TNF-alpha-induced osteoclasts.

We found that while SOCS3 mRNA is undetectable in macrophages, TNF-alpha-induced osteoclasts express SOCS3, and TGF-beta upregulates this expression. To determine if SOCS3 plays a role in TNF-alpha-induced osteoclast differentiation we expressed SOCS3 in precursors using a retroviral system. We found that osteoclast differentiation was significantly enhanced in SOCS3-infected precursors compared with controls, and SOCS3 expression prevented the inhibitory effect of IFN-beta on formation. Moreover, specific antisense knockdown of SOCS3 suppressed osteoclast formation and the enhancing effect of TGF-beta was blunted in precursors expressing the SOCS3 retrovirus.

This data suggests that TGF-beta-induced expression of SOCS3 may represent one mechanism by which TGF-beta suppresses inhibitory cytokine JAK/STAT signaling, priming precursors for a role in bone resorption rather than alternative inflammatory macrophage lineages.

P8 (OP8)

A ROLE FOR RETINOIC ACID IN REGULATING OSTEOCLAST FUNCTION IN REGENERATING DEER ANTLERS

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We have recently used the deer antler model to show that retinoids may play a role in regulating adult mammalian bone regeneration (1). Retinoic acid (RA) is synthesised in regions of the antler where RA receptors are expressed and RA controls chondrocyte differentiation. The rapid rate of endochondral growth in antlers requires extensive remodelling of cartilage matrix and cells of the osteoclast lineage can be identified in perivascular tissues in antler cartilage (2), a site of RA synthesis. Micromass cultures of cells from antler cartilage support OC differentiation in the absence of exogenous factors (2). The present study explores the hypothesis that osteoclastogenesis in antler is also regulated by RA. The specific objectives were: (i) to determine whether RA regulates osteoclastogenesis and to establish whether these effects are mediated by receptor activator of NF-kB ligand (RANKL), (ii) to correlate sites of RANKL expression in antler tissues in vivo with sites of OC differentiation and RA synthesis (as determined by expression of RALDH2, a RA synthesising enzyme).

Addition of all-trans-RA (100nM) to cartilage micromass cultures for 7-19 days stimulated the differentiation of osteoclast-like multinucleated cells, as determined by TRAP staining. The magnitude of this increase ranged from 2-40 fold. Resorption area on dentine slices also increased ($P<0.0005$) in cultures treated for 14 days. RA increased RANKL mRNA expression, as determined by semi-quantitative PCR analysis. This induction of RANKL expression did not require de novo protein synthesis, as it could not be blocked with cycloheximide. However, it was dependent upon retinoic acid receptor (RAR) signalling as RANKL expression could be abrogated by the RAR antagonist Ro41-5253. Addition of osteoprotegerin, the decoy receptor for RANKL, inhibited RANKL's effect on OC formation. In tissue sections, RANKL was immunolocalised in cells in the perivasular region of antler cartilage the site where osteoclasts differentiate and where RALDH2 was also immunolocalised. In conclusion, these results show that retinoic acid regulates osteoclast formation during bone regeneration and demonstrate that the effects of RA on antler osteoclasts are mediated by RANKL.

(1) Allen SP et al. (2002) Dev Biol. 251:409-23 (2) Faucheux C et al. (2001) J Exp Biol. 204:443-55.

P9 (OP9)

OLDER PEOPLE IN CHINA AND UK DIFFER IN THE RELATIONSHIPS BETWEEN PARATHYROID HORMONE, VITAMIN D AND BONE MINERAL STATUS

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Hip fracture incidence in China is lower than in Britain. To explore whether there are differences in relationships between PTH, vitamin D and bone mineral status, 352 healthy volunteers, 60-83 years old, were studied in Cambridge, UK (67 men, 67 women) and Shenyang, PR China (108 men, 110 women) in late winter (Feb-April). An early-morning, fasting blood was analysed for plasma 25(OH)D and PTH. Hip bone mineral status was measured using Lunar DPX (cross-calibration by European phantom). Statistical analysis was by multiple linear regression with continuous variables except age converted to natural logarithms.

There were significant differences ($p<0.001$) in PTH and 25OHD concentrations between Shenyang and Cambridge [PTH pg/ml: Shenyang = 34.3 (SD13.4), Cambridge = 25.2 (SD11.0); 25(OH)D nmol/L: Shenyang = 29.0 (SD12.7), Cambridge = 35.7 (SD12.9)]. PTH was negatively related to 25OHD. The relationship was exponential and best described by an inverse linear log-log equation. There was no evidence of curvature on this line, indicating that the exponential curve did not tend towards a low plateau. The relationship was significantly different in the two populations. In Shenyang, PTH was higher for a given 25OHD and decreased less with increasing 25OHD than in Cambridge (after age and sex adjustment: country effect = 27.6% (SE4.1), $p<0.0001$; country*ln25OHD interaction ($p=0.0005$)]. Women had a higher PTH for a given 25OHD than men (13.6% (3.9)), but there was no evidence that this difference varied between country (sex*country interaction, $p=0.54$).

After adjusting for bone area, weight, height, age and sex, hip BMC was significantly related to PTH in Cambridge but not in Shenyang (neck coefficient: Cambridge = -0.061 (SE0.027) $p=0.025$; Shenyang = -0.027 (SE0.028) $p=0.33$; trochanter coefficient: Cambridge = -0.112 (SE0.035) $p=0.0015$; Shenyang = -0.019 (SE0.027) $p=0.49$). There was a significant country*lnPTH interaction at the trochanter ($p=0.03$), but not at the neck ($p=0.74$). 25OHD was not a significant predictor of size-adjusted BMC, independent of PTH.

These data suggest that although PTH increases when 25(OH)D decreases, and Chinese people have a higher PTH for a given 25OHD, older Chinese adults may be more resistant than Britons to the effects of PTH on bone.

P10 (OP10)

CORTICAL POROSITY DURING FAST GROWTH IN THE IMMATURE SKELETON: THE ROLE OF PERIOSTEAL OSTEOBLAST PROLIFERATION

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In response to skeletal loading, bones increase their diameter through the incorporation of periosteal blood vessels and the formation and infilling of primary osteons. In the immature skeleton the influence of growth rate on this process is unclear. Comparing chickens with fast and slow growth potential, we have previously reported that the fast growing birds had increased cortical porosity which, we speculated, may account for the lowered tibial mechanical properties observed in these birds.

To investigate underlying mechanisms for this increased porosity we have completed further morphometric analysis of tibiae from chickens with fast (F) and slow (S) growth potentials (porosity;F=38%;S=30%,$P<0.001$) (body weights at 21 days; F=440g and S=224g).

Staining for reversal lines indicated the absence of primary osteon remodelling in the periosteal region There was no difference in osteon area between strains so that increased porosity was the result of a slower infilling of the primary osteons in the rapidly growing birds (% unfilled;F=42.5%;S=27.0%,$P<0.01$). Osteocyte density within the circumferential lamellae was also higher within the rapidly growing birds (F=5.3/mm2; S=1.8mm2;, $P<0.01$), but unchanged within the newly laid down bone of the primary osteons (F=2.73/mm2;S=2.86/mm2;,$P<0.01$) Proliferating pre-osteoblast cells within the osteogenic layer of the periosteum had a lower labelling index in the rapidly growing birds seen across four circumferential areas of the periosteum (F=20.47%;S=32.17%,$P<0.001$), even though the osteogenic layer of the periosteum was thicker in the fast strain (F=20.57/microns2;S=15.54/ microns2;,$P<0.001$). Blood vessel numbers within the periosteum was similar between strains but differed between regions habitually loaded in tension (anterior: 3.82/mm2) or in compression (posterior: 6.14/mm2, $P<0.01$)

In conclusion, no evidence was obtained to suggest that osteonal remodelling or periosteal blood vessel number were a determinant for primary osteon size. However, the lower labelling index at the periosteum and increased osteocyte density within the circumferential lamellae of the fast strain suggests an increase in transit time through the osteoblast lineage at the periosteal surface. This would account for the reduced primary osteon infilling but as osteocyte density within primary osteons was similar in both strains other mechanisms such as reduced osteoblast incorporation into primary osteons and increased apoptotic rates may be responsible.

P11 (OP11)

ADHESION OF BREAST AND PROSTATE CANCER CELLS TO EXTRACELLULAR MATRIX PROTEINS IS DEPENDENT ON GROWTH FACTOR RECEPTOR EXPRESSION AND INHIBITED BY ZOLEDRONIC ACID

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Breast and prostate cancer preferentially metastasise to bone, however the mechanisms involved in this process are inadequately understood. Bisphosphonates including zoledronic acid (ZA) are known to limit skeletal related morbidity in patients with metastatic bone disease and have direct apoptotic effects against breast and prostate cancer cells in vitro. This study aimed to determine whether the breast cancer cells lines MCF-7, MDA-MB-231 and SKBr3 and the prostate cancer cell line DU-145 adhere with equal avidity to the extracellular matrix proteins fibronectin, vitronectin, laminin, collagen I and collagen IV. We also investigated whether ZA was able to inhibit adhesion to these proteins. Finally we aimed to investigate the role of aberrant Ras and her2 signalling on breast cancer cell adhesion. Equal concentrations of cells were seeded onto multiwell plates coated with a variety of extracellular matrix proteins for 1 hour, washed, fixed, stained, the dye eluted and read in a platereader at 550nm. The breast cancer cell line MDA-MB-231, which harbours the Ki-ras mutation, adhered to all matrices with significantly greater avidity than any other cell line investigated. Furthermore each cell line exhibited preferential adhesion to different proteins. MCF-7 cells were then exposed to increasing doses of ZA for 24 hours before following the same protocol. ZA impaired adhesion of MCF-7 cells to all matrices with maximal inhibition occurring at 1 nanomolar ZA. Adhesion of MDA-MB-231 and DU-145 cells was also significantly impaired by treatment with 1 nanomolar ZA but adhesion of the her2 overexpressing cell line SKBr3 was unaffected by this treatment. Finally we demonstrated that forced her2 receptor overexpression in the normal breast epithelial cell line, Hb4a, resulted in significantly increased adhesion efficiency, as did an activating Ras mutation in this same cell line. These findings confirm that cancer cells adhere to a variety of extracellular matrix proteins and demonstrate that this is inhibited by ZA which may account for its known anti-cancer effects. Moreover, our results suggest that increased growth factor receptor signalling confers an increased ability to adhere to mineralised matrices and may be associated with relative resistance to conventional therapeutic intervention with agents such as bisphosphonates.

P12 (OP12)

A NOVEL APPROACH TO THE GENERATION OF ANTIBODIES AGAINST ADULT HUMAN MESENCHYMAL STEM CELLS

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Adult marrow contains rare clonogenic precursors (CFU-F) which form colonies in vitro and differentiate into multiple stromal cell types in vivo. CFU-F and their immediate progeny are not identifiable morphologically and there are no definitive markers. Our aim is to use antibody phage display to generate monoclonal antibodies against CFU-F enriched fractions of bone marrow mononuclear cells (BMMNC), to facilitate the identification and isolation of CFU-F within human marrow stroma.

To identify an antibody suitable for CFU-F enrichment, the colony forming efficiency (CFE) of different, antibody-defined (CD49a, STRO-1, CD45) sub-populations was determined. BMMNC, labelled with monoclonal antibody and magnetic beads, were passed over a magnetic column (MACS). Positive and negative fractions were plated at 20 000 cells/cm2, fed twice weekly and after 14 - 21 days, colony formation assessed. When compared with unseparated BMMNC, CFE was greatest in the CD49a+ fraction with an enrichment factor of 20 fold. The corresponding figure for glycophorin A- CD45-, and glycophorin A- STRO-1+ fractions were 0 and 9 and fold respectively.

We will use the highly diverse Griffin.1 phagemid library to generate phage antibodies against CFU-F enriched BMMNC. Preliminary studies using MG-63 cells have generated of an antibody clone (JL4a) after 4 rounds of selection. The cell and tissue specificity of this clone is currently being investigated, but results have highlighted the need for a simultaneous positive/negative selection procedure to ensure appropriate specificity. Therefore, library phage are incubated with unseparated BMMNC, and bound phage subsequently recovered from the magnetically separated, antibody defined subpopulations. By optimising binding and washing conditions, a mean phage titre of 200 000 colony forming units was obtained from CD49a positive BMMNC after the first round of selection.

In summary, anti-CD49a has been shown to give good enrichment of CFU-F, and by optimising phage selection conditions, we have succeeded in obtaining sufficient clones from CD49a+ BMMNC to perform multiple rounds of selection. Production of antibody clones against CFU-F enriched BMMNC, and screening at the molecular and cellular level is now in progress. We anticipate that phage antibodies generated in this way will provide an invaluable and much-needed resource for the study of mesenchymal stem cell differentiation.

P13

THE ISSUE OF BIORESORPTION OF THE BIO-OSS XENOGENEIC BONE SUBSTITUTE IN BONE DEFECTS

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Bone grafts and bone substitute biomaterial implemented in guided tissue regeneration should undergo the process of biological decomposition in the recipient's system. The aim of this work is the presentation of current views concerning the issue of Bio-oss bovine bone bioresorption and their juxtaposition with the results of the author's own research.

The work presents histopathological and immunohistochemical tests of the xenogeneic Bio-oss preparation from biopsies carried out 30 months after implantation. It was observed that the preparations contained correct bone neighbouring remnant particles of Bio-oss, intratrabecular fibromatosis around the implant, abundant vascularisation, absence of osteoid and of active inflammatory process. A small number of T and B lymphocytes was detected.

The results obtained in the above-described cases testify to the descending character of the inflammatory infiltration 30 months after the implementation of Bio-oss and efficient restoration of the bone.

The prevalent view in literature is that Bio-oss is resorbable biomaterial. However, there are also reports questioning this view as remnants of Bio-oss have been detected even 44 months after implantation into the bone defect. It seems that although the creation of new bone structure is indisputable, nevertheless the process of biological decomposition of Bio-oss should be described as slow bioresorption.

P14

MORPHOLOGY OF ROOT CANALS CROSS-SECTIONS OF RESECTED ROOTS OF FIRST AND SECOND LOWER MOLARS

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Precise preparation of root canal ostium with a view to hermetical closing with retrograde root filling is an important prognostic factor determining the success of the procedure of tooth resection. Root canals interconnected with a narrow isthmus may cause problems both in endodontic treatment and in retrograde filling. The aim of this work is the research of the transverse cross-section of root canals of first and second lower molars on the resection model.

The research encompasses 100 randomly selected molar teeth: 50 first and 50 second lower molars. The tooth root apexes were cut 3mm below the apex and examined under an electron microscope, special attention being paid to the shape of root canal cross-sections.

In the group of first molar teeth, in 20 % the presence of an isthmus between canals in the proximal roots was observed; in the group of second molar teeth an isthmus between the canals of proximal roots occurred in 18 % of the cases.

It seems that the relatively high percentage (20 %-18 %) of the occurrence of an isthmus, 3mm below the root apex, between two elongated transverse cross-section proximal root canals of first and second molars should encourage particular caution in retrograde filling of the above-mentioned canals during the procedure of resection.

P15

MINERAL BIOMIMICRY- GENERATION OF BIOMIMETIC MICROPOROUS CALCIUM CARBONATE SPHERES FOR SKELETAL REGENERATION

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The development of self assembling biomineral complexes for cell growth, growth factor and gene delivery offers tremendous opportunities for skeletal repair. Natural biological ceramic structures possess arrangements of structural elements that govern and optimise tissue function, nutrition and organisation may provide an innovative strategy to address this clinical need. The aim of this study was to fabricate biomineral microporous shells with highly complex forms and to examine their ability to interact with human osteoprogenitor cells as cell and growth factor delivery vehicles.

Microporous vaterite shells were generated using a synthetic in-solution mineralisation technique in which mineral is spontaneously deposited around vesicular templates (Walsh et al 1999)#. Porous and textured self-organising hollow microspheres (5-20 microns) were generated expressing controlled and uniform shapes. These micropores puncture the surface at high densities and are interconnected throughout the sphere. Primary human bone marrow cells labelled with Cell Tracker Green (CTG) and ethidium homodimer-1 fluorescent labels and osteoprogenitors transfected with an adenoviral vector expressing Green Fluorescent Protein (AdGFP) were cultured with vaterite shells over three weeks.

Cell biocompatibility of these biomimetic spheres was confirmed by confocal fluorescence and light microscopy in primary human bone marrow cultures labelled with CTG and bone marrow cultures transfected with AdGFP. At three weeks microspheres were encapsulated and integrated with osteoprogenitor cells. Histological analysis confirmed expression of alkaline phosphatase, extracellular matrix synthesis and the capacity for extensive mineralisation. Examination by fluorescent, SEM and light microscopy showed that the growth of osteoprogenitors transfected with AdGFP encapsulated and integrated with vaterite sphere in pellet culture and integration of vaterite spheres within the osteoprogenitor cell matrix indicating the potential of growth factor delivery. To determine the potential of the spheres to encapsulate selected proteins, microporous spheres were incubated with bovine haemoglobin. FITC microscopic examination showed haemoglobin could be entrapped inside the spheres and between the biomineral crystal plates during self-assembly.

In conclusion, these studies demonstrate the development of facile techniques for the generation of porous microsphere scaffolds that are biocompatible, aid mineralisation with potential for cell and growth factor delivery. These biomineral complexes present an innovative material for skeletal regeneration and for tissue engineering.

ref:- #Walsh, D., Lebeau, B., & Mann, S. Morphosynthesis of calcium carbonate (vaterite) microsponges. Adv. Mater, 1999; 11; 324-328.

P16

INTERLEUKIN-10 DIRECTLY INHIBITS RANKL-INDUCED OSTEOCLAST FORMATION FROM MONONUCLEAR PHAGOCYTE PRECURSORS

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IL-10, a T-cell derived cytokine synthesis inhibitory factor has important effects on bone cell physiology, in addition to its pleiotropic activity. Previous studies have reported IL-10 to inhibit osteoclastogenesis in murine haemopoietic co-culture systems. However, due to the presence of numerous cell types in these systems it is unclear whether IL-10 acts directly on osteoclast precursors or indirectly via another cell type. Since the role of RANKL in the mechanism of osteoclastogenesis was elucidated, osteoclasts can now be cultured without the confounding influence of other cell types. Therefore, to investigate the cellular mechanism through which IL-10 acts we compared its ability to inhibit RANKL-induced osteoclast formation in co-cultures of UMR and bone marrow cells, stromal-depleted mononuclear phagocyte precursors and the murine monocytic cell line RAW 264.7.

Murine non-adherent M-CSF dependent precursors were treated with MCSF, RANKL and recombinant murine IL-10 (1, 10, 100 ng/ml) with or without the prior addition of UMR cells. We found that IL-10 inhibited the formation of RANKL-induced TRAP-positive osteoclasts in a dose-dependent manner from mononuclear phagocyte precursors. This effect was not enhanced by the addition of UMR cells, suggesting that the anti-osteoclastic action of IL-10 is not mediated through an indirect osteoblastic effect. Furthermore, in cultures of RAW 264.7 cells IL-10 suppressed osteoclast differentiation to the same extent as that of co-cultures, suggesting that IL-10 acts directly on haemopoietic precursors.

These findings indicate that IL-10 inhibits RANKL-induced osteoclast differentiation by acting directly on non-committed mononuclear phagocyte precursors. This observation may have important implications for the understanding of inflammatory bone pathology.

P17

DURATION AND DEPTH OF RESORPTION DURING CANCELLOUS BONE REMODELING CAN BE SIMULATED BY CHANGES IN CELLULAR ACTIVITY USING MICHAELIS-MENTEN EQUATIONS AND THE INTERACTIVE EFFECTS OF RANKL, RANK, OPG AND TGF-BETA1 ON OSTEOCLAST SURVIVAL AND FUNCTION

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There is an increased interest in using mathematical models in pharmaceutical research for pre-clinical testing the effectiveness of new drugs. Therefore the objective of this work was to construct a mathematical model to simulate cellular activity during resorption in the remodeling of normal, healthy cancellous bone, as a first stage in the development of models to simulate the disruption of cellular processes that occurs with disease.

Model equations were formulated using information drawn from published information on histomorphometric data and the physiological interactions between bone matrix and marrow tissue. Osteoclastic activity was simulated by the competitive inhibition of OPG on the interaction between RANKL and RANK. The Michaelis-Menten equations that describe enzyme kinetics were adapted to simulate cellular activity, a method recently successfully applied by other researchers in other fields of biology (e.g. Sunray et al., 2002). TGFbeta1 released from the matrix by resorption causes OPG production by marrow stromal cells, resulting in a decreased rate of osteoclastic resorption (negative feedback effect). Apoptosis of osteoclast nuclei and cessation of osteoclastic resorption is assumed to occur once TGFbeta1 reaches a threshold amount. The Michaelis-Menten equation is also used to describe the rate of collagen removal by mono-nuclear lining cells during the second phase of resorption (see Everts et al., 2002). Model parameter values were estimated from published data by regression analysis (e.g. Eriksen et al., 1984; Parfitt et al., 1996; Fuller et al., 1998).

Preliminary results of sensitivity analysis showed relatively conservative changes in resorption depth with changes in most model variables (e.g. less than 6% change in depth per 30% change in model parameter), apart from TGFbeta1 in matrix and the apoptosis threshold, which had greater, and opposing, effects on depth. However, resorption depth was conserved when TGFbeta1 and apoptosis threshold were altered simultaneously. Meanwhile, the duration of resorption phase showed greatest sensitivity to the maximum rate of mono-nuclear cell activity in removal of collagen fibrils.

This study shows that this model, based on equations formulated from published data, is a valuable tool for investigating the complex interactive processes that control resorption depth and duration during cancellous bone remodeling.

P18

RATES OF BONE FORMATION AND MINERALIZATION DURING CANCELLOUS BONE REMODELING CAN BE SIMULATED BY CELL PROLIFERATION AND MICHAELIS-MENTEN EQUATIONS THAT DESCRIBE OSTEOBLAST ACTIVITY

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Interest in the use of mathematical modeling as a tool for the pre-clinical testing of new pharmaceutical drugs is increasing. Therefore the objective of this work was to construct a mathematical model to simulate the cellular activity during the reversal, formation and mineralization phases of remodeling in normal, healthy cancellous bone as part of a longer term plan to simulate the disruption of these cellular processes with disease.

The model was constructed using information collected from published data of histomorphometric measurements and the physiological interactions that occur within the bone microenvironment during bone formation.

Proliferation of pre-osteoblasts is simulated by a relationship that has been successfully used to describe the growth of muscle stem cell numbers in the presence of growth factors (Deasy et al. 2002). As proliferation is delayed until the committed osteogenic cells have made contact with the matrix, the model includes the potential effects of growth factors embedded within the matrix on cell number.

The Michaelis-Menten kinetic equations that describe enzyme and cellular activity (for example Sunray et al., 2002), are adapted to simulate the rates of both osteoid formation and mineralization. Cellular activity is simulated by both the number and activity of cells of the osteoblast lineage. Two feedback effects that occur during the mineralization process are included in the model: a reduction in number of active cells via osteocyte formation, and a reduction in the substrate available for mineralization, as more osteoid becomes mineralised. After model equations were formulated, the parameter values were estimated by fitting simulations to published data of temporal changes in osteoid volume, osteoid seam width, osteoid formation rate and rate of mineralization (Eriksen et al., 1984).

Because the model is fitted to variations in several measured histomorphometric parameters and based on knowledge of mechanisms underlying cellular processes during bone formation, sensitivity analysis of model parameters will elucidate the relative importance of factors controlling bone formation.

This study supports the use of mathematical models to simulate complex interactive processes during the reversal, formation and mineralization phases of cancellous bone remodeling as an aid to identifying questions for further research.

P19

CHONDROCYTES FROM THE OSTEOPETROTIC MUTATION TOOTHLESS (TL)/ CSF-1 NULL RAT DIFFERENTIATE IN VITRO

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The toothless (tl) mutation in the rat is a naturally occurring, autosomal recessive mutation resulting in profound deficiencies of bone-resorbing osteoclast. The tl rats have severe, unrelenting osteopetrosis with a highly sclerotic skeleton, lack of marrow spaces, the failure of tooth eruption and a progressive, severe growth plate chondrodystrophy. We have recently found a 10-base insertion near the beginning of the open reading frame of the Csf-1 gene that yields a truncated, nonfunctional protein and an early stop codon, thus rendering the tl rat CSF-1 null. Injections of CSF-1 increases bone resorption, growth and tooth eruption in tl rats but does not improve the growth plate phenotype. In order to investigate how CSF-1 affects the differentiation of chondrocytes we have developed a primary cell culture model using costochondral chondrocytes from both normal and mutant animals. Both normal- and tl-derived chondrocytes differentiate to hypertrophy and are capable of forming mineralized cartilage nodules as assessed by alizarin red staining. These cultures also express other chondrocyte specific markers such as the transcription factors sox 9, aggrecan and collagen type II mRNA. As it appears that normal culture conditions are sufficient for differentiation of the tl-derived chondrocytes, the severe growth plate chondrodystrophy of the tl rat does not result from an intrinsic chondrocyte defect.

P20

THE PROMISING IMPLANTABLE MATERIAL THE FLUORINATED 'LITAR'

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Efficiency of the results in reconstructive maxillofacial surgery depends to a large measure on the postoperative time period. The reduction of the rehabilitation time period can be achieved either by the use of nonmetal and ceramic materials (constructions) or by the biotransformation time period reduction of the implantable materials. Of the 3 existing types of materials: biodegradable, bioresorbable and integrowable ones the most efficient materials are the implantable materials. They dissolve rapidly in the living body, thus this fact will assist in regenerating the native bone tissue in the defective part, including the facial skeleton. Ensuring the biodegradation of the composite materials is achieved by a high degree of the structural integration of the components and considerable porosity of the material.

The collagen-apatite composite material 'LitAr' has porosity of 75%. The consequence of this property is the unique clinical effect according to the bone tissue regeneration time period in the defective upper jaw parts for about 30 days and of the low jaw for 45 ÷ 50 days.

The author of the present investigation forecast the further reduction of the regeneration time period at the expense of introducing the fluoride-ion into the material 'LitAr'.

The synthesis of the material 'LitAr' which contains fluoride has been performed in conformity with the general principles of the technological plan of making the composite material. The quality of the material to be made and the availability of possible admixtures was checked by the X-ray-phase analysis and infrared spectroscopy. We could not reveal the other saline component with the other exception of fluoroapatite. The content of fluorine in the material bulk to be implanted once did not exceed the quantity.

We used fluorinated material 'LitAr' for substituting the jaw defects which could result in consequence of removing the cysts, impacted and dichotomy teeth. The postoperative time period was taking a normal course in all the cases.

The biotransformation of the material was checked by radiology, computed tomography in the course of 2, 7, 30, 60, 90 days after performing the operations. The regeneration of the native bone tissue was checked on the 30 ÷ 40 days.

P21

EFFECT OF HYDROCORTISONE ON CORTICOSTEROID RECEPTOR MRNA LEVELS IN CULTURED HUMAN OSTEOBLASTS DEPENDS ON THE STATE OF DIFFERENTIATION OF THE CELLS

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Osteoblasts in neonatal and adult bone and in culture have glucocorticoid and mineralocorticoid receptors (GR, MR). Thus, both types of receptor may mediate the effects of glucocorticoids on bone. We added hydrocortisone to osteoblast growth medium to give concentrations representing those found in normal plasma and in plasma from patients receiving oral glucocorticoid therapy and measured the effects on GR and MR mRNA levels in cultured osteoblasts from one young and two older female donors.

Osteoblasts were grown in proliferation medium containing 15 nanomolar cortisol from 10% human serum. On day zero, this medium was replaced with medium containing either 4 micromolar cyclodextrin carrier or 200 nanomolar or 4 micromolar water-soluble hydrocortisone. The medium was changed every two days until day 8 when the cells were harvested for total RNA extraction. Levels of mRNAs for GAPDH, ALP, COL1, GR alpha, GR beta and MR were measured using quantitative real-time RT-PCR. The levels of specifc mRNAs were corrected using the levels of GAPDH mRNAs and then normalised to the carrier controls.

Levels of ALP mRNA relative to GAPDH mRNA varied in the different cell lines. Levels were higher in the cells from the two older donors with a maximum 10-fold difference between old donor and young donor controls. A dose-dependent rise in ALP mRNA levels was seen in all cell lines ($p<0.05$) whereas changes in COL1 mRNA levels with hydrocortisone dose varied in the different cell lines. In contrast to the other cell lines, in which both GR alpha and MR mRNA expression was significantly inhibited by added hydrocortisone, the cells with highest ALP mRNA expression had unchanged GR alpha mRNA but significantly increased levels of MR mRNA with increased hydrocortisone concentration ($p<0.05$). GR beta mRNA was detectable only by nested PCR, resulting in an assay of insufficient precision to detect changes in GR beta mRNA comparable to those seen GR alpha mRNA.

Thus, eight days culture in added hydrocortisone resulted in down-regulation of GR alpha and MR mRNA only in relatively undifferentiated cultured human osteoblasts. In more differentiated cells, MR mRNA levels were elevated in response to increased hydrocortisone concentration.

P22

ASSOCIATIONS IN BONE TURNOVER BETWEEN THE ENDOCORTICAL AND CORTICAL COMPARTMENTS OF THE HUMAN FEMORAL NECK

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Hip fracture is characterised by the loss of cortical and not cancellous bone. Mechanisms leading to increased cortical thinning and porosity are therefore important determinants of femoral neck fragility. In this study we compared intra-cortical and endocortical bone remodelling in the femoral neck of female fracture cases (n=12, mean age±SE = 81.3±1.3) and age/gender matched post-mortem controls (n=12; 81.4±1.8) in order to determine possible associations between these bone compartments.

Biopsies were fixed in 4% paraformaldehyde and dehydrated in ethanol for embedding in methyl methacrylate. Sections (10 microns) were stained with solchrome cyanine R for quatification of osteoid bearing canals and the extent of endocortical osteoid surface (%OS/BS). The osteonal wall thickness (W. Th.) and endocortical bone packet W.Th were measured under polarised light. Eroded cortical canals and endocortical surfaces (%ES/BS) were quantified on Goldner's stained sections. Over the whole biopsy, comparison of cortical and endocortical %OS/BS (Spearman-Rho analysis of mean ranked data) showed a significant correlation ($P=0.041$) between the endocortical and intra-cortical compartments. Regional analysis revealed significant correlations in the anterior ($P=0.026$), inferior ($P=0.0006$) and posterior ($P=0.047$) quadrants. With regards %ES/BS, only in the inferior region was there a significant correlation ($P=0.031$) between compartments. Endocortical W.Th. data was compared with cortical osteon W.Th where the canals were sub-divided into simple (canal surrounded by a single undisrupted cement line) and composite systems in which the osteon is comprised of at least 2 separate bone packets. Endocortical mean W.Th (31 microns ± 0.4) was identical to that in composite osteons (31 microns ± 0.9) but in both cases this was markedly lower ($P<0.0001$) than that in simple osteons (46 microns ± 1.8) as shown by contrast analysis within a regression model .

In conclusion, remodelling rates between the intra- and endocortical compartments are significantly associated suggesting that both are controlled by a common regulatory system. Furthermore, the W.Th. of endocortical bone packets was the same as that in composite osteons. Therefore it is possible to hypothesise that the formation of composite osteons, presumably by the merging of Haversian systems, is the initial step in cortical trabecularisation which eventually results in cortical thinning.

P23

REGULATION OF OSTEOBLASTS BY ACTIVATORS OF THE LXR AND FXR NUCLEAR RECEPTORS

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Previously we have shown,using both the CFU-f assay and an intact male rat model, that activators of the PPARalpha and/or PPARdelta receptors cause an increase in bone formation. Activation of the PPAR by its ligand, allows it to heterodimerise with retinoid X receptors and bind to the PPAR-responsive element on target genes. Other nuclear receptors also heterodimerise with the RXR and therefore we investigated whether activation of these receptors had an effect on CFU-f formation.

Briefly, bone marrow cells were isolated from rat tibias and femurs. 106 nucleated BMC were plated out in 55 cm2 petri dishes in DMEM containing 10% FCS, 10-8 M dexamethasone and 50 µg/ml ascorbic acid. The cells were treated until day 5 with agonists for the liver-activated receptor (LXR) or the farnesol-activated receptor (FXR). The medium was changed after 5 days and thereafter twice weekly. The cultures were maintained for 12 days after which the cells were washed with PBS and fixed by the addition of cold ethanol. After fixation, the cultures were sequentially stained for alkaline phosphatase (ALP), calcium and collagen-positive colonies.

Briefly, oxysterols, which act via the LXR receptor, either caused an increase in ALP expression, collagen expression and subsequent calcification, or had no effect on colony formation. Bile acids, which act via the FXR receptor, either caused an inhibition of ALP expression, collagen expression and subsequent calcification, or had no effect on colony formation.

These data suggest that the nuclear receptors LXR and FXR may also play a role in the regulation of bone formation.

P24

THE DIFFERENTIAL EFFECTS OF PHOSPHATIDYLINOSITOL 3-KINASE ON OSTEOBLASTIC DIFFERENTIATION BY PROSTAGLANDIN E2

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Prostaglandin E2 (PGE2) is a potent osteogenic stimulus in vivo and in vitro that induces the activation of specific signalling cascades. Previous studies have established a role for phosphatidylinositol 3-kinase (PI3K) in both osteoblast proliferation and differentiation and this study examines its role in transducing the osteogenic effects of PGE2. Bone marrow-like (BM) cells and osteoblast-like cells (OB) derived by outgrowth of rat femoral bones, were cultured in DMEM (10% FCS), until confluent. To examine the role of PI3K in PGE2-induced OB proliferation and differentiation, cells were treated with PI3K inhibitor, LY294002 (10 µM) for 1h, followed by a 72h incubation ± 1 µM PGE2 (DMEM + 3% FCS). Cell proliferation (DNA per well, Hoescht Assay) and differentiation (alkaline phosphatase activity, ALP, p-nitrophenol assay, ALP activity/ng DNA) were measured.

In BM cells, LY294002 inhibited basal proliferation and ALP by 22.5±2.0% ($p<0.01$), and 30.4±7.6% ($p<0.01$) respectively. Under these conditions, PGE2 alone had no effect on proliferation, but enhanced ALP by 134.8±56% ($p<0.01$). This PGE2-induced increase in ALP was inhibited by LY294002 (decreased by 32.9±6.8%, $p<0.01$). These results suggest that PI3K plays a role in both proliferation and differentiation of otherwise unstimulated BM cells, and that PGE2-induced increases in ALP are also PI3K-dependent. In OB, LY294002 had no effect on proliferation, but inhibited ALP by 18.3±3.7% ($p<0.05$) and PGE2 alone had no effect on proliferation, but enhanced ALP (22.1±2.8%, $p<0.05$). In marked contrast to BM, however, LY294002 treatment increased PGE2-induced ALP in OB by 35±3.0% ($p<0.01$).

Our results suggest that PI3K exerts differential effects on the differentiation of BM-derived and long bone-derived cells induced by PGE2. They also highlight that in contrast to BM cells, long bone-derived osteoblast proliferation is PI3K-independent. Thus, despite similar roles for PI3K in promoting differentiation in these cells under basal conditions, our results suggest that PI3K exerts differential effects by promoting BM-derived, and restricting long bone-derived, PGE2-induced differentiation. Further investigations into these differential effects of PI3K are currently ongoing.

P25

PROTEIN KINASE C MODULATES THE ROLE OF EXTRACELLULAR SIGNAL-REGULATED PROTEIN KINASES IN PROSTAGLANDIN E2-INDUCED OSTEOBLAST DIFFERENTIATION

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Prostaglandin E2 (PGE2) is produced by osteoblasts in response to mechanical stimuli and is thought to stimulate osteogenesis via activation of downstream signals. A role for extracellular signal-regulated protein kinases 1/2 (ERK1/2) in such responses, as well as their role in proliferation/differentiation is well described. In this study we examine whether ERK1/2 regulate PGE2-induced osteoblast proliferation and differentiation and determine whether protein kinase C (PKC) contributes to these responses.

To study the effect of PGE2 on ERK1/2 activation, osteoblast-like cells (from rat femurs) were incubated without serum for 1h with the PKC inhibitor (bisindolylmaleimide I, Bis I, 300nM), ± an inhibitor of MEK (ERK1/2's upstream activator, 1µM U0126) ± PGE2 (1µM) and activation (10min) assessed using phospho-specific (active-ERK) antibodies and Western blotting. We found that PGE2 and Bis I each independently induced ERK1/2 activation that was inhibited by U0126, and that Bis I further increased PGE2-induced ERK activation. The role of ERK1/2 and PKC during long term effects of PGE2 on OB proliferation/differentiation (DNA/well and ALP activity/ng DNA) were examined in cells pre-treated as described (above) followed by 72h incubation in DMEM (1% or 3% FCS). We found that U0126, but not PGE2 or Bis I, inhibited proliferation in 1% serum, whilst none modified proliferation in 3% serum. In contrast, ALP was unaffected by U0126, but inhibited by Bis I (16.7%, $p<0.05$) under basal conditions (1% serum). PGE2-induced increases in ALP (84.7%, $p<0.01$) were unaffected by U0126, but inhibited by Bis I (34.4%, $p<0.01$). In the presence of Bis I, however, PGE2-induced ALP was decreased by U0126 (20.8%, $p<0.01$). In 3 % serum, PGE2 had no effect, while Bis I inhibited ALP (33.8%, $p<0.01$).

These results suggest that ERK activation by PGE2 depends on the 'classical' MAPK cascade and that PKC is non-essential to this response. However, whilst PGE2's effects on OB differentiation appear independent of MEK, these exhibit a greater MEK-dependency when PKC activation is modulated, suggesting that PKC modifies ERK1/2's role during PGE2-induced osteoblast differentiation.

P26

SOYBEAN ISOFLAVONES AND RALOXIFEN IN POSTMENOPAUSAL WOMEN WITH BONE MASS LOSS

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Statement of purpose: determining if there are diferences in using soybean isoflavones and raloxifen on bone mass loss in postmenopausal women.

Statement of method: we studied for 20 months 24 women who were 45 to 62 years old at base line, were within 1 and 10 years of menopause, and had a bone mineral density at the lumbar spine between 150 mg/cc and 50 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 12 were assigned to therapy with soybean isoflavones 80 mg and 12 were treated with raloxifene HCL 60 mg. The SPSS programme was used for statiscal analysis.

Summary of results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 1 and 3 DS below the mean value for 30 years old normal premenopausal women. After a treatment statistically significant difference was found among the groups as for the bone mineral density at the lumbar spine.

Conclusions: it is necessary to carry out a wider study but it seems that raloxifene HCL contribute advantages versus isoflavones therapy to decrease the bone mass loss in postmenopausal women at least at lumbar spine.

P27

AUTOTRANSPLANTED TOOTH FROM TISSUE BANK AS ABUTMENT FOR PERMANENT PARTIAL DENTURE - DESCRIPTION OF A CASE

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Cryopreservation of teeth at low temperatures is currently not widely implemented despite the fact that in some cases (extractions for orthodontic, surgical and prosthetic reasons) tooth freezing is the only method of its salvation. The aim of this work is the evaluation of the possibility of using a fully impacted tooth cryopreserved at a low temperature in accordance with the Schwartz method as a bridge abutment.

This work describes a rare case of autotransplantation where a fully impacted canine has been transplanted in the place of a molar as a permanent denture abutment. The transplanted tooth 13 had a fully formed root and was stored in a Tissue Bank for 4 months after its surgical removal. After its de-freezing, autotransplantation into position 36 with the implementation of the two-stage method was performed. The tooth was treated endodonticaly and used as a bridge abutment.

The observation period of two-and-a half years demonstrated correct in-healing of the autotransplant retaining its full functionality. The tooth did not cause any clinical problems, neither parodontium pathology nor transplant mobility were noticed.

The authors presume that a tooth stored at a low temperature in a Tissue Bank and then re-planted may be used as an abutment for permanent dentures. The above case is treated as a preliminary report requiring further research.

P28

BONE TURNOVER IN MALES WITH BETA-THALASSAEMIA MAJOR

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Osteoporosis in beta-thalassaemia major is a well recognised complication affecting males worse than females probably due to enhanced genetic susceptibility of Sp1 polymorphisms interacting with risk factors. Chronic anaemia with ineffective erythropoesis and marrow expansion and iron overload from repeated transfusions impair osteoblast/ osteoclast function. Additionally, desferrioxamine itself inhibits DNA synthesis. Previous studies indicate a predominantly increased bone resorption responsive to iv pamidronate. We describe 3 post-pubertal active affected hypogonadal males on adequate testosterone replacement over the past 15 years, yet with osteoporosis and accelerated bone loss as determined by % change in BMD. Case 1 had severe osteoporosis as defined by DXA but no clinical fractures, with reduced bone resorption as reflected by low serum NTx and urine DPD. He was marginally hypocalcaemic with a raised total alkaline phosphatase (ALP), 66% bone isoform and had suboptimal vitamin D levels. He was also hypo-parathyroid presumably due to iron overload. However cases 2 and 3, were osteoporotic and osteopenic respectively and had accelerated bone loss over 4 years as measured by DXA Bone turnover markers, DPD and NTx were at the upper quartile for normal males indicating increased bone resorption, supporting published data as the mechanism. Adequate testosterone replacement over years in these cases did not halt bone loss, raising the question of the role of testosterone on bone turnover. They were hypogonadotrophic, with adequate growth hormone levels and IGFs and BP3. Case 2 was on adequate thyroxine replacement. Whilst bisphosphanates are not contraindicated in cases 2,3, their use in case 1 may result in an adynamic bone state. Bone biopsy would help in their management, but is invasive in patients already needle averse, biochemical markers of bone turnover may help in individualising osteoporosis treatments.

P29

COMPARISON OF PTH SECRETION AND BONE METABOLISM IN ADULT GROWTH HORMONE DEFICIENT PATIENTS WITH NORMAL AND REDUCED BONE MINERAL DENSITY

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AGHD is associated with reduced bone mineral density (BMD) and turnover. Target-organ insensitivity to effects of PTH may contribute to development of AGHD-related osteopaenia. Growth Hormone Replacement (GHR) results in increased PTH target-organ sensitivity, bone turnover and BMD, but some patients remain osteopaenic/porotic. Previous reports have not correlated BMD, in AGHD, with abnormalities in PTH target-organ sensitivity. Therefore, we compared markers of bone metabolism in GH naïve (GHN) and GHR AGHD patients, with normal and reduced BMD.

43 AGHD patients adequately replaced with pituitary hormones were hospitalised for 24 hours. 25 patients were GHN: 13 had reduced BMD (median hip T-score(range) -1.8(-3.1 to -1.0)), and 12 normal BMD (0.6(-0.8 to 2.2)). The other 18 patients were GHR for 2 years: 10 had reduced BMD (-1.5(-1.9 to -1.0)), and 8 normal BMD (0.5(-0.1 to 2.2)). Half-hourly blood and 3-hourly urine samples were collected for PTH, calcium, phosphate, nephrogenous cAMP (NcAMP, marker of renal PTH activity), CTx (bone resorption marker) and PINP (bone formation marker). Serum calcium was adjusted for albumin (ACa). Results are expressed as mean±SEM.

24-hour mean PTH was higher in the reduced BMD GHN than normal GHN group (6.63plus/minus0.15pmol/L versus 3.91plus/minus0.15pmol/L, $p<0.001$), while NcAMP (20.6plus/minus3.1nmol/LGFR versus 31.5plus/minus2.2nmol/LGFR, $p=0.004$) and ACa (2.29plus/minus0.004mmol/L versus 2.32plus/minus0.004mml/L, $p<0.001$) were lower. In the GHR group, PTH (3.55plus/minus0.15pmol/L versus 4.52plus/minus0.18pmol/L), NcAMP (21.9plus/minus1.9nmol/LGFR versus 32.7plus/minus2.4nmol/LGFR) and ACa (2.35plus/minus0.004mmol/L versus 2.37plus/minus0.005mmol/L) were lower ($p<0.01$) and serum phosphate higher (1.14plus/minus0.01mmol/L versus 1.13plus/minus0.01mmol/L, $p=0.03$) in reduced BMD than normal patients. CTx was higher in both GHN (0.41plus/minus0.06ng/ml) and GHR (0.39plus/minus0.06ng/ml) reduced BMD patients than GHN (0.21plus/minus0.06ng/ml) and GHR (0.29plus/minus0.07ng/ml) normal patients ($p<0.02$), but PINP was similar ($p=0.50$).

Relative PTH target-organ insensitivity and increased bone resorption exists in reduced BMD GHN compared to normal GHN patients, factors which may contribute to AGHD-related osteopaenia/porosis. In GHR patients, NcAMP, ACa and serum PO4 are appropriate to PTH levels, indicating restoration of PTH sensitivity. However, PTH activity is lower and bone resorption is higher in reduced BMD GHR than normal GHR patients. A mechanism such as delayed response to GHR may be important in the persistence of low BMD in some GHR AGHD.

P30

MARKERS OF BONE TURNOVER AND DISEASE STATUS IN PROSTATE CANCER

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Markers of bone turnover are elevated in metastatic bone disease and may be useful in tracking disease progression. The aims of this study were to determine which markers of bone turnover best indicated disease status in newly diagnosed patients and indicated relapse after treatment, with androgen ablation, in patients with prostate cancer.

Newly diagnosed patients were classified as having localised disease (T1/2)(n = 30, mean age 64.6 years) or nonlocalised disease (T3/4) (n = 27, mean age 74.2 years). Patients treated with androgen ablation were classified as responders (n = 12, mean age 75.8 years), borderline responders (n = 11, mean age 76.2 years) or refractory (n = 25, mean age 74.8 years).

Serum formation markers measured were osteocalcin (OC), procollagen type I N terminal propeptide (PINP) and bone alkaline phosphatase(bone ALP). Serum resorption markers measured were C terminal cross-linking telopeptide of type I collagen (betaCTX), C terminal cross-linking telopeptide of type I collagen generated by MMPs (CTX-MMP), and tartrate resistant acid phosphatase 5b (TRACP 5b)

In patients with nonlocalised disease at diagnosis median serum levels of four of the markers measured were significantly higher than in patients with localised disease at diagnosis: PINP (51.3 vs 37.7 ng/ml, $P<0.01$), bone ALP (28.5 vs 22.7 U/L, $P<0.05$), betaCTX (0.255 vs 0.135 ng/ml, $P<0.05$), and CTX-MMP (5.29 vs 3.53 ng/ml, $P<0.001$). In patients with advanced disease being treated by androgen ablation there was no significant difference in serum levels of any of the makers between the group who were responding and the group who were only showing a borderline response. In the group who had become refractory to treatment PINP, bone ALP , beta CTX and CTX-MMP were higher than in the responding group however only the difference in bone ALP (89.0 vs 27.2 U/L, $P<0.01$) and CTX-MMP (8.72 vs 4.57, $P<0.05$) reached significance. (T tests followed by Bonferroni correction)

We conclude that different markers maybe useful in assessing skeletal involvement at different stages of disease. Longitudinal studies are required to investigate whether any of the markers of bone turnover can predict disease progression disease progression.

P31

BONE MASS ACQUISITION DURING CHILDHOOD IS RELATED TO TOTAL ENERGY INTAKE IN INFANCY

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Environmental exposures in early life may influence peak bone mass development by causing irreversible changes in the mechanisms which control the trajectory of bone development in childhood. To investigate the role played by early life nutrition, we examined whether diet at age 18 months is related to bone mass as measured eight years later at age 9.5. A preliminary analysis was performed based on 757 children randomly drawn from the Avon Longitudinal Study of Parents and Children (ALSPAC). The latter is a population-based prospective cohort study consisting of children born in Avon with an expected delivery date between April 1991 and December 1992. Diet was assessed at age 18 months by three-day diet diaries completed by the mother, and the following variables were subsequently coded: total energy intake (TEI), and intake of fat, carbohydrate, protein, calcium and vitamin D. Total body bone mineral density (TBBMC) was measured by Lunar Prodigy at a mean age of 9.5 years. The following dietary variables were found to be positively related to age-adjusted TBBMC on partial correlation analysis: total energy intake (TEI) (r=0.11, p=0.003), carbohydrate intake (r=0.11, p=0.004), fat intake (r=0.07, p=0.03) and protein intake (r=0.10, p=0.008). However, on multiple regression analysis, TEI was the only dietary variable found to be independently related to TBBMC. We then investigated whether our results reflected a relationship between TEI and skeletal envelope size and/or bone mineral density. Although an equivalent association was found between TEI and skeletal area to that observed for TBBMC, when the latter was adjusted for skeletal size by dividing by skeletal area, a significant relationship with TEI was no longer seen. We conclude that TEI as assessed at the age of 18 months is significantly related to TBBMC at age 9.5, reflecting an association between energy intake in infancy and subsequent bone growth. These findigs suggest that it may be possible to improve understanding of how early life factors influence the trajectory of bone development in childhood, by investigating the determinants of food intake in infancy.

P32

BONE DENSITY, BODY COMPOSITION AND BONE TURNOVER IN HEALTHY AND OSTEOPOROTIC MEN

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Previous work showed that lumbar spine (LSBMD) and femoral neck bone density (FNBMD) were lower, and sex hormone binding globulin (SHBG) and urinary free deoxypyridinoline/creatinine (fDPD/Cr) were greater in men with idiopathic vertebral osteoporosis (IVO). The latter suggests increased catabolism. It has been suggested that elevated SHBG corresponds with decreased insulin-like growth factor (IGF-I). This may modulate body mass in men with IVO.

We have measured whole body bone mineral content (WBBMC), lean body mass (LBM), total body mass (TBM), fat mass (FM), LSBMD, FNBMD, IGF-I, IGF binding protein 3 (IGFBP3), fDPD and uCr in 142 healthy men (age; mean± SD, 52.6±16yr) and in 21 MEN with IVO (defined by vertebral fracture of 20% or more deformity) (60.6±13yr). We have also explored changes in fDPD and fDPD/Cr in 9 men with IVO following bisphosphonate treatment for 8.4±2yr.

LSBMD (0.879±0.18g/cm2 vs 1.055±0.15g/cm2), FNBMD (0.713±0.14 g/cm2 vs 0.844±0.13g/cm2), WBBMC (2258±481g vs 2731±356g) and LBM (55327±7436g vs 60131±7275g) were all lower in men with IVO ($p<0.05$). Differences in TBM and FM were not significant. fDPD (38.6±23 vs 26.6±15nmol/d) and fDPD/Cr (6.43±4 vs 3.15±1nmol/mmol) were greater ($p<0.01$), and uCr lower (6.6±2.4 vs 8.4±3.4mmol/l) in men with IVO ($p<0.05$). In limited number of subjects IGF-I and IGFBP3 were not significantly different in healthy and IVO men, and did not correlate with fDPD. In healthy men both IGF-I and IGFBP3 declined with age ($p<0.05$) but neither fDPD nor fDPD/Cr changed significantly. In the healthy men, FNBMD was related to IGF-I (r2=0.26) and IGFBP3 (r2=0.31) ($p<0.01$). In preliminary studies bisphosphonate treatment decreased fDPD in men with IVO (56.3±15 vs 17.6±6nmol/d) and fDPD/Cr (10.2±5 vs 5.8±2nmol/mmol respectively).

These early data suggest that as well as LSBMD and FNBMD being low, WBBMC and LBM are also decreased in IVO, suggesting a more generalised effect on body composition. However IGF-system components are unchanged. In men with IVO IGF-I correlated with FNBMD but not with bone resorption. The higher fDPD levels, restored to normal by bisphosphonates, suggest that enhanced bone breakdown may be important in IVO.

P33

BONE TURNOVER FOLLOWING TIBIAL SHAFT FRACTURE

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Tibial shaft fractures are common, particularly in young men. Up to 35% of these fractures are complicated by delayed union. Currently there is no method of predicting which patients will develop this complication. The aim of this study was to measure the biochemical markers of bone turnover following fracture and to compare the results in those with delayed and normal union.

Biochemical markers of bone turnover were measured over a 24 week period in 19 subjects (mean age 34, range 18-78) treated with either a cast or intramedullary nail following a tibial shaft fracture. The bone formation markers were bone alkaline phosphatase (Bone ALP), osteocalcin (OC) and procollagen type I N-terminal peptide (PINP). The bone resorption marker was serum C-telopeptides of type I collagen (s-beta CTX). A marker of collagen III turnover, procollagen type III N-terminal peptide (PIIINP) was also measured as type III collagen is initially present in fracture callus and is later replaced by type I collagen.

A significant rise was seen in all the markers with levels remaining elevated at 24 weeks following fracture except for OC where no change occurred. Seven subjects (37%) with radiologically determined delayed union, all of whom were treated with an intramedullary nail, had a larger increase in area under the curve measurements of OC up to week 2 ($p=0.04$) and PINP up to week 4 ($p=0.04$) compared to subjects with normal union.

Following tibial shaft fracture a high turnover state occurs with the maximal mean percent increase in bone formation and resorption markers within the first 4 weeks with levels remaining elevated even after union has occurred. In subjects with delayed union a larger increase is seen for the bone formation markers OC and PINP in the first 2-4 weeks compared to those with normal union. This increase may reflect an exaggerated response to an unfavourable healing environment or may simply reflect the additional surgical insult.

P34

ACCURACY AND PRECISION OF PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY MEASUREMENTS AT THE TIBIAL METAPHYSIS

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Peripheral quantitative computed tomography (pQCT) is an alternative technique to dual-energy X-ray absorptiometry (DXA) for measuring bone mineral density. In contrast to DXA, pQCT measures the true volumetric BMD (mg/cm3) and allows for separate assessment of trabecular and cortical bone. The accuracy and precision of pQCT at measuring cortical and trabecular bone separately has not been reported.

The accuracy of a peripheral quantitative computed tomography (pQCT) scanner, the Stratec XCT-2000 was evaluated by comparing the bone mineral content (BMC) with the ash weight at three different metaphyseal regions (proximal 5% and 10%, distal 4%) using five tibias from four young adult cadavers (ages 36-44). The accuracy of 30 pQCT software analysis modes at discriminating cortical from trabecular bone was also determined by comparing the cortical and trabecular area measured by each analysis to the area measured using a radiographed section of the cadaver. The precision of pQCT was calculated using duplicate measurements made at all three regions in a cross-sectional study of the non-fractured limbs of 28 subjects (mean age 41, range 17-78) with tibial shaft fractures.

Highly significant correlations (r, 0.71-0.98) and moderate accuracy (CV, 5-22%) was found between ash weight and BMC for the most accurate analysis mode with similar results for total, cortical and trabecular bone. All the software analysis modes had a good accuracy when measuring total bone area and a poor accuracy when measuring cortical bone area at the proximal 5% and distal 4% regions. For trabecular bone measured at all the regions and cortical bone area measured at the proximal 10% region the Stratec peel mode 5 was the most accurate analysis mode. Precision of bone mineral density (BMD) measurements was good in all regions (total, CV 2-5%, trabecular, CV 2-5%, cortical, CV 4-6%).

pQCT is a moderately accurate, precise method of measuring cortical, trabecular and total BMC at the tibial metaphysis. The authors recommend caution when interpreting results for separate cortical and trabecular BMD as cortical area measurements are inaccurate and less precise especially at sites rich in trabecular bone.

P35

VISUAL IDENTIFICATION OF PREVALENT VERTEBRAL FRACTURES: COMPARISON BETWEEN METHODS

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The aims of this study were to test agreement between different visual approaches, evaluate causes of disagreement and identify the optimum approach.

We studied 372 women from local General Practice lists. Spinal radiographs were assessed independently by two readers for vertebral fracture and non-fracture deformity. Reader 1, an experienced clinical radiologist performed a qualitative assessment (Qual) with no pre-defined criteria for diagnosis. Reader 2, a research radiologist used two methods (1) a semi-quantitative approach (SQ), and (2) visual diagnosis according to predefined qualitative criteria for vertebral fracture and vertebral deformity (PQual). Readers 1, 2 and an experienced clinical investigator adjudicated all fractures not confirmed by all methods.

Women with vertebral fractures identified by consensus reading (either by confirmation by the 3 methods, or by adjudication) had lower bone mineral density (BMD) in comparison to those without fractures. The difference in BMD between women with and without vertebral fractures was greater when fractures were identified by Qual and PQual than by SQ. Non-fracture deformities identified by Qual and PQual were not associated with low BMD. The prevalence of vertebral fracture by PQual was similar (6.7%) to consensus reading (6.7%), but was higher for Qual (10.5%) and SQ (23.7%). PQual had better agreement (k =1.00) with the consensus reading (for identification of patients with fracture) than Qual (k =0.66) and SQ (k =0.36). All fractures in consensus reading involved fracture of the cancellous endplates, with or without fracture of the cortex of the vertebral body. False positives were mainly due to wedge deformities (Qual) and inclusion of non-fracture deformities (SQ). Degenerative changes (mainly following Schuermann's disease) accompanied non-fracture deformity.

The optimum approach for visual identification of prevalent vertebral fracture is PQual. Our results indicate that better understanding of the structural changes involved in osteoporotic vertebral fractures will improve their identification.

P36

VITAMIN D STATUS OF DEPRIVED CHILDREN IN DELHI, INDIA: SEASONAL VARIATION & THE EFFECT OF ORAL VITAMIN D SUPPLEMENTATION

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We have previously reported that children living in an area of high atmospheric pollution in Delhi had significantly lower serum concentrations of 25-hydroxyvitamin D (25OH D; a marker of an individual's vitamin D status) than those living in an area of the city with low atmospheric pollution #. The aims of this study were: (1) to examine if there was a seasonal variation in serum 25OHD concentrations in children living in an area of high atmospheric pollution in Delhi (latitude 28.30 degrees North). (2) To examine the increase in serum 25OHD2 concentrations after oral administration of a single dose of 50,000 IU vitamin D2, in winter.

Serum concentrations of 25OHD3 & 25OHD2 were measured in early spring (March 2001; n=13) and early fall (August 2001; n=23) in children whose median age was 24 months (10 to 36). These children were administered 50 000 IU of Vitamin D2 or placebo (n=18 in each group) in October and their serum concentrations of 25OHD3 & 25OHD2 measured 12 weeks later.

The mean (SD) 25(OH)D3 concentration in spring was 15.2ng/ml (13.7) while in summer, it was 30.2ng/ml (6.7)($p<0.001$). Serum 25(OH)D2 concentration increased by 3.2 ng/ml (SD 0.7) ($p<0.01$) in those who received a single dose of 50,000 IU vitamin D2. Six children in the supplementation group had no measurable 25(OH)D2.

Even in sunny Delhi, there was a seasonal variation in 25(OH)D3 concentrations. A single oral dose of 50,000 IU of vitamin was inadequate in raising the winter levels of vitamin to those found in summer. A further supplementation study, using a higher dose of oral vitamin is needed.

Reference; #Agarwal KS, et al. Arch Dis Child 2002; 87:111-113

P37

THE EFFECT OF CALCIUM SUPPLEMENTATION ON LEPTIN LEVELS, FAT ACCUMULATION AND MENARCHE

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We have previously shown that calcium supplementation in adolescent girls (11-12 years) is associated with an earlier menarche. In an 18 month, randomised, controlled trial, the mean age at menarche was four months earlier for subjects receiving a daily supplement of 792 mg of calcium, as calcium citrate malate (CCM), compared with a control group. Calcium has the potential to affect pubertal development through influencing leptin secretion by adipocytes. The aim of this study was to explore if leptin levels and fat mass accumulation were congruent with the earlier menarche in the supplemented group.

Serum levels of leptin (pg/ml) were measured at baseline and at 6 and 12 months. Total fat mass (g) was measured at the same time points using DXA. The % change in leptin and fat mass at 6 and 12 months were calculated, using the baseline measurement as the denominator. These % change values were plotted and the area under the curve (AUC) calculated.

For leptin, the AUC for the supplemented group was 429.2 (S.D. 839.2) and for the control group 479.7 (S.D. 639.0). For fat mass, the AUC for the supplemented group was 59.3 (S.D. 100.2) and for the control group 95.0 (S.D. 124.6). A t-test showed that there was no significant difference between the supplemented and the control group in the AUC for leptin ($p>0.05$). Similarly, there was no significant difference for fat mass ($p>0.05$). However, there was a trend for fat mass accumulation to be less in the supplemented group, which concurs with the published data on dietary calcium and body composition (Heaney, 2002). We conclude that the effect of CCM supplementation on menarche is unlikely to be mediated by leptin. An alternative explaination is that the effect is mediated by the action of calcium on the hypothalamic-pituitary-ovarian axis (Thys-Jacobs, 2000)and we plan to investigate this next.

References:

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P38

SEMI-AUTOMATED METACARPAL MORPHOMETRY (METACARPAL CORTICAL INDEX) PREDICTS FRACTURE RISK IN ELDERLY WOMEN

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Metacarpal cortical index (MCI) is known to predict future fracture risk. We have evaluated a new, rapid semi-automated technique to derive MCI from hand radiographs using a digitising tablet.

We studied 4929 women aged 75 years or older participating in the MRC HIPS study which was designed to evaluate risk factors for fracture combined with a placebo-controlled trial of oral clodronate (Bonefos) for fracture prevention. Bilateral hand radiographs were obtained at baseline and the measurements were captured using a transparent cross-hair cursor with the films placed on a backlit digitising tablet. The length, diameter and cortical widths of the second, third and fourth metacarpals were measured by a single operator (LR) and stored automatically in an electronic database. The MCI was calculated for both hands separately and an average value was also derived (AMCI). This analysis remains blinded to the treatment allocation.

During a median follow-up of 4 years, 792 women sustained at least one fracture; of these 180 sustained hip fractures and 658 sustained non-hip fractures. At baseline, these women had significantly lower weight, total hip BMD, left hand MCI, right hand MCI and AMCI (all P= or <0.001). In univariate analysis the gradient of risk of fracture (odds ratio, 95% confidence interval) for 1 standard deviation decrease in AMCI was 1.42, 1.22-1.65 for hip fractures; 1.24, 1.14-1.35 for non-hip fractures; and 1.30, 1.20-1.40 for all fractures (all P<0.001). The gradients of risk with AMCI were either similar or higher than with left or right MCI alone. The corresponding ORs for total hip BMD were 2.09, 1.80-2.43; 1.46, 1.34-1.49; and 1.61, 1.49-1.74 respectively. After adjusting for age and body weight in forward-conditional regression, AMCI remained significantly associated with both hip and non-hip fractures.

We conclude that metacarpal cortical index (AMCI) computed using this rapid technique is an indicator of future hip and non-hip fracture risk in elderly women in the community. As hand radiographs are inexpensive and easy to access, this technique could have wide applicability in screening and management of osteoporosis in the community, especially where access to DXA is limited.

P39

VITAMIN D STATUS ACROSS EUROPE : IMPACT OF GEOGRAPHICAL LOCATION, SEASON, AND BODY WEIGHT

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Although it is commonly assumed that latitude is an important factor influencing vitamin D status, these geographical differences are poorly characterised. Most studies are biased by non-concurrent sample collection or biochemical measurement. It has also been suggested that obese individuals may be more susceptible to Vitamin D deficiency. We examined sources of variation in plasma 25(OH)Vitamin D (25D) within the general population in 5 European centres (Aberdeen, Berlin, Kiel, Paris, Sheffield). Participants were a subset of women in the Osteoporosis and Ultrasound study (OPUS). Women were selected at random from population or general practice registers. Parathyroid hormone (PTH) and 25(OH) Vitamin D (25D) were measured in 1169 women (mean age 60 years, range 20 to 80). Collection was completed over 24 months ending April 2001, serum was collected between 12:00 and 15:00, and assays were performed in a single center in a randomised order.

In contrast to expectations, 25D was unrelated to latitude, and was slightly lower in Paris (41.1 plus/minus 1.9SEM nmol/L) than in the other 4 centers (ANOVA P<0.01; Aberdeen 50.4 plus/minus 2.0, Berlin 54.0 plus/minus 2.3, Kiel, 52.3 plus/minus 1.9, Sheffield 49.9 plus/minus 1.8; ANOVA P <0.001; Post-hoc Scheffe test). These differences persisted after correction for season and menopausal status. Time of year accounted for 12.8% of the variance in 25D (Cosinor analysis, P< 0.0001, amplitude 20.8%, peak at day 240). Mean body mass index (BMI) was 26.2 kg/m2 (95% range 18.9 - 36.6). BMI accounted for a small but highly significant component of variation in 25D (r=-0.13; R2=1.7%; P<0.001) and PTH (r=0.15; R2=2.2%; P<0.001) which persisted after correction for between center and seasonal differences in body weight.

In conclusion: Sunshine behaviour and skin pigmentation are likely to be more important determinants of 25(OH)D than latitude. Increased body weight may be associated with lower 25D due to decreased sunshine exposure or increased volume of distribution.

P40

DIGITAL X-RAY RADIOGRAMMETRY OF THE METACARPALS IN PAEDIATRIC SUBJECTS

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The aim of this study was to evaluate age-related changes in metacarpal morphometry and areal bone mineral density (aBMD) in normal paediatric subjects using digital X-ray radiogrammetry (DXR). From digitised hand radiographs, this method analyses the second, third and fourth metacarpals and gives an assessment of the metacarpal index (MCI), cortical thickness (cm) (CT), outer bone width (cm) (BW) and areal bone mineral density (aBMD) (g/cm2).

Non-dominant hand radiographs of 119 healthy Caucasian children (mean age 11.3 +/- 3.6, range 5.4-19.0 years) were assessed using the Sectra Pronosco X-posure system(TM) (version 2). Measurements of height, weight, grip strength and pubertal stage were made. Statistical analysis was according to age, gender, pre-pubertal or pubertal status and in females, Tanner stage.

CT, BW, MCI and aBMD increased with age in males and females (p<0.05-0.001). Females had a higher MCI but a lower BW compared to males (p<0.001). CT was higher in females overall (p<0.05), but this difference was not significant when the genders were divided into pre-pubertal or pubertal groups. There was no gender difference demonstrated in aBMD. MCI, CT and aBMD increased in females at Tanner stage 3. Positive correlations were found for all subjects for age, height, weight, grip strength, MCI, CT, BW and aBMD. However, MCI did not correlate significantly with grip strength in pubertal males.

DXR is a useful method for the evaluation of age- and gender-related changes in metacarpal morphometry and aBMD in normal paediatric subjects. This valuable information can be utilised for the study of paediatric conditions associated with increased fracture risk.

P41

SERUM INTACT AND C-TERMINAL FGF-23 FOLLOWING ACUTE AND CHRONIC PHOSPHATE SUPPLEMENTATION

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Hypophosphatemia and hyperphosphaturia in oncogenic osteomalacia, X-linked hypophosphatemia and autosomal dominant hypophosphatemic rickets may be mediated by fibroblast growth factor (FGF-23). The role of FGF-23 as a physiological phosphatonin is uncertain. We have previously shown that serum C-terminal FGF-23 is increased in response to chronic phosphate supplementation in human subjects (weeks). However, it is not clear 1) whether this reflects secretion of biologically active 'intact' FGF-23 or 2) whether FGF-23 responds to acute alterations in dietary phosphate intake. We evaluated the response of FGF-23 to phosphate supplementation using an ELISA which requires the presence of N-terminal and C-terminal portions of FGF-23 (iFGF-23), and compared this to a C-terminal fragment detecting assay (cFGF-23). Samples were taken from twelve healthy men (ages 19 - 38 years) whose phosphate intake was increased over a five week period. For the first week, participants were studied on their habitual diet. Participants were then studied for 4 weeks on a standardised diet containing 1000mg/d phosphate and 1000mg/d calcium. No supplement was given for one week, followed by 1000mg/d, 1500mg/d and 2000mg/d elemental phosphate on successive weeks (total dose 1000mg/d, 2000mg/d, 2500mg/d, 3000mg/d respectively). Blood samples were collected before breakfast at the end of each week. Serum intact FGF-23 was measured using iFGF-23 and cFGF-23 assays. We also evaluated the response to a single oral dose of phosphate (2000mg at 9am, following an overnight fast) in comparison with placebo over 4 hours (n=10).

Fasting serum FGF-23 increased in response to escalating-dose phosphate supplementation with both cFGF-23 and iFGF-23 assays(increase 36.8%±21SEM and 43.0%±18SEM at the highest dose respectively; Repeated measures ANOVA for trend, both P<0.01). However there was no response to acute supplementation. We conclude that chronic phosphate loading in healthy adults results in a modest causes an increase in intact as well as C-fragment FGF-23. FGF-23 may be involved in long-term regulation of phosphate metabolism, but is unlikely to mediate acute renal tubular adaptation to phosphate intake.

P42

RELATIONSHIP BETWEEN BONE MARKERS, AGE AT START OF TRAINING, AND RISK OF FRACTURE AND FATIGUE INJURY OF THE THIRD METACARPAL BONE IN TWO YEAR OLD RACEHORSES

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The objective of this study was to determine if biochemical markers of bone cell activity measured before the start of training are associated with increased risk of fracture and fatigue injury of the third metacarpal bone (specifically dorsal metacarpal pain or 'sore shins') in two year old racehorses.

Blood samples were collected from 164 thoroughbreds in 10 racing stables during November/December 1998 before animals had undertaken any training. Age range was 18 to 23 months. Osteocalcin (OC) and the carboxy-terminal propeptide of type I collagen (PICP) were measured as markers of bone formation, and the carboxy-terminal telopeptide of type I collagen (ICTP) as a marker of bone resorption. Records of veterinary treatment over the following training/racing season were kept for each horse.

During the season there were 20 confirmed fractures (12.2%) and 24 cases of metacarpal fatigue injury (14.6%). Biochemical marker concentrations in horses with a confirmed fracture were not significantly different from those that didn't fracture. Neither was there any significant difference in age between these two groups. In contrast, OC and ICTP were significantly increased in animals that went on to have an episode of dorsal metacarpal pain. Mean OC concentrations were 38.55 v 33.97ng/ml (P = 0.017), and mean ICTP concentrations 13.42 v 12.6ug/L (P = 0.019) in horses with and without metacarpal fatigue injury respectively. Age was also significantly higher in the animals with metacarpal fatigue injury (P = 0.023). When animals were divided into two equal groups on the basis of age at start of training the prevalence of metacarpal fatigue injury was 8.5% in animals aged up to 20 months old and 20.7% in animals over 20 months old, with an odds ratio of 2.73.

In conclusion, bone markers measured before the start of training are higher in animals that go on to suffer fatigue injury of the third metacarpal bone. Age at start of training is also associated with an increased risk of developing this condition. However, age and bone marker concentrations before the start of training appear to be of less value in identifying animals that will subsequently go on to fracture.

P43

BONE MINERAL DENSITY (BMD) IN CHILDHOOD SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) TREATED WITHOUT CRANIAL IRRADIATION (XRT)

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We have previously shown that adult survivors of childhood ALL treated with multi-agent chemotherapy, steroids and cranial XRT have a highly significant reduction in volumetric spinal BMD. As cranial XRT has been implicated as a factor for reduced BMD in ALL survivors, we examined the BMD of the distal radius and the lumbar spine (LS) in 56, [25 males] childhood survivors of ALL who had completed their treatment for ALL at least one year previously without cranial XRT.

In this cross sectional study, we measured bone mineral content (BMC) and bone area (BA) of L1-L4 vertebrae (LS) using the Hologic QDR-4500 dual energy x-ray absorptiometry scanner, and distal radius volumetric trabecular BMD (DRvTBMD; mg/cm^3) using the Stratec XCT 2000 peripheral quantitative computer tomography scanner. The volumetric or bone mineral apparent density (BMAD; g/cm^3) of LS was calculated by dividing LS BMC by LS BA^1.5 (*Carter DR et al J Bone Miner Res. 7: 137–145, 1992*). LS BMAD and DRvTBMD in each childhood survivor of ALL was compared with that of gender and age matched control, using the Wilcoxon test.

The table shows age, height, weight, BMI, LS BMAD & DRvTBMD in the two groups, expressed as median and inter-quartile range. There were no gender differences in the LS BMAD & DRvTBMD among the childhood survivors of ALL.

	Childhood Survivors of ALL	Matched Control	P (95% CI for median difference)
Age	11.1 (8.9 to 13.4)	11.0 (9.0 to 13.6)	
Height	145 (33 to 158)	146 (136 to 158)	0.57 -3.1 to 1.7
Weight	42.9 (32.6 to 53.5)	39.6 (31.5 to 54.4)	0.83 -2.2 to 5.3
BMI	19.5 (17.8 to 22.1)	18.62 (16.3 to 21.0)	0.061 -0.06 to 2.5
LS BMAD	0.21 (0.19 to 0.23)	0.21 (0.20 to 0.23)	0.14 -0.02 to 0.003
DRvTBMD*	172 (1578 to 188)	179 (160 to 217)	0.012 -23.7 to - 3.1

* one missing value n=55

We found significantly reduced DRvTBMD among childhood survivor of ALL treated without XRT, compared with healthy controls. As BMD is a surrogate measure of bone strength, childhood survivor of ALL may be at increased risk of sustaining distal forearm fractures.

P44

SOURCE OF MATERNAL SERUM OSTEOPROTEGERIN DURING HUMAN PREGNANCY

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Animal studies have indicated that serum osteoprotegerin (OPG) is increased in pregnancy and might prevent excessive maternal bone resorption. The physiological role and tissue source of circulating OPG in human pregnancy is uncertain. The breast, foetus, placenta and skeleton are amongst the potential sources. Serum OPG exists in several forms and it is also possible that the apparent increase during pregnancy is dependent on the choice of assay.

We assessed serum OPG in a longitudinal study of planned human pregnancy and lactation (n=17), in human neonates (n=22), human breast milk (n=5), placental tissue and arterial/venous umbilical cord serum (n=9). Fasting morning blood samples and 24 hour urine samples were collected before conception, at 16, 26 and 36 weeks gestation, and at 2 and 12 weeks postpartum. OPG was measured using three different immunometric ELISA assays. Results were similar using all three assays.

Serum OPG increased to 210%±16 (P< 0.001) by 36 weeks gestation. Human breast milk contained substantial amounts of OPG (162000pg/mL SEM 58000, n=5 at 1-6 days postpartum vs. 863pg/mL SEM 97 in maternal serum). However serum OPG declined rapidly postpartum (P<0.01) and was close to pre-conception values by 2 weeks postpartum. This decline was independent of lactation. Serum OPG was lower in neonates than in premenopausal women (289±91SD pg/mL vs. 420±167 pg/mL; P<0.001) making a foetal source of OPG unlikely. Western blot analysis of human placental tissue indicated the presence of 2 immunoreactive OPG components (minor 66kDa, major 36kDa). OPG in venous and arterial umbilical cord blood (foetal circulation) was not elevated relative to maternal serum OPG at 36 weeks gestation and there was no atrerio-venous difference. There was no correlation between the change in OPG and bone turnover (uNTX, sCTX, bone ALP) at 36 weeks gestation.

The tissue source of circulating OPG in pregnancy remains uncertain, but a placental source seems likely. The rapid postpartum decline in maternal OPG, and low levels of OPG in neonates suggest that the breast and fetus are unlikely sources.

P45

ROLE OF OSTEOPROTEGERIN GENE POLYMORPHISMS IN OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common joint disease. The major focus of OA research has been on articular cartilage degeneration since this is considered the initial event in the disease process. However, OA is invariably associated with sclerotic changes in the subchondral bone. It is clear that OA has a genetic component and one approach has been the analysis of single nucleotide polymorphisms (SNPs) in candidate genes implicated in OA pathogenesis. Osteoprotegerin (OPG) is a soluble receptor for RANKL and acts as an inhibitor of osteoclast formation and activity. The aim of this study was to evaluate the potential role of SNPs in the OPG gene as a candidate for mediating bone changes seen in OA.

DNA was extracted from blood of 180 patients with primary OA (140 knee and 40 hip) and 193 controls who consisted of healthy Caucasian blood donors from the South-West region. Four previously described SNPs of the OPG gene (T950-C, A163-G, G1181C, C4441-T; Wuyts et al. Bone 2001,28:104; Langdahl et al. J Bone Miner Res 2002,17:1245) were determined using induced heteroduplex generators. The presence of a novel microsatellite dinucleotide (CA) repeat polymorphism with 5 alleles in intron 3 of which 3 were sequenced (Genbank Accession numbers AY168337, AY168338, AY168339), was detected using Polymerase Chain Reaction Single Strand Conformation (PCR-SSCP) analysis. Haplotype loci analysis of the allele frequencies were determined using PHASE software (Bayesian statistics). Differences in 5-locus haplotype frequencies between controls (n=386) and patients (n=360) were determined by Chi-squared or Fisher's Exact analyses.

Analysis revealed the presence of 21 and 18 haplotypes in controls and OA patients respectively, 15 of which were represented in both groups. The frequency of a CG2C2 haplotype was significantly different (P=0.007) between controls (n=0) and patients (n=7) and the frequency of a CA2C2 haplotype was greater (p=0.06) in controls (n=5) than in patients (n=0). The CG2C2 haplotype may be a risk factor within this small group of patients whereas the CA2C2 haplotype may act as a protective genotype. This study suggests that a more detailed analysis of SNPs in the OPG gene in patients with OA is warranted.

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THE RELIABILITY OF FOLTIN'S CLASSIFICATION FOR ASSESSING THE BONE QUALITY OF THE PROXIMAL TIBIA.

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Radiogrametry (cortical thickness, trabecular architecture) has a significant role in assessing bone quality and has been used in several skeletal sites (hip, tibia, Calcaneus). Singh's classification of osteoporosis using proximal femoral radiographs deleted has only a moderate reproducibility but is still popular due to its low cost, availability and simplicity. A similar classification using the proximal tibia [Foltin 1987, 1986] has been used to predict failure of fixation in tibial plateau fractures. However, its reliability has not yet been assessed.

The purpose of this study is to assess the inter and intraobserver reproducibility of Foltin's classification.

Following ethical committee approval, fresh frozen human cadaver tibias were collected from subjects without a medical history of skeletal pathology. Bi-planar radiographs were used to exclude those specimens with other pathological lesions.

All radiographs were taken in a standard way, 1m above the specimen with the same rotation for all the specimens.

Using Foltin's classification, seven observers' classified 32 radiographs of 16 human tibial cadavers (AP and Lateral) on two occasions. Three observers were consultant orthopaedic surgeons with an interest in knee surgery. Two observers were consultant radiologists with an interest in the musculo-skeletal system. The other two were senior orthopaedic residents. All observers worked independently and repeated their measurements three weeks later without reference to the previous assessments. Intra- and inter-observer agreement was evaluated using the weighted kappa (k) coefficient of Cohen.

The inter-observer agreements were very poor, almost non-existent. The intra-observer agreements were better and ranged from slight to moderate but were still not good. There was no relationship between status or speciality of the observer and the level of agreement.

The study followed a strict methodology, which involved a laboratory set-up in producing optimal radiographs to avoid confounding factors. Senior doctors in two related specialities assessed the radiographs. In spite of this, the results

suggest very poor reproducibility. Therefore we feel that this classification is not reliable and its use in research or clinical practice should be questioned.

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HOMOCYSTINURIA PRESENTING WITH PREGNANCY ASSOCIATED OSTEOPOROSIS

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Mrs J.S dob 16.5.62 developed severe thoracic pain during her first pregnancy at the age of 28 and she had a second pregnancy at the age of 29 with continuing spinal pain.Conceptions occured after in vitro fertilisation.Her pain was treated conservatively with little success but it was not until 1994 at the age of 32 that osteoporosis was diagnosed with loss of height of 6 cm and the presence of vertebral body fractures at T10,T12 and L2.Examination was otherwise normal with normal body phenotype.Previous medical history included menarche at age 11,no secondary amenorrhoea but highly selective vagotomy for peptic ulcer at age 21 followed by postoperative pancreatitis.Further investigations included a negative screen for underlying causes of osteoporosis and malabsorption and normal 25-OH-vitamin D3 of 18.1 mcg/L.Lumbar spine L1-4 T score was -2.12 and left total femoral T score was -0.84 in 1994. Cyclical etidronate and calcium was administered from 1994 to 1996.In 1996,alendronate was substituted.In 1999,vitamin B12 deficiency and positive helicobacter serology were identified but malabsorption was again excluded.There has been a steady improvement in bone density.(Lumbar spine T score -1.44; left femoral T score -0.28 in 2002).No further fractures have occurred.Back pain is minimal.

In 2002,Mrs J.S reported that her sister(who is different phenotypically from her - tall,long limbed and lens subluxation) has homocystinuria.Mrs J.S was therefore investigated for the disease.Urine homocystine excretion was 1052 micromoles/L (normal undetectable).Plasma homocystine was 46 micromoles/L and methionine 92 micromoles/L consistent with classical homocystinuria.Mrs J.S's sister has the pyridoxine (vitamin B6)responsive form and it is therefore likely that Mrs J.S will also be pyridoxine responsive.

The cause of pregnancy associated osteoporosis is unknown. Homocystinuria ia an autosomal recessive condition with an incidence of 1 in 200 000 - 300 000. It is characterised by cystathionine synthase deficiency. Phenotypic changes include lens subluxation,thrombo- embolic disease and osteoporosis.Pyridoxine responsiveness is associated with an improved vascular prognosis.

The link between pregnancy associated osteoporosis and homocystinuria has not been reported previously. We now plan to screen our patients with pregnancy associated osteoporosis for homocystinuria.

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SEVERE BONE DISEASE IN PRIMARY HYPERPARATHYROIDISM IN A BRITISH ASIAN ADOLESCENT

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Primary hyperparathyroidism is rare in adolescence but can be severe when it occurs. We describe an extreme case of primary hyperparathyroidism in an adolescent female that resulted in gross skeletal complications. A 17 year old female Asian was referred because a pelvic radiograph suggested generalised loss of bone density and alteration of normal trabecular pattern. She had developed shin pain whilst running 18 months earlier, and more recently developed knee and groin pain. Three months prior to her referral she developed difficulty in walking after a trivial fall. She had sustained a clavicle fracture 2 years previously. Her only other symptom was nausea. There was no family history of parathyroid or endocrine problems and no evidence of malabsorption. Although her development was normal, she was substantially shorter than her parents and younger sister. Before review in clinic she had a further fall that resulted in a fractured neck of femur, which required operative fixation. Investigations at this time demonstrated abnormal bone biochemistry (calcium 3.7mmol/L, phosphate 0.45mmol/L, alkaline phosphatase 3121U/L, parathyroid hormone (PTH) 2240ng/L [NR 12-72]). She was treated initially with fluids and IV pamidronate. Subsequent parathyroidectomy identified 2 glands, one of which harboured a parathyroid adenoma (weight 3g). Following removal of the adenoma PTH fell from 2850 to 360ng/L over 10 min. Histological examination confirmed parathyroid adenoma. Post-operatively she developed symptomatic hypocalcaemia that was treated with alpha-calcidol and oral calcium supplements. In the 6 months following surgery, calcium has remained in the low normal range and the alkaline phosphatase has fallen but remains substantially elevated. The latest PTH is 140ng/L probably reflecting resolving osteomalacia. Isolated parathyroid adenoma is the commonest cause of primary hyperparathyroidism in childhood/adolescence and is likely to have been the cause in this patient. Hyperparathyroidism and secondary vitamin D deficiency during adolescence probably reduced her final height and accrual of bone mineral.