Abstracts from the Bone and Tooth Society Annual Meeting 2001

4-5 July 2001

University of Warwick
UK

Organiser

Professor Tim Skerry
University of York

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.

President

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Annual Meeting 2001

Venue

The meeting will take place at the University of Warwick.

The Society gratefully acknowledges the support of the following (list as at March 2001):

Major Sponsors

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CME

The meeting is approved for 14 CME credits for full attendance.

Meeting Organiser

For further information please contact our Meeting Organiser:
Janet Crompton, Conference Organiser
Tel: +44 (0)1453 549929, Fax: +44 (0)1453 548919
Email janetcrompton@compuserve.com

Future Bone and Tooth Society Meetings

24-26 June 2002, Cardiff, UK
Organiser: Dr Bronwen Evans

Web Site

www.batsoc.org.uk

Programme

All sessions in Arts Centre Conference Room except where otherwise stated

Wednesday 4th July 2001

10.00 *Registration and coffee*
(Arts Centre Foyer)

10.30 **Oral communications – Osteoclast and Bone Resorption**

Chairs: Tim Chambers/Dana Gaddy-Kurten

10.30 OC1 FLT3 LIGAND CAN SUBSTITUTE FOR MACROPHAGE COLONY STIMULATING FACTOR IN SUPPORT OF OSTEOCLAST DIFFERENTIATION AND FUNCTION
JM Lean, K Fuller, TJ Chambers

10.42 OC2 DISCOVERY OF A NOVEL INHIBITOR OF RAB GERANYLGERANYL TRANSFERASE THAT INHIBITS BONE RESORPTION BY SELECTIVELY PREVENTING PRENYLATION OF RAB PROTEINS IN OSTEOCLASTS
FP Coxon, B Larijani, J Dunford, MC Seabra, FH Ebetino, MJ Rogers

10.54 OC3 THE TEMPO OF IN VITRO OSTEOCLASTOGENESIS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS
D O'Gradaigh, K Bayley, TJ Chambers

11.06 OC4 THE EFFECTS OF OESTROGEN ON OSTEOPROTEGERIN, RANKL AND ESTROGEN RECEPTOR EXPRESSION IN HUMAN OSTEOBLASTS
S Bord, DC Ireland, SR Beavan, JE Compston

11.18 OC5 IL-6 INDUCES HUMAN OSTEOCLAST FORMATION AND BONE RESORPTION INDEPENDENT OF RANK/RANKL INTERACTION
O Kudo, A Sabokbar, Y Fujikawa, T Torisu, NA Athanasou

11.30 OC6 HYPOXIA IS A POWERFUL STIMULATOR OF BONE RESORPTION
DC Gibbons, S Meghji, A Hoebertz, M Rosendaal, TR Arnett

11.42 OC7 EXTRACELLULAR NUCLEOTIDE EFFECTS ON BONE RESORPTION AND FORMATION INVOLVE DIFFERENT P2 RECEPTOR SUBTYPES
A Hoebertz, S Mahendran, G Burnstock, TR Arnett

11.54 OC8 NITROSYLATED NSAIDS ARE POTENT INHIBITORS OF BONE RESORPTION IN VITRO
AII Mohamed, KJ Armour, P del Soldato, SH Ralston, RJ van't Hof

12.06 OC9 CELLULAR MECHANISMS OF OSTEOCLAST FORMATION AND BONE RESORPTION IN RHEUMATOID ARTHRITIS
L Danks, A Sabokbar, NA Athanasou

12.18 OC10 HUMAN AND BOVINE MILK CONTAINS THE OSTEOCLASTOGENESIS INHIBITORY FACTOR, OSTEOPROTEGERIN
JM Kanczler, T Bodamyali, TM Millar, JG Clinch, CR Stevens, DR Blake

12.30 *Lunch and posters (odd numbered boards)*
(Lunch in Rootes Building, Posters in Arts Centre Ensemble Room)

14.00 **Update on Clinical Guidelines**

- Malignant bone disease, Stuart Ralston
- Paget's disease, Peter Selby
- Primary hyperparathyroidism, David Hosking
- Ongoing activities, Juliet Compston

14.30 **Oral communications – Miscellaneous**

Chairs Colin Farquharson/David Hosking

14.30 OC11 BISPHOSPHONATES INDUCE APOPTOSIS IN BREAST CANCER CELLS IN A CASPASE-DEPENDENT MANNER BY INHIBITION OF THE MEVALONATE PATHWAY
SG Jayasundara, KW Colston

14.42 OC12 SYNTHESIS OF 1,25-DIHYDROXYVITAMIN D3 BY HUMAN ENDOTHELIAL CELLS IS REGULATED BY INFLAMMATORY CYTOKINES: A NOVEL AUTOCRINE DETERMINANT OF VASCULAR CELL ADHESION
D Zehnder, R Bland, RS Chana, D Wheeler, PM Stewart, M Hewison

14.54 OC13 CORTICAL POROSITY AND ITS RELATIONSHIP TO REMODELLING CLUSTERS (SUPER-OSTEONS)
N Loveridge, KL Bell, J Reeve, CDL Thomas, SA Feik, JG Clement

15.06 OC14 REDUCED BONE MASS AND ALTERED GROWTH PLATE MORPHOLOGY IN AGED RATS EXPOSED TO INTRAUTERINE PROTEIN RESTRICTION
G Mehta, HI Roach, S Langley-Evans, P Taylor, R Oreffo, AA Sayer, NMP Clarke, C Cooper

15.18 OC15 EXPRESSION OF HYPOXIA-RESPONSIVE GENES DURING CHONDROCYTE DIFFERENTIATION
B Houston, C Farquharson

15.30 *Tea and posters*
(Arts Centre Studio and Ensemble Room)

16.00 Minisymposium/debate
Are cell lines representative of osteoblasts and osteocytes?

Chair/moderator: **Larry Suva**
University of Arkansas, Little Rock, USA

17.00 **Clinical lecture**
Chair: Richard Eastell

MALE OSTEOPOROSIS: ROLE OF ANDROGENS
Roger Bouillon
Katholieke Universiteit Leuven, Belgium

19.30 ***Reception and Annual Dinner***
(Rootes Building)

Thursday 5th July 2001

08.00 **Oral communications – Osteoblasts and Bone Formation**

Chairs: Agi Grigoriadis/Lance Lanyon

08.00 OC16 MATERNAL PROTEIN DEFICIENCY AFFECTS MESENCHYMAL STEM CELL POPULATIONS IN THE DEVELOPING OFFSPRING
B Lashbrooke, NMP Clarke, C Cooper, ROC Oreffo

08.12 OC17 OSTEOBLASTIC EXPRESSION OF ADAMTS FAMILY MEMBERS
TF Lind, N McKie, MA Birch

08.24 OC18 THE ROLES OF THE OSTEOBLAST-STIMULATING FACTOR-1 DURING ENDOCHONDRAL OSSIFICATION
RS Tare, H Rauvala, T Hashimoto-Gotoh, ROC Oreffo, NMP Clarke, HI Roach

08.36 OC19 OSTEOCYTES EXPRESSING ENOS ARE PERIPHERALLY LOCATED IN CORTICAL BMU'S: A ROLE IN MAINTAINING CORTICAL INTEGRITY?
N Loveridge, S Fletcher, J Power, A-M Caballero, J Reeve, A Pitsillides

08.48 OC20 CELL CYCLE CONTROL DURING OSTEOBLAST DIFFERENTIATION AND POST-CONFLUENCE GROWTH REGULATION BY C-FOS
A Sunters, DP Thomas, D Harmey, A Tumber, K Beedles, WA Yeudall, AE Grigoriadis

09.00 OC21 MALE OESTROGEN RECEPTOR-BETA KNOCKOUT MICE SHOW INCREASED SENSITIVITY TO OESTROGEN-INDUCED BONE FORMATION
KE McDougall, MJ Perry, JH Tobias

09.12 OC22 INDUCTION OF HUMAN OSTEOPROGENITOR CHEMOTAXIS, PROLIFERATION AND DIFFERENTIATION BY A NOVEL BONE FACTOR, OSTEOBLAST STIMULATING FACTOR-1
XB Yang, HI Roach, NMP Clarke, SM Howdle, KM Shakesheff, ROC Oreffo

09.24 OC23 ISOLATION OF OSTEOPROGENITOR CELLS FROM SKELETAL MUSCLE
L Reading, A Scutt

09.36 OC24 PHOSPHORYLATION OF PROTEIN KINASE B BY MECHANICAL STRAIN IN OSTEOBLASTS - A MECHANISM FOR ACTIVATION OF NITRIC OXIDE SYNTHASE IN THE ADAPTIVE RESPONSE
TS Grewal, RJ Tolley, TM Skerry

09.48 OC25 CALCIUM DEPENDENT PROTEIN KINASE II ACTIVITY IS OBLIGATE FOR BONE FORMATION IN VITRO
GJ Spencer, TS Grewal, PG Genever, TM Skerry

10.00 *Coffee and posters (even numbered boards)*
(Arts Centre Studio and Ensemble Room)

10.30 **Clinical case presentations**
Chairs: Trevor Stamp/Roger Smith

10.30 CC1 OSTEOPOROSIS SECONDARY TO SYSTEMIC MASTOCYTOSIS - A LONG TERM FOLLOW UP STUDY OF TREATMENT WITH PAMIDRONATE AND ALDENDRONATE
AYN Lim, AJ Crisp

10.45 CC2 SPORADIC, ADULT-ONSET, HYPOPHOSPHATAEMIC OSTEOMALACIA - A CASE REPORT
AK Brown, SM Doherty

11.00 CC3 DIFFICULTIES WITH BIOCHEMICAL ASSESSMENT OF X-LINKED HYPOPHOSPHATAEMIA IN ADULTS
JA Clowes, D Hughes, R Eastell, NFA Peel

11.15 CC4 HAEMOCHROMATOSIS PRESENTING AS RECURRENT TIBIAL PLATEAU FRACTURES
JH Tobias

11.30 **Poster Discussion session**
Chairs: Jon Tobias/Mike Rogers

11.30 P1 INVOLVEMENT OF RANKL-DEPENDENT/INDEPENDENT MECHANISMS IN OSTEOCLAST FORMATION IN ASEPTIC LOOSENING
A Sabokbar, E Schulze, O Kudo, DW Murray, NA Athanasou

11.35 P2 COOPERATIVITY BETWEEN C-FOS AND FGF RECEPTOR SIGNALLING IN CHONDROCYTE DIFFERENTIATION AND TRANSFORMATION
DP Thomas, A Sunters, I Anagnostopoulos, AE Grigoriadis

11.40 P3 OSTEOBLASTIC 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1: INDUCTION BY GLUCOCORTICOIDS AND METABOLISM OF PREDNISONE/PREDNISOLONE
MS Cooper, EH Rabbitt, P Goddard, W Bartlett, M Hewison, PM Stewart

11.45 P4 POLYMORPHISMS IN THE 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 GENE ASSOCIATE WITH LOW BONE MINERAL DENSITY: A ROLE FOR AUTOCRINE GLUCOCORTICOID METABOLISM IN BONE PHYSIOLOGY
MS Cooper, N Draper, EL Duncan, JAH Wass, MC Sheppard, M Hewison, PM Stewart

11.50 P5 DIFFERENTIATION-DEPENDENT REGULATION OF 'PRESYNAPTIC' SNARE PROTEINS IMPLICATED IN GLUTAMATE SIGNALLING IN OSTEOBLASTS
PS Bhangu, PG Genever, TM Skerry

11.55 P6 EFFECTS OF INSULIN LIKE GROWTH FACTORS (IGF-I AND -II) DURING OSTEOINDUCTION
E Damien, T MacInnes, PA Revell

12.00 P7 IDENTIFICATION OF A BISPHOSPHONATE WHICH INHIBITS FPP SYNTHASE AND IPP ISOMERASE
K Thompson, FP Coxon, JE Dunford, FH Ebetino, MJ Rogers

12.05 P8 HIP AXIS LENGTH, A PREDICTOR OF HIP FRACTURE IN ELDERLY WOMEN IN ENGLAND
K Kayan, RU Ashford, A Dey, D Charlesworth, J Bostock, EV McCloskey

12.10 P9 AGE-DEPENDENT ANDROGEN RECEPTOR EXPRESSION IN HUMAN FRACTURE CALLUS
G Batra, L Hainey, G Andrew, AJ Freemont, JA Hoyland, IP Braidman

12.15 P10 EFFECTS OF RISEDRONATE, ALENDRONATE AND ETIDRONATE ON VIABILITY AND ACTIVITY OF RAT STROMAL CELLS IN VITRO
K Still, RJ Phipps, A Scutt

12.20 P11 COLLAGEN TYPE I ALPHA 1 GENE POLYMORPHISM AT THE SPI BINDING SITE AND THE RISK OF OSTEOPOROSIS IN MEN
A Rogers, D Fatayergi, YM Henry, JA Sorrell, R Eastell

12.25 P12 IS PAGET'S DISEASE SEVERITY DECREASING IN NORTH WEST ENGLAND?
GI Al-Bahrani, RS Lawson, MC Prescott, PL Selby

12.30 *Lunch*
(Rootes Building)

13.30 **Plenary session**
Chairs: Juliet Compston/Tim Skerry

THE ESTROGEN RECEPTOR: STRUCTURE, FUNCTION AND BEYOND

13.30 **Rod Hubbard**
University of York, UK
What can structure tell us about the function of the estrogen receptor?

13.55 **Jan Åke Gustafsson**
Karolinska Institute, Huddinge, Sweden
Regulation of transcription by different Estrogen receptors

14.20 **Isobel Braidman**
University of Manchester, UK
Impaired estrogen receptor expression in men with osteoporosis

14.45 **Lance Lanyon**
Royal Veterinary College, London, UK
Evidence for involvement of the ER in responses of bone to mechanical loading

15.10 **Dana Gaddy-Kurten**
University of Arkansas, Little Rock, USA
Beyond Estrogen - Regulation of osteoblast and osteoclast differentiation by activin and inhibin

15.35 *Tea*

15.45 Bone and Tooth Society AGM

16.05 Prize giving (New Investigator Awards and Registrar Prizes)

PRIZE WINNERS – 2001

Congratulations to the following, who were awarded prizes for their work as shown.

ORAL PRESENTATION

FRASER COXON:
DISCOVERY OF A NOVEL INHIBITOR OF RAB GERANYLGERANYL TRANSFERASE THAT INHIBITS BONE RESORPTION BY SELECTIVELY PREVENTING PRENYLATION OF RAB PROTEINS IN OSTEOCLASTS

LYNETT DANKS:
CELLULAR MECHANISMS OF OSTEOCLAST FORMATION AND BONE RESORPTION IN RHEUMATOID ARTHRITIS

ORAL POSTER

MARK COOPER:
POLYMORPHISMS IN THE 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 GENE ASSOCIATE WITH LOW BONE MINERAL DENSITY: A ROLE FOR AUTOCRINE GLUCOCORTICOID METABOLISM IN BONE PHYSIOLOGY

JAMIE BHANGU:
DIFFERENTIATION-DEPENDENT REGULATION OF 'PRESYNAPTIC' SNARE PROTEINS IMPLICATED IN GLUTAMATE SIGNALLING IN OSTEOBLASTS

POSTER

KARINA STEWART:
FURTHER CHARACTERISATION AND MOLECULAR CLONING OF THE ANTIBODY H8G

ALISON EAGLETON:
DIFFERENT RESPONSE OF SERUM BETA-CROSSLAPS AND SERUM NTX TO SUBCUTANEOUS OESTRADIOL IMPLANT

CLINICAL CASE

ANITA LIM:
OSTEOPOROSIS SECONDARY TO SYSTEMIC MASTOCYTOSIS - A LONG TERM FOLLOW UP STUDY OF TREATMENT WITH PAMIDRONATE AND ALDENDRONATE

16.15 *Close of meeting*

Contents

OC13, CORTICAL POROSITY AND ITS RELATIONSHIP TO REMODELLING CLUSTERS (SUPER-OSTEONS)
N Loveridge, KL Bell, J Reeve, CDL Thomas, SA Feik, JG Clement

OC14, REDUCED BONE MASS AND ALTERED GROWTH PLATE MORPHOLOGY IN AGED RATS EXPOSED TO INTRAUTERINE PROTEIN RESTRICTION
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Posters

P1, INVOLVEMENT OF RANKL-DEPENDENT/INDEPENDENT MECHANISMS IN OSTEOCLAST FORMATION IN ASEPTIC LOOSENING
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E Damien, T MacInnes, PA Revell

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K Thompson, FP Coxon, JE Dunford, FH Ebetino, MJ Rogers

P8, HIP AXIS LENGTH, A PREDICTOR OF HIP FRACTURE IN ELDERLY WOMEN IN ENGLAND
K Kayan, RU Ashford, A Dey, D Charlesworth, J Bostock, EV McCloskey

P9, AGE-DEPENDENT ANDROGEN RECEPTOR EXPRESSION IN HUMAN FRACTURE CALLUS
G Batra, L Hainey, G Andrew, AJ Freemont, JA Hoyland, IP Braidman

P10, EFFECTS OF RISEDRONATE, ALENDRONATE AND ETIDRONATE ON VIABILITY AND ACTIVITY OF RAT STROMAL CELLS IN VITRO
K Still, RJ Phipps, A Scutt

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A Rogers, D Fatayergi, YM Henry, JA Sorrell, R Eastell

P12, IS PAGET'S DISEASE SEVERITY DECREASING IN NORTH WEST ENGLAND?
GI Al-Bahrani, RS Lawson, MC Prescott, PL Selby

P13, DIAGNOSIS OF OSTEOPOROSIS IN CLINICXAL PRACTICE: A COMPARISON OF SPINE AND PROXIMAL FEMUR T SCORES
I Debiram, D O'Gradaigh, S Love, J Compston

P14, FURTHER CHARACTERISATION AND MOLECULAR CLONING OF THE ANTIBODY H8G
K Stewart, S Gronthos, S Bord, A Zannettino, P Simmons, JN Beresford

P15, A COMPARISON OF STRO-1, HOP-26, CD166 AND CD49A AS DEVELOPMENTAL MARKERS FOR HUMAN CELLS OF THE OSTEOGENIC LINEAGE
K Stewart, JN Beresford

P16, SYSTEM AND ORGAN CONSERVATION OF BONE MASS
L Klein

P17, A RAPID METHOD FOR DETECTING THE COL1A1 SP1 POLYMORPHISM
DC Ireland, RL Harrall, JE Compston, C Speed

P18, USE OF THE OESTROGEN-TREATED MOUSE AS AN ANIMAL MODEL FOR STEROID-INDUCED OSTEOPOROSIS
JM Bright, MJ Perry, RL Gibson, GL Wakley, JH Tobias

P19, OPTIMISATION OF SELECTION PROCEDURES FOR THE ISOLATION OF PHAGE ANTIBODIES FROM STRO-1+ CELL POPULATIONS
J Letchford, C Jefferiss, S Walsh, K Stewart, J Beresford

P20, EXPRESSION OF ESTROGEN RECEPTORS IN CULTURED CELLS OF OSTEOBLAST LINEAGE
E Sherrif, L Hainey, PKT Saunders, JA Hoyland, IP Braidman

P21, 2-METHOXYESTRADIOL INDUCES APOPTOSIS IN CELLS OF THE OSTEOBLASTIC LINEAGE
M Naylor, N Scutt, A Scutt

P22, AGONISTS FOR PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR INCREASE OSTEOGENESIS IN VITRO
K Still, A Scutt

P23, THE IMPACT OF ATMOSPHERIC POLLUTION RELATED HAZE ON VITAMIN D STATUS OF TWO-YEAR-OLDS IN DELHI, INDIA
MZ Mughal, K Agarwal, P Upadhyay, EB Mawer, JL Berry, JM Puliyel

P24, EFFECT OF CHRONIC METHOTREXATE TREATMENT ONBONE METABOLISM IN STEROID-DEPENDENT ASTHMATICS
A Nowkeji, BH Shakur, RJ Shiner, PW Ind, SI Girgis

P25, DIFFERENT RESPONSE OF SERUM BETA-CROSSLAPS AND SERUM NTX TO SUBCUTANEOUS OESTRADIOL IMPLANT
AC Eagleton, CA Pereda, RA Hannon, KE Naylor, R Eastell, A Blumsohn

P26, OESTROGEN INDUCED SUPPRESSION OF MATRIX METALLOPROTEINASE-2 IN MURINE LONG BONES
SJ Yarram, N Longdon, C McDougall, M Perry, J Tobias, J Sandy, J Mansell

P27, THE EFFECTS OF DIET ON HIP BONE LOSS: A PROSPECTIVE STUDY
S Kaptoge, AA Welch, N Dalzell, A McTaggart, AM Mulligan, R Luben, K-T Khaw, SA Bingham, J Reeve

P28, EVIDENCE THAT P1-PURINOCEPTORS OF THE A1- AND A2-SUBCLASSES MEDIATE OPPOSING EFFECTS ON THE PROLIFERATION OF MG-63 CELLS
M Shafique, AM Caswell

P29, THE APPLICATION OF DIGITAL X-RAY RADIOGRAMMETRY TO THE DIAGNOSIS OF OSTEOPOROSIS
KA Ward, JE Cotton, JE Adams

P30, BONE MINERAL DENSITOMETRY OF THE TIBIA AND LUMBAR SPINE USING THREE-DIMENSIONAL QUANTITATIVE COMPUTED TOMOGRAPHY IN CHILDREN WITH DISABLING CONDITIONS
KA Ward, CW Alsop, MZ Mughal, JE Adams

P31, EXPRESSION OF MINERALOCORTICOID RECEPTORS BY HUMAN OSTEOBLASTS IN RESPONSE TO STEROID TREATMENT
SR Beavan, DC Ireland, S Bord, JE Compston

P32, DETERMINANTS OF VERTEBRAL FRACTURE IN MEN ATTENDING AN OSTEOPOROSIS CLINIC
K Chatterjee, JE Adams, PL Selby

P33, WHO SHOULD TREAT PAGET'S DISEASE OF BONE?
PL Selby, M McCallum, SH Ralston

P34, STABILITY OF URINARY NTX
RA Hannon, IB Catch, A Price, R Eastell

P35, STRUCTURAL AND CHEMICAL ANALYSIS OF OSTEOPETROTIC (OP/OP) MOUSE BONE
T Sakae, Y Sato, H Okada, H Yamamoto, Y Kozawa]

P36, THE EMPLOYMENT OF THE COLLAGEN-APATITE MATERIAL "LITAR" IN PERIODINTOLOGY.
I Rakhimov, S Litvinov, V Sherbakha, O Krasnobayeva, V Danilov, I Kislitsin, V Chegarin

P37, EFFECT OF SEASON, MEASUREMENT INTERVAL AND RANDOM VARIABILITY ON REPRODUCIBILITY OF NEW SERUM MARKERS OF BONE TURNOVER
AC Eagleton, RA Hannon, R Eastell, A Blumsohn

P38, DISTRIBUTION OF BMD AND BMC AND HANDEDNESS
S Lekamwasam

P39, DO HOLOGIC AND LUNAR HIP STRENGTH ANALYSIS SOFTWARES GENERATE COMPARABLE DATA ON THE FEMORAL NECK?
S Lekamwasam, N Crabtree, K-T Khaw, D Anderson

P40, BONE MINERAL DENSITY OF THE HEEL IN WOMEN WITH ACUTE AND CHRONIC STROKE
MJ Haddaway, N Bainbridge, MWJ Davie, S Hill

P41, STABLE ISOTOPES FROM BONE AND TOOTH COLLAGEN REVEAL - DIETARY PATTERNS AND THE AGE OF WEANING AT THE MEDIEVAL VILLAGE SITE OF WHARRAM PERCY
B Fuller, MP Richards, S Mays

P42, IMPORTANCE OF THE ALTERED PHENOTYPE OF "OSTEOARTHRITIC" CHONDROCYTES IN THE AETIOLOGY OF OSTEOARTHRITIS
HI Roach, ROC Oreffo, NMP Clarke

P43, COMPARISON OF PA AND LATERAL LUMBAR SPINE BONE MINERAL DENSITY MEASUREMENTS FOR THE DISCRIMINATION OF WOMEN WITH VERTEBRAL FRACTURE
L Ferrar, NAB Barrington, R Eastell

P44, ANKLE FRACTURE IS ASSOCIATED WITH NORMAL BONE MINERAL DENSITY BUT DISSOCIATION OF ULTRASOUND PROPERTIES OF BONE
BM Ingle, R Eastell

P45, THE PREVALENCE OF OSTEOPOROSIS AMONG PATIENTS WITH GASTROINTESTINAL DISEASES REFERRED FOR BONE DENSITOMETRY: AN AUDIT
S Lekamwasam, S Love, JE Compston

P46, CAVEOLIN-1 EXPRESSION IN HUMAN OSTEOBLASTS, BONE MARROW AND PERIPHERAL BLOOD MONONUCLEAR CELLS
GA Burke, S McHenry, D Marsh, G Li

P47, THE HISTORY AND THE REALITY OF COLLAGEN-APATITE MATERIALS
SD Litvinov

P48, EFFECT OF PRESSURE AND PARTICLE ON OSTEOLYSIS
K Sampathkumar, M Jeyam, C Evans, C Ward,T McVoy, G Andrew

P49, CHARACTERISATION OF THE EFFECTS AND MECHANISM OF ACTION OF DEXAMETHASONE ON MOUSE BONE
F McLaughlin, J MacKintosh, B Hayes, A McLaren, PL Salmon, JA Humphreys, G Brown, S Farrow

IS01

MALE OSTEOPOROSIS : ROLE OF ANDROGENS

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The role of estrogen deficiency is well-established in postmenopausal osteoporosis. In men, androgens are crucial for both gain of bone mass during puberty and maintenance of bone mass after puberty. Both aspects of androgen action protect men against osteoporosis. Indeed, failure and perhaps even delay of androgen action during puberty may permanently limit bone size, resulting in less mechanical strength. Moreover, ongoing testosterone (T) secretion in elderly men may also protect against osteoporosis by reducing the slope of male bone loss. Unfortunately, it remains unclear how much T the male skeleton really needs. A definition of such a threshold, however, is clinically important with respect to possible indications for androgen replacement in men with both 'subnormal' bone density and T concentrations.

Androgens have the unique feature that they may be aromatized in estrogens, which have powerfull skeletal action in both humans and animals of both gender. The importance of this aromatization for male skeletal homeostasis was demonstrated in animals by knockout of one or both estrogen receptors (ER) in mice and by administration of a specific aromatase inhibitor or estrogen antagonists and agonists in rats. Furthermore, men suffering from inactivating mutations of the aromatase gene or the estrogen receptor-alpha gene show extreme delay of maturation.

A better understanding of these ER-dependent actions of androgens could lead to more efficient and safer treatment modalities for male and possibly even female osteoporosis.

IS02

WHAT CAN STRUCTURE TELL US ABOUT FUNCTION IN THE OESTROGEN RECEPTORS?

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Advances in a range of molecular biology and biophysics techniques are rapidly expanding the scope of Structural Biology. This is giving us not only a detailed structural description of the molecules and mechanisms that maintain living organisms, but also provides information that can be used in both ligand and protein design.

In this talk, I will describe our work over the past few years on the oestrogen receptor and discuss how structural work on various forms of the ligand binding domain of this receptor have had a major impact on our understanding of how the receptor works at the molecular level.

The oestrogen receptor (ER) is a ligand-inducible transcription factor that controls expression of a number of genes in a wide variety of tissues. Binding of the natural hormone, oestradiol, triggers dimerisation and nuclear location of the receptor and assembly of a functional transcriptional complex through recruitment of various coactivators.

We have determined the structure of the ligand binding domain of the alpha form of the receptor (ERa) complexed to oestradiol and the selective ER modulator raloxifene. This has provided insights at the molecular level into some key aspects of the pharmacology and function of this molecule.

- Understanding the distinctive ERa pharmacophore
- A rationale for the large range of molecules that bind to ERa
- Description of the biologically active dimer
- Structural basis for the antagonist action of molecules such as raloxifene
- Indications of the site of interaction with some co-activators

(see Brzozowski et al, Nature (1997), 389, 753-758 and 106. Pike, Brzozowski and Hubbard, J of Steroid Bioch and Mol. Biol (2000), 74, 261-268)

This presentation will review these structural results and discuss recent work including structural studies of the beta isoform of the receptor (ERb) (see Pike et al (1999), Embo J, 18, 4608-4618) and of complexes probing the interactions between co-activators and ligand binding domains.

IS03

Abstract not available

IS04

IMPAIRED ESTROGEN RECEPTOR EXPRESSION IN MEN WITH OSTEOPOROSIS

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Osteoporosis affects 1 in 12 of men in the UK. While there are known causes for the disease in men, approximately 30% of cases are idiopathic, a significant proportion of whom are aged below 55 years. Estrogen is important for skeletal development and maintaining adult bone mineral density, in both men and women. Previous studies show that estrogen levels are reduced in some patients with male idiopathic osteoporosis (MIO), not in others. We hypothesised that in some patients with normal estrogen, estrogen receptor (ER) expression might be defective, leading to impaired response of bone cells to the hormone. There are two known isoforms of ER; ER alpha, which is expressed in “classic” reproductive tissues like uterus and breast, while ER beta is in estrogen responsive tissues, with low ER alpha expression, e.g. ovary and prostate. We examined bone biopsies from MIO patients (aged 33 – 61 years) for ER alpha expression, using indirect immunofluorescence and in situ RT- PCR. There was a marked loss of osteoblast and osteocyte ER alpha protein expression in these men, although receptor mRNA was still detectable. We are now investigating in vivo ER beta expression, by indirect immunoperoxidase. In both men and women, ER alpha and ER beta are expressed in osteoblasts, osteocytes and osteoclasts. Both isoforms are clearly localised to small chondrocytes, but not hypertrophic chondrocytes. Expression of ER alpha is more restricted, and is low in some mesenchymal cells, involved in active osteogenesis, where ER beta expression is high. Thus, in contrast to reproductive tissues, which express predominately either ER alpha or beta, both isoforms are expressed in bone. This is particularly significant for men, in whom bone cells respond to low estrogen levels. Expression of both isoforms might be essential for this process, either by expression in different cell types, or, as exemplified by osteoclasts, within the same cell, through receptor co-operativity, possibly by heterodimerisation. Loss of ER alpha expression in men, from mature bone cells, might be sufficient to impair their response to estrogen and could therefore explain osteopenia in MIO. Expression of ER beta in MIO is currently under investigation.

IS05

INVOLVEMENT OF THE ESTROGEN RECEPTOR IN BONE CELLS' EARLY RESPONSES TO MECHANICAL STRAIN: IMPLICATIONS FOR THE AETIOLOGY OF POST-MENOPAUSAL OSTEOPOROSIS

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Bones' structural competence is achieved and maintained by a homeostatic mechanism in which bone cells adjust bone architecture to control the strains engendered by functional loading. The mechano-responsive cells are most likely osteocytes and osteoblasts.

Post-menopausal osteoporosis is characterised by a decline in bone mass despite continued functional activity, and an increase in fracture incidence. This implies reduced responsiveness to the increased strains which the bone loss should engender.

In cultures of rat primary osteoblasts, and in ROS 17/2.8 cells, both a short period of strain and estrogen stimulate proliferation. The size of the proliferative response to strain and estrogen together equals the addition of the two individual maximal responses separately.

The estrogen antagonist ICI 182,780 reduces, and tamoxifen abolishes the proliferative response to strain and reduces that due to estrogen. ICI 182,780 similarly blocks strain-related proliferation in TE85 cells and human primary osteoblasts but tamoxifen does not.

In ROS cells transfection with ERα increases the proliferation stimulated by both estrogen and strain, both of which are inhibited by ICI 182,780. Strain and estrogen both upregulate ERE activity.

In rat primary osteoblasts and ROS17/2.8 cells estrogen and strain cause phosphorylation of serine ^{118} in the amino terminal of ERα which is the consensus phosphorylation site for MAPK. This response is eliminated by inhibitors of ERK-1 and ERK-2, the immediate upstream activators of MAPK.

Thus in osteoblast-like cells early responses to strain and estrogen appear to involve the MAPK pathway, Estrogen Receptor α and EREs.

At the cell level strain and estrogen neither enhance nor obstruct each other's effects on osteoblast proliferation although at the tissue and organ level there may be some synergism. Proliferation of osteoblasts is only one of resident bone cells' adaptive responses to strain but it may be representative of others.

The etiology of post-menopausal osteoporosis could be explained by less effective strain-related control of bone architecture associated with the down-regulation of ER number which others have reported following the withdrawal of estrogen.

IS06

BEYOND ESTROGEN-REGULATION OF OSTEOBLAST AND OSTEOCLAST DIFFERENTIATION BY ACTIVIN AND INHIBIN

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It is widely accepted that estrogen (E) plays a critical role in the maintenance of bone homeostasis, and that E deficiency in post-menopausal women results from de-repression of both osteoblast (OBL) and osteoclast (OCL) development. However, in peri-menopausal women, a time when E levels have not yet begun to diminish, markers of bone resorption are already elevated. This increased resorption is best correlated with elevated FSH, due to de-repression by another gonadal hormone, inhibin B. Inhibins and their related peptides, activins, exert opposing effects on reproductive tissues and hematopoietic cell development. In some situations, activin has also been shown to stimulate bone formation. Thus, we postulated that cells within the bone marrow directly respond to changes in the activin/inhibin ratio by altering osteoblastogenesis and osteoclastogenesis. Using bone marrow cell cultures, we have demonstrated that inhibin suppresses and activin stimulates both OBL and OCL formation. This activin action (whose synthesis in bone marrow cultures is autoregulated, stimulated by BMP and blocked by inhibin) is required to achieve the full osteoblastic phenotype in vitro. Furthermore, ~30% of the stimulatory activin effect on OCL formation is RANKL-independent. However, the stimulatory effects of locally produced activin on OBL and OCL development are not likely to be dominant over the suppressive effects of gonadally derived inhibin. The suppressive effect of inhibin is maintained in the presence of either activin or BMP, suggesting the presence of a distinct, inhibin-specific receptor. We have identified a novel inhibin binding protein, p120, in bone that may mediate these suppressive effects. p120 is expressed during marrow cell osteoblastogenesis, during which time inhibin blocks BMP induction of OBL formation. In contrast, p120 is not expressed in C2C12 cells in which inhibin does not block the osteogenic effect of BMP. Taken togethor, the direct regulation of OBL and OCL development by inhibin and activin suggests that changes in the inhibin/activin ratio detected by bone marrow cells during the peri-menopausal transition contributes to altered cell differentiation and the associated increased bone resorption observed at this time.

CC1

OSTEOPOROSIS SECONDARY TO SYSTEMIC MASTOCYTOSIS - A LONG TERM FOLLOW UP STUDY OF TREATMENT WITH PAMIDRONATE AND ALDENDRONATE

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Systemic mastocytosis is a disease of mast cell proliferation with involvement of the reticuloendothelial systems including skin,bone,gastrointestinal tract,liver,lungs,spleen and lymph nodes.Radiological findings include osteosclerotic lesions or a mixture of osteosclerotic and osteolytic lesions.However,patients can have pure diffuse osteoporosis with or without vertebral fractures.We report three patients with systemic mastocytosis and severe osteoporosis who have received intravenous Pamidronate infusions during a follow up period of six,nine and eleven years respectively.In these patients,oral Alendronate was substituted after four,eight and eleven years respectively.Serial bone densities were measured annually.

In the first patient,RL,the lumbar spine (L1 to L4) bone mineral density (BMD) has increased 18.9 percent since commencement of annual infusions of Pamidronate in 1995 to 1997.Despite a further Pamidronate infusion in 1998, a fall in BMD precipitated change of therapy to Alendronate and this has resulted in a rise in lumbar spine BMD of 9.2 percent in the past two years. In the second patient,MN,there has been a 29.7 percent increase in lumbar spine BMD over a period of four years with annual infusions of Pamidronate.There has been a 16.5 percent fall in these measurements over the past three years,hence she has recently been started on Alendronate. In the third patient,BM,there has been a 20.9 percent increase in her lumbar spine BMD over a period of five years.The slow but steady decline in her BMD by 14 percent over the subsequent five years may partly be attributed to a concomitant diagnosis of mild primary hyperparathyroidism made in 1997.She has also recently been started on Alendronate.

We conclude that our three patients with systemic mastocytosis have benefited from long term bisphosphonate therapy as they have not sustained any further fractures since starting treatment.Their lumbar spine densities have improved. An added benefit was significant relief of bone pain.

CC2

SPORADIC, ADULT-ONSET, HYPOPHOSPHATAEMIC OSTEOMALACIA - A CASE REPORT.

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History & examination: A previously healthy 50 year-old caucasian man presented with a seven-year history of progressive joint pains involving hips, feet, ankles, left shoulder and radicular ribcage pain. His debility necessitated walking with the aid of a stick. He denied significant alcohol intake or relevant family history. On examination, he had proximal muscle weakness, generalized joint stiffness involving the cervical spine, shoulders and hips and tender metatarsophalangeal (MTP) joints.

Investigations: Spinal x-rays: multiple concave osteopenic vertebrae; Hand and feet x-rays: juxta-articular osteopenia, healing fractures of the right and left third MTP's; Chest x-ray: old rib fractures. Isotope bone scan: symmetrical increased uptake in hips, knees, ankles, spine, multiple ribs, hands and feet. Blood investigations: low phosphate 0.57mmol/l (N 0.70-1.40), raised alkaline phosphatase 196iu/l (N 30-100), raised parathyroid hormone (PTH) 75iu (N 12-72) and normal adjusted calcium 2.29mmol/l (N 2.20-2.60). Urine electrolytes: normal calcium and phosphate but reduced phosphate glomerular filtration (TmP/GFR) 0.3mmol/l (N 0.8-1.35). Dual-energy x-ray absorptiometry (DEXA) bone mineral density (BMD) femoral neck 0.69g/cm2 (T-score -2.9) and L2-L4 1.05g/cm2 (T-score -1.6). Magnetic Resonance Imaging: Looser's zones in the right distal femur, medial aspect of left tibia and at the tibial metaphysis of left ankle. Bone biopsy confirmed severe longstanding osteomalacia.

Management & follow-up: Treatment for hypophosphataemic osteomalacia was commenced with alfacalcidol 4mg od,
phosphate-sandoz 4 tablets od and calcichew D3 2 tablets od. Following six months of treatment the musculoskeletal symptoms and biochemical parameters had almost normalised. Serum calcium 2.41mmol/l, phosphate 1.56mmol/l, alkaline phosphatase 100iu/l and PTH 35iu. Repeat isotope bone scan now shows normal appearances of the large joints and only a few isolated areas of increased uptake in the ribs. Six month follow-up DEXA scan was normal with BMD at the femoral neck of 0.97g/cm2 (T-score -0.8) and at L2-L4 of 1.44g/cm2 (T-score +1.7).

Conclusion: Sporadic adult-onset hypophosphataemic osteomalacia is rare. Differential diagnoses were underlying malignancy and X-linked hypophosphataemic osteomalacia. Full malignancy screen was negative, there was no family history and onset was relatively late. The condition classically presents with an indolent onset and initial symptoms can be rather non-specific which may result in a delay in diagnosis. However, appropriate treatment leads to a marked clinical, biochemical, BMD and scintographic response

CC3

DIFFICULTIES WITH BIOCHEMICAL ASSESSMENT OF X-LINKED HYPOPHOSPHATAEMIA IN ADULTS

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Treatment for X-linked hypophosphataemia (XLH) is frequently withdrawn once skeletal maturity is reached. Treatment using phosphate and vitamin D metabolites is generally given only to individuals with biochemical, radiological or symptomatic osteomalacia. The requirement for treatment may be determined by observing for increases in serum alkaline phosphatase or PTH. We describe two patients in whom this approach has proved insensitive. Case 1 is a 36 year old caucasian male in whom XLH was diagnosed at the age of 3. He was treated with calciferol and phosphate to the age of 16 and subsequently observed annually off treatment. Serum biochemistry was stable with borderline low calcium, normal AP and PTH at the upper limit of the reference range. Bone biopsy, obtained due to a small increase in PTH (79 ng/L, 8-63) showed marked accumulation of osteoid with evidence of secondary hyperparathyroidism. Case 2 is a caucasian male who presented at 44 years with hip pain, short stature and genu varum. Radiographs showed severe Looser's zones of the femoral neck and investigations confirmed XLH. Serum and urine biochemistry normalised with treatment with 1? calcidol and phosphate. Bone mineral density (BMD) increased by 12% at the lumbar spine (LS) and 22% at the total hip (TH) indicating remineralisation. Treatment was continued with 1? calcidol alone. One year later BMD had decreased by 23% at LS and 10% at TH. During this time the only change in biochemical parameters was a decrease in serum phosphate. Investigations revealed no additional underlying cause for bone loss. We suggest that biochemical monitoring is not adequate to identify adult patients with XLH requiring treatment, and that it may be inappropriate to withdraw therapy once skeletal maturity is reached.

CC4

HAEMOCHROMATOSIS PRESENTING AS RECURRENT TIBIAL PLATEAU FRACTURES

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A 61 old male was referred to the osteoporosis clinic following a seven year history of recurrent fractures of the left and right tibial plateaus. The patient had sustained five tibial plateau fractures during this time. Other than a distal radial fracture in childhood, he had no other history of fractures, and no clinical features to suggest spinal osteoporosis. He had been treated for insulin dependent diabetes mellitus for twenty years, and had suffered from impotence for the past 15 years. No other risk factors were osteoporosis were present, and no abnormal features were elicited on clinical examination. DXA scan showed striking generalised bone loss (lumbar spine L2-L4 T score –4.7, femoral neck T score –4.5). Investigations for secondary osteoporosis revealed low serum 25-hydroxyvitamin D3, hypogonadotrophic hypogonadism (FSH and LH were both undetectable), and abnormal liver function tests. On subsequent review, the patient revealed that he had recently undergone a liver biopsy for investigation of abnormal liver function tests elsewhere, which was found to reveal haemochromatosis complicated by cirrhosis. Literature review revealed that osteoporosis is a recognised complication of haemochromatosis, due to two distinct causes both of which were present in our patient, i.e. hypogonadotrophic hypogonadism and vitamin D malabsorption secondary to liver cirrhosis. The patient was subsequently commenced on alendronate and vitamin D supplements, referred for pituitary function tests, and screening of first degree relatives for haemochromatosis arranged. Unfortunately, he died shortly afterwards following a diagnosis of hepatoma secondary to liver cirrhosis.

OC1

FLT3 LIGAND CAN SUBSTITUTE FOR MACROPHAGE COLONY STIMULATING FACTOR IN SUPPORT OF OSTEOCLAST DIFFERENTIATION AND FUNCTION

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Although bone resorption and osteoclast numbers are reduced in osteopetrotic (op/op) mice, osteoclasts are nevertheless present and functional, despite absence of macrophage colony stimulating factor (M-CSF). This suggests that alternative factors can partly compensate for the crucial actions of M-CSF in osteoclast-induction. We found that when non-adherent bone marrow cells were incubated in RANKL with flt3 ligand (FL) without exogenous M-CSF, TRAP-positive cells were formed, and bone resorption occurred. Without FL, only macrophage-like tartrate-resistance acid phosphatase (TRAP)-negative cells were present. GM-CSF, stem cell factor (SCF), IL-3 and vascular endothelial growth factor (VEGF) could not similarly replace the need for M-CSF. TRAP-positive cell-induction in FL was not due to synergy with M-CSF produced by the bone marrow cells themselves, since FL also enabled their formation from the haemopoietic cells of op/op mice, which lack any M-CSF. FL appeared to substitute for M-CSF by supporting the differentiation of adherent cells that express mRNA for RANK and responsiveness to RANKL. To determine whether FL can account for the compensation for M-CSF-deficiency that occurs in-vivo, we blockaded FL signaling in op/op mice by injection of soluble recombinant Flt3. We found that the soluble receptor induced a substantial decrease in osteoclast number, strongly suggesting that FL is responsible for the partial compensation for M-CSF-deficiency that occurs in these mice.

OC2

DISCOVERY OF A NOVEL INHIBITOR OF RAB GERANYLGERANYL TRANSFERASE THAT INHIBITS BONE RESORPTION BY SELECTIVELY PREVENTING PRENYLATION OF RAB PROTEINS IN OSTEOCLASTS

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Nitrogen-containing bisphosphonates such as risedronate inhibit bone resorption by inhibiting farnesyl diphosphate (FPP) synthase, resulting in loss of isoprenoid lipids required for prenylation of small GTPases. Replacement of a phosphonate group of risedronate with a carboxylate moiety gives rise to a less potent anti-resorptive phosphonocarboxylate compound, NE10790. We examined whether the anti-resorptive property of this analogue could also be attributed to inhibition of FPP synthase.

NE10790 was less potent than risedronate at reducing cell viability of J774 macrophages (IC50 ~1200microM vs ~27microM respectively) and less potent at inhibiting bone resorption by rabbit osteoclasts (IC50 ~900microM vs ~15microM respectively) in vitro. Whereas risedronate inhibited the incorporation of [14C]mevalonate into all prenylated (farnesylated and geranylgeranylated) proteins in J774 cells, NE10790 inhibited only the prenylation of proteins of molecular mass 22-26kDa that were not affected by FTI-277 or GGTI-298, peptidomimetic inhibitors of farnesyl transferase (FTase) and geranylgeranyl transferase (GGTase) I, respectively. Furthermore, when purified osteoclasts or J774 cells were labelled with [3H]geranylgeraniol, NE10790 specifically inhibited geranylgeranylation of 22-26kDa proteins that were not affected by GGTI-298. Together, these observations suggest that NE10790 inhibits Rab GGTase, the enzyme which geranylgeranylates 22-26kD Rab proteins. This was confirmed directly by immunoprecipitation of Rab6, Ras or Rho A from J774 cells labelled with [14C]mevalonate. NE10790 inhibited incorporation of [14C]mevalonate into geranylgeranylated Rab6, but not into farnesylated Ras or geranylgeranylated Rho A. Finally, NE10790 inhibited the activity of rhRab GGTase in vitro (IC50 ~600microM), whereas risedronate had no effect. Neither NE10790 nor risedronate affected the activity of either rhFTase or rhGGTase I. Whilst risedronate was a potent inhibitor of rhFPP synthase (IC50 10nM), NE10790 was far less potent (IC50 ~900microM).

These data demonstrate that NE10790 is a newly-identified inhibitor of Rab GGTase. Although NE10790 is also a weak inhibitor of FPP synthase, treatment of intact cells with NE10790 results in the selective loss of prenylation of Rab proteins; NE10790 is the only known prenylation inhibitor to show this specificity. The anti-resorptive activity of NE10790 is likely due to disruption of intracellular membrane trafficking in osteoclasts for which Rab function is crucial.

OC3

THE TEMPO OF IN VITRO OSTEOCLASTOGENESIS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Osteoclasts (OC) can derive in vivo from circulating precursor cells. Yet significant bone resorption typically requires prolonged incubation (14-21 days) of large numbers (2x10^6/ml) of human peripheral blood mononuclear cells (PBMCs). In contrast, OC can appear within 2 days in vivo. Even in vitro, human bone-marrow cells show a maximal rate of bone resorption by 10 days. Poor performance by PBMCs could be explained if (i) osteoclasts normally derive in vivo from a committed but rare precursor in blood; or (ii) if osteoclastogenic conditions in vitro are suboptimal for haematogenous precursors; or (iii) if the haematogenous route is not the usual one for osteoclast supply.

To detect the existence of rare committed precursors, we examined the entire surface of multiple cortical bone slices by scanning electron microscopy for resorption by osteoclasts derived from PBMCs incubated for 48hrs under various conditions. No resorption was ever detected. In 2- to 21-day cultures, we studied various conditions: induced (UMR-106+PTH) or constitutive (retrovirus-transformed UMR-106 cell-line) membrane-expressed RANKL versus soluble RANKL (sRANKL) in a standard (30ng/ml) or high (100ng/ml) dose; addition of TGF-beta1; the timing of exposure to M-CSF, RANKL, TGF-beta1. We found no increase in osteoclast number or in amount of bone resorption, and no decrease in time to first detecting bone resorption, compared with M-CSF and sRANKL alone. Time-course studies demonstrated falling numbers of TRAP +ve cells before bone resorption occurred, an exponential increase in bone resorption between day 10 and 17, and no detectable osteoclast activity from day 2 to 7.

These findings indicate that the slow tempo of in vitro osteoclastogenesis is best explained by the hypothesis that in vitro osteoclasts derive from a sub-population of PBMCs that probably includes cells which do not normally become osteoclasts in vivo. This has important implications for the interpretation of studies using osteoclasts generated in vitro (e.g.Fox et al, J Cell Physiol, 2000; 184:334-340).

OC5

IL-6 INDUCES HUMAN OSTEOCLAST FORMATION AND BONE RESORPTION INDEPENDENT OF RANK/RANKL INTERACTION

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Osteoclast progenitors can differentiate into mature bone resorbing osteoclasts in the presence of macrophage colony stimulating factor (M-CSF) and RANK ligand (RANKL), which is expressed on bone stromal/osteoblastic cells. Osteoprotegerin (OPG) inhibits RANKL-induced osteoclast formation and bone resorption. It has been reported that tumour necrosis factor-a (TNF-alpha), a potent cytokine involved in regulation of osteoclast activity via a primary effect on osteoblasts, can directly (in the presence of M-CSF) induce the differentiation of osteoclast progenitors into mature osteoclasts. These studies revealed that TNF-alpha-induced osteoclast formation is independent of RANK/RANKL interaction. In the present study we sought to determine whether IL-6, another potent stimulator of bone resorption, can similarly induce osteoclast formation and bone resorption in vitro. To address this, mononuclear cells were isolated from peripheral blood of healthy volunteers and cultured for up to 3 weeks on glass coverslips and dentine slices in the presence of: (i) RANKL and M-CSF; (ii) IL-6 (plus soluble IL-6 receptor), M-CSF ± OPG; (iii) IL-6 (plus soluble IL-6 receptor), M-CSF ± anti gp130. The extent of osteoclast formation and bone resorption was determined by generation of TRAP-positive multinucleated cells on glass coverslips and lacunar resorption on dentine slices. We noted that addition of IL-6 (plus soluble IL-6 receptor), in the presence of M-CSF, but in the absence of RANKL, was sufficient to induce the formation of TRAP-positive multinucleated osteoclast-like cells which were capable of lacunar resorption in vitro. The addition of OPG to the cultures containing IL-6 and soluble IL-6 receptor did not inhibit osteoclast formation and bone resorption, thus suggesting that IL-6 induces osteoclast formation in manner independent of the RANK/RANKL mechanism. IL-6-induced osteoclast formation was significantly inhibited in cultures containing anti-gp130 antibodies. Our results indicate that IL-6, which is thought to play a role in several osteolytic bone disorders (e.g. Paget's disease) and is known to be produced in excessive amounts in inflammatory and neoplastic lesions, can directly induce osteoclast precursors to differentiate into active bone resorbing osteoclasts.

OC4

THE EFFECTS OF OESTROGEN ON OSTEOPROTEGERIN, RANKL AND ESTROGEN RECEPTOR EXPRESSION IN HUMAN OSTEOBLASTS

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The cellular responses of osteoblasts and osteoclasts to oestrogen are initiated via two high affinity receptors (ERs). Osteoblasts synthesize RANKL (receptor activator of NF kappa B ligand), necessary for osteoclast formation and function, and osteoprotegerin (OPG), its decoy receptor. To investigate the effects of oestrogen on expression levels of OPG, RANKL and ERs by human osteoblasts, cells were cultured with physiological (100pM) and saturating (100nM) doses of beta-estradiol for 24 and 48 hours. Proteins and corresponding mRNAs were determined by immunohistochemistry and quantitative RT-PCR.

The percentage of osteoblasts expressing OPG was significantly increased at 24 hours by 3 fold and 8 fold with 100pM (p=0.02) and 100nM (p=0.001) estradiol respectively compared to untreated cells. The intensity of staining in cells also showed significant increases (p=0.01). Similar but smaller increases were seen at 48 hours (p=0.01). Osteoblasts treated with physiological doses of estradiol demonstrated a transient increase both in the number of positive cells and intensity of staining for RANKL at 24 hours (p=0.02). However, at saturating concentrations of estradiol these increases were not seen, and by 48 hours estradiol treated cells expressed less RANKL than untreated cells, with the greatest change seen with high-dose estradiol.

ER alpha and ER beta were expressed in a small percentage of untreated cells but demonstrated a significant oestrogen dose-dependent rise in the percentage of cells expressing protein at 24 and 48 hours (p=0.01).

The mRNA data were in concordance with the changes seen in all proteins examined.

These results suggest that oestrogen may exert its anti-resorptive effect on bone, at least in part, by stimulating ER and OPG expression in osteoblasts. These effects, together with the suppression of RANKL are enhanced in the presence of high-dose oestrogen.

OC6

HYPOXIA IS A POWERFUL STIMULATOR OF BONE RESORPTION

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We investigated the effects of oxygen tension (PO2) on bone resorption using two systems. Firstly, mouse marrow cells, free of stromal cells, were cultured 7d on dentine discs in medium supplemented with 10 ng/ml RANKL and 30 ng/ml M-CSF in atmospheres containing 20%, 12.5% or 5% O2 (all with 5% CO2, balance N2). Culture medium was acidified to pH 7.0 for the final 2 days to activate resorption by osteoclasts (OC); PO2, PCO2 and pH were monitored by blood gas analyser. In 5% O2, a 4-fold stimulation of TRAP-positive OC formation was observed, compared with 20% O2. However, OC formed in 20% O2 were generally small (1-2 nuclei), whereas those generated in 5% O2 were large, with multiple nuclei, resulting in striking increases in resorption pit formation (>15-fold). Secondly, ½ calvaria of 5d old mice were incubated 3d in 2% or 20% O2 atmospheres on steel grids and Ca2+ release into culture medium was measured colorimetrically. OC-mediated Ca2+ release was >5-fold greater in 2% O2, equivalent to the maximum stimulation resulting from prostaglandin E2. Increased OC resorption in bones exposed to 2% O2 was evident in TRAP-stained whole mount preparations. Hypoxia-stimulated resorption was completely blocked by 0.1 microM indomethacin, implying prostaglandin mediation, and was strongly inhibited by an IL-1 receptor antagonist protein. In calvarial but not marrow cultures, hypoxia was associated with significant culture medium acidification, consistent with increased anaerobic metabolism. In atmospheric air, arterial and venous blood, PO2 is 160, ~95 and ~40 mmHg, respectively (20%, 12% & 5%).

Interstitially, PO2 is <40 mmHg, whereas in hypoxic environments such as the poorly vascularised yellow fatty marrow associated with ageing, or in inflamed tissue, tumours and fracture sites, PO2 may be <10-20 mmHg. These pathophysiological states are associated with increased bone resorption and also local acidosis, which is now recognised as the key requirement for OC activation. Our experiments reveal a new control mechanism for bone resorption of major importance. The stimulation of OC function in hypoxia may be mediated via increased production of angiogenic cytokines such as TNFalpha, IL-1 and VEGF; the role of RANK/RANKL is presently under investigation.

OC7

EXTRACELLULAR NUCLEOTIDE EFFECTS ON BONE RESORPTION AND FORMATION INVOLVE DIFFERENT P2 RECEPTOR SUBTYPES

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There is increasing evidence that extracellular nucleotides act on bone cells via multiple P2 receptors, a receptor family divided into ionotropic P2X receptors and metabotropic P2Y receptors. The naturally occurring ligand ATP is a potent agonist at all receptor subtypes, whereas ADP and UTP only act at specific receptor subtypes. We recently reported that extracellular ADP, the first degradation product of ATP, is a powerful stimulator of bone resorption at nanomolar concentrations, probably acting via the P2Y1 receptor subtype.

In the present study we investigated the actions of ADP on osteoclast recruitment and osteoblastic function. In 10 day, stromal cell-free mouse marrow cultures in MEM supplemented with RANKL and M-CSF, ADP and ATP at 0.2 to 2 microM, but not 20 microM, stimulated osteoclast formation both on dentine discs and on plastic 48-well plates. In cultures of primary rat calvarial osteoblasts maintained for 16-21 days in DMEM supplemented with ascorbic acid, beta-glycerophosphate and dexamethasone, ADP and the selective P2Y1 agonist 2-methylthioADP were without effect on bone nodule formation at concentrations between 1 and 125 microM. However, both UTP, a P2Y2 and P2Y4 receptor agonist, and ATP strongly inhibited bone nodule formation at >=10 microM, concentrations somewhat lower than those reported previously. Using in situ hybridization, we have shown that rat osteoclasts and rat osteoblastic cells express P2Y1 and P2Y2 receptors, respectively, but in contrast to other studies we found no evidence for the P2Y4 receptor.

Thus, the low-dose effects of extracellular nucleotides on bone resorption and formation appear to be mediated via different P2 receptor subtypes: ADP, signalling through the P2Y1 receptor, is a powerful stimulator of osteoclast formation and activity, whereas UTP (which does not affect osteoclast function), signalling via the P2Y2 receptor, could play a role as a negative modulator of bone formation by osteoblasts.

OC8

NITROSYLATED NSAIDS ARE POTENT INHIBITORS OF BONE RESORPTION IN VITRO

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Non-steroidal anti-inflammatory drugs (NSAIDs) that contain a nitric oxide (NO)-donor group (NO-NSAID) exhibit anti-inflammatory properties without causing the gastro-intestinal side effects usually associated with NSAIDs. As NO can both stimulate and inhibit bone resorption, the NO-NSAIDs may affect the bone loss observed in inflammation. We therefore studied the effect of the NO-NSAID nitrosylated flurbiprofen (HCT1026), and the NSAID Flurbiprofen, on osteoclast formation and bone resorption.

Osteoclasts (OC) were generated in 10-day co-cultures of osteoblasts and spleen cells, or in 14-day human bone marrow cultures, stimulated with RANKL and M-CSF. Drugs and/or IL-1 (10 U/ml) were present during the last 3 days of culture. OCs were identified by TRAcP staining and resorption was measured using reflected light microscopy.

Both compounds tested inhibited IL-1-stimulated OC formation and resorption in the mouse co-cultures. However, the NO-NSAID (IC50 30 µM for OC formation, 15 µM for resorption) was much more potent than the Flurbiprofen (IC50 190 µM for OC formation, 150 µM for resorption). As the resorption was completely blocked by HCT1026, even though there were still OCs present, we studied the effect of this drug on actin ring formation as an indicator of OC activity. HCT1026 significantly decreased the percentage of active OCs within 4 hours and all actin rings had disappeared after 24 hours. When the HCT1026 was added during the first 3 days of the co-culture only, when no mature OCs are present and the system has not yet been stimulated with IL-1, OC formation and resorption were virtually completely abolished, indicating an effect on OC-precursors, whereas Flurbiprofen had no effect. In human bone marrow cultures, a non-inflammatory test system, HCT1026 strongly inhibited OC formation and bone resorption, whereas Flurbiprofen had no effect.

In conclusion, HCT1026 is a potent inhibitor of OC formation and bone resorption in vitro, more potent than its non-nitrosylated parent compound. The mechanism of action is also distinct from the parent compound, and involves inhibition of osteoclast activity, survival and formation. This NO-NSAID derivative may therefore represent a promising new therapeutic agent for treating both inflammatory and non-inflammatory bone loss.

OC9

CELLULAR MECHANISMS OF OSTEOCLAST FORMATION AND BONE RESORPTION IN RHEUMATOID ARTHRITIS

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BACKGROUND AND OBJECTIVES: Pathological bone resorption (marginal erosions and juxta-articular osteoporosis) by osteoclasts is commonly seen in rheumatoid arthritis (RA). Osteoclasts are multinucleated cells that are formed by the fusion of mononuclear precursors that circulate in the monocyte fraction. The nature of the mononuclear precursor from which osteoclasts are formed at sites of bone resorption in RA, however, is uncertain. We have shown that, in the presence of soluble RANKL (sRANKL) and M-CSF, mononuclear inflammatory cells isolated from the RA synovium can differentiate into osteoclasts. In this study we have determined whether osteoclasts are formed from the macrophage fraction of the inflammatory cell population in RA and the role of CD14-negative inflammatory/stromal cells on osteoclast differentiation in RA and osteoarthritis (OA).

METHODS: Synovium from knees and hips was taken during joint replacement surgery from 9 RA patients and 13 OA patients (OA cases were then subdivided into inflammatory OA or non-inflammatory OA based on histological examination). Mononuclear cells were obtained by enzyme digestion of the synovial membrane; this mixed cell population consists of cells which express the CD14+ monocyte/macrophage marker and other inflammatory/stromal cells which are CD14-. CD14+ and CD14- cell populations (separated using the MiniMACS magnetic sorting system) as well as mixed CD14+/ CD14- cells were cultured on coverslips and dentine slices for up to 14 days with sRANKL and M-CSF.

RESULTS/DISCUSSION: Tartrate resistant acid phosphatase (TRAP) and vitronectin receptor (VNR) positive multinucleated cells capable of lacunar resorption were only formed in cultures of CD14+ and mixed CD14+/CD14- synovial cell populations. There was no difference in the extent of osteoclast formation noted in cultures of CD14+ cells isolated from RA, inflammatory OA and non-inflammatory OA synovium. However, significantly more TRAP and VNR positive cells and lacunar resorption was noted in cultures of the mixed synovial cell populations from RA ($p=0.0008$) and inflammatory OA ($p=0.0001$) than from non-inflammatory OA. Our findings indicate that osteoclast precursors are CD14+ cells. The increase in osteoclast formation from the mixed CD14+/CD14- synovial cell populations in RA and inflammatory OA points to a role for CD14- cells in inflamed synovial tissue.

OC10

HUMAN AND BOVINE MILK CONTAINS THE OSTEOCLASTO-GENESIS INHIBITORY FACTOR, OSTEOPROTEGERIN

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Milk is recommended as an excellent calcium source for maintaining strong healthy bones. Milk is also deemed to contain an array of growth factors such as IGF, FGF, TGF beta and other factors that help maintain a healthy skeleton. Osteoprotegerin (OPG), a tumour necrosis factor (TNF) receptor family member, is a critical regulator of bone resorption. It is an important inhibitor of the terminal differentiation and activation of osteoclasts and helps increase bone mineral density and bone volume. In this present study, we investigated whether OPG protein is expressed in human breastmilk, fresh, unpasteurised bovine milk, colostrum, buttermilk and whey.

Twenty micrograms of total protein from each of the human breastmilk, fresh unpasteurised bovine milk (first colostrum-104days), buttermilk and whey samples were subjected to western blot analysis. The blots were incubated with antibodies to human OPG and the protein signal was detected by enhanced chemiluminescence on an autoradiography film. A 55kDa protein band (OPG) was detected in the human breastmilk and the various milk fractions that we analysed. Breastmilk samples showed a slight decrease in OPG protein levels over a period of 5 days. Bovine colostrum samples showed an approximate 3-fold increase in OPG levels compared to bovine milk samples taken from latter time points. Analysis of the whey protein fraction showed a 5-fold increase in OPG protein levels compared to that of unpasteurised buttermilk and a commercial brand of semi-skimmed milk.

Our results show that osteoprotegerin protein is expressed in varying concentrations in differing types of milk. A milk-binding protein from the whey fraction from bovine milk has been shown to suppress osteoclastic-mediated bone resorption and osteoclastic cell formation and to protect bone loss in ovarectomised rats. Additionally, a recent study has shown that the addition of recombinant OPG to women suffering from osteoporosis stabilises the loss of bone. We propose the presence of OPG in milk indicates a physiological role in the development and maintenance of mature mineralised bone and may be a source of natural and combatible OPG that could be isolated and used as a treatment of osteoporosis and other bone resorptive diseases.

OC11

BISPHOSPHONATES INDUCE APOPTOSIS IN BREAST CANCER CELLS IN A CASPASE-DEPENDENT MANNER BY INHIBITION OF THE MEVALONATE PATHWAY

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We have previously shown that aminobisphosphonates (N-BPs) pamidronate and zoledronic acid (ZOL) inhibit cell proliferation and induce apoptosis in breast cancer cells. The aim of this study was to identify the signalling pathways involved. We have previously reported that incubation of cell cultures with N-BPs leads to decreased expression of bcl-2 and also our initial studies suggested a role for caspase activation. We determined the effect of forced expression of the anti-apoptotic protein bcl-2 in MDA-MB-231 breast cancer cells treated with N-BPs. Cells stably transfected with a pUSEamp (+) plasmid containing wild-type mouse bcl-2 were protected against loss of cell viability and induction of DNA fragmentation in response to 100µM ZOL after 3 days treatment. Furthermore, effects of pamidronate (35µM) on these indices of apoptosis could also be prevented by preincubation of cells with the broad spectrum caspase inhibitor z-VAD-fmk (50µM) implicating a caspase-dependent mechanism. Rescue of cells from ZOL-induced apoptosis by preincubation with a caspase-3 selective inhibitor (0.5µM), and by demonstration of cleavage of intact pro-caspase-3 (40kD) into 17 and 11kD fragments after 3 days treatment with 100µM ZOL, suggests that at least one of the caspases activated is caspase-3.

It has been previously suggested that N-BPs induce apoptosis in osteoclasts and myeloma cells via inhibition of the mevalonate pathway. This results in impaired generation of intermediates including farnesyl pyrophosphate (FPP) which are required for post-translational lipid modification of small G-proteins such as Ras. To investigate this potential mechanism, we first identified that co-treatment with farnesol (40µM of which is converted to FPP intracellularly), protects against N-BP-induced apoptosis in MDA-MB-231 cells. Secondly, using Western blot analysis, we have demonstrated a reduction in membrane bound Ras and an increase in its cytosolic localization, after 3 days treatment with 100µM ZOL. Thirdly we compared the ability of ZOL and manumycin A, a farnesyl transferase inhibitor which prevents Ras prenylation, to induce cell death in three breast cancer cell lines. Similar order of sensitivity (Hs 578T> MDA-MB-231>MCF-7) was seen with both compounds. These observations lend support to the suggestion that N-BP induced apoptosis in breast cancer cells is caspase dependent and may be related to impaired post-translational modification and cellular localisation of Ras.

OC12

SYNTHESIS OF 1,25-DIHYDROXYVITAMIN D3 BY HUMAN ENDOTHELIAL CELLS IS REGULATED BY INFLAMMATORY CYTOKINES: A NOVEL AUTOCRINE DETERMINANT OF VASCULAR CELL ADHESION

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Recent studies have shown that increased circulating concentrations of active vitamin D, 1,25-dihydroxyvitamin D3 (1,25D3), reduce the risk of coronary calcification of atherosclerotic vessels. Furthermore, low 1,25D3 serum levels in patients with renal failure appear to increase vascular calcification. The precise mechanism for this remains unclear, although data have highlighted a possible role for 1,25D3 as a modulator of cell adhesion and angiogenesis. Previous studies have demonstrated the presence of vitamin D receptors and the enzyme 1alpha-hydroxylase (1-HYD) in bovine endothelial cells. Here we have used immunohistochemical, and in situ hybridization analyses to show for the first time the expression of 1-HYD mRNA and protein in human endothelial cells from kidney, spleen and lymph nodes. RT-PCR and Western analyses using primary cultures of human umbilical vein endothelial cells (HUVEC) confirmed expression of mRNA and protein corresponding to the reported renal 1-HYD. Enzyme activity assays in HUVEC using 3H-25D3 as substrate showed basal conversion of 318 ± 56 (±SD) fmoles 1,25D3/hr/mg protein which increased 3-4-fold following 24 hr treatment with TNFalpha (1054 ±166) or lipopolysaccharide (1381 ±88). Forskolin (554 ±56) increased only slightly the production of 1,25D3, and incubation with 1,25D3 itself (475 ±111) did not suppress 1-HYD activity, as is classically observed with the renal enzyme. Functional analyses showed that exogenously added 1,25D3 or precursor 25-hydroxyvitamin D3 significantly increased monocyte adhesion to HUVEC in a similar fashion to inflammatory agents such as TNFalpha; parallel ELISA studies revealed that this was not due to increased expression of ICAM-1 or VCAM-1. These data suggest that vitamin D metabolism and function in human endothelia is similar to that described previously for macrophages, with inflammatory cytokines showing greater up-regulation of endothelial 1-HYD activity than classical calcitropic regulators. We therefore propose that in endothelial cells 1-HYD acts as a novel autocrine/paracrine immunomodulatory mechanism. In particular, the synthesis of 1,25D3 by blood vessels during inflammation may play an important role in leukocyte adhesion. Further analysis of this process may help to elucidate a mechanism for the impact of vitamin D on cardiovascular disease.

OC13

CORTICAL POROSITY AND ITS RELATIONSHIP TO REMODELLING CLUSTERS (SUPER-OSTEONS)

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The increased cortical porosity in the fractured femoral neck is primarily dependent on canals whose diameter exceeds the normal mean +3SD (385µm). These "giant" canals were unlikely to have been formed by random remodelling events but were positively associated with spatially clustered remodelling osteons (super-osteons). We have now investigated whether super-osteons are skeletal site-, age- or gender-specific.

Microradiographic images of the cross-section of the mid-femoral shaft from 33 male and 33 female non-pathological subjects aged 20-91y at death were analysed using NIH Image. The number, size and locations of all Haversian canals were mapped and an edge-detection algorithm used to identify the most recently remodelled osteons. Cluster analysis (0.75mm diameter) then identified the number and size of super-osteons in the complete cortex, circumferentially divided into periosteal (P, 1.5 mm from the periosteal surface), endosteal (E, 1.5 mm from the endosteal surface) and intra-cortical (I-C, between periosteal and endosteal regions) regions.

Cortical remodelling was greater in females (+34% p=0.034). As in the femoral neck, remodelling osteons were more clustered than would be predicted by chance ($p<0.0001$) but this was not affected by age (p=0.59) or gender (p=0.51). The density of super-osteons was highest in the cortex closest to the periosteal surface (periosteal: 0.043± 0.004/mm2; intra-cortical 0.028±0.003; endosteal 0.017±0.002. P>I: $p<0.0001$, I>E: p=0.002). Cortical porosity increased with age in both males and females and the proportion of giant canals was age (20-40y: 0.22±0.05%, >80y: 1.18±0.3%, $p<0.01$) but not gender dependent. Furthermore, the density of giant canals was negatively related to the density of remodelling clusters (adj $r2 = 0.24$, $p<0.0001$).

In conclusion, these data suggest that near simultaneous activation of cortical remodelling occurs within adjacent osteons. This study also indicates that the presence of super-osteonal remodelling is not skeletal site- or gender-specific and occurs throughout adult life. In the non-pathological femoral shaft, clustering is negatively associated with the development of giant canals. This suggests that our previous observation of a positive association in the femoral neck cortex in hip fracture might result from a focal loss of the control of resorption depth within super-osteons, analogous to trabecular fenestration seen in post-menopausal osteoporosis.

OC14

REDUCED BONE MASS AND ALTERED GROWTH PLATE MORPHOLOGY IN AGED RATS EXPOSED TO INTRAUTERINE PROTEIN RESTRICTION

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Cohort studies in humans have suggested that the peak bone mass attained at skeletal maturity may be programmed in utero. In this study, a rodent model was used to test the hypothesis that maternal undernutriton during pregnancy is associated with reduced bone mass and altered growth cartilage morphology in adult offspring.

Ten female rats were mated and fed diets containing 180g casein/kg (control), or 90g casein/kg (low protein) throughout pregnancy. 23 offspring of the low protein group, and 31 offspring of the control group were maintained on a normal chow diet throughout life and were allowed to die without intervention if there was no evidence of pain, distress or discomfort. Bone mineral measurements were obtained by dual energy X-ray absorptiometry, and tibiae were removed and processed for histology.

Offspring in the low protein group had significantly lower whole body bone area (p=0.01), and lower whole body bone mineral content (p=0.06). There was no difference in whole body bone mineral density (p=0.98). Histological analysis was performed on 16 male offspring, matched for age at death. In rats, growth plates remain until old age, but these are stationary after growth has ceased. Quantitative measurements demonstrated that the heights of the proximal tibial growth plate were significantly greater ($p<0.001$) among offspring of the low protein group. In addition, the following age-related morphological changes were identified in the growth plates: i) presence of large acellular areas of cartilage matrix; ii) resorption of irregular areas within the cartilage and deposition of bone matrix. Semi-quantitative analysis of these features demonstrated that the growth plates from the low protein group had larger areas of acellularity as well increased remodelling than those of the controls.

These findings are consistent with the hypothesis that growth trajectory and the accrual of bone mineral are programmed in early life. The increased height of the growth plate in maternally undernourished animals may reflect a cessation of growth at an earlier age. The increased irregularity of the growth plate in this group may infer an earlier onset of age-related changes within the growth cartilage.

OC15

EXPRESSION OF HYPOXIA-RESPONSIVE GENES DURING CHONDROCYTE DIFFERENTIATION

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Acquisition of the terminally differentiated (hypertrophic) phenotype by growth plate chondrocytes appears to be essential for vascular invasion from the metaphysis and the subsequent resorption of the growth plate and its replacement by bone. In order to identify genes that may be involved in the regulation of growth plate vascularisation/resorption, we performed an agarose differential display analysis on chick growth plate chondrocytes that had been separated into five phenotypically distinct populations by centrifugation through Percoll density gradients. Using this strategy we identified and cloned a 1500 bp cDNA derived from a transcript more abundantly expressed in hypertrophic chondrocytes compared to proliferating cells. Sequence analysis indicated 100% identity to the chick endothelial domain-1 (EPAS-1) gene. EPAS-1 is a transcription factor which is upregulated in response to hypoxia and which subsequently regulates the expression of a number of hypoxia-inducible genes. Using semi-quantitative RT-PCR assays we confirmed that EPAS-1 is upregulated as growth plate chondrocytes become terminally differentiated. In contrast, the related transcription factor HIF1-alpha, which also regulates the expression of hypoxia-inducible genes, was expressed uniformly throughout the growth plate.

A potential target for EPAS-1 in the growth plate is vascular endothelial growth factor (VEGF) whose expression is upregulated in hypertrophic chondrocytes and which is essential for vascular invasion into the growth plate. VEGF is a member of a family of at five structurally proteins, but the role of these other VEGF-related proteins in the growth plate has not been investigated. Using the murine cell line ATDC5, which differentiate into chondrocytes that ultimately hypertrophy and elaborate a mineralised matrix, we detected expression of VEGF, VEGF-B, VEGF-D and placental growth factor. We also observed the expression of VEGF-C in Percoll-fractionated chick growth plate chondrocytes. We also detected transcripts for two of the receptors that mediate the effects of the VEGF family, VEGFR-2 and VEGFR-3, with expression being highest the proliferating chondrocytes. These results suggest that the role of the VEGF family members in the growth plate is not confined to the stimulation of vascular invasion but may involve an autocrine/paracrine loop functioning within the growth plate itself.

OC16

MATERNAL PROTEIN DEFICIENCY AFFECTS MESENCHYMAL STEM CELL POPULATIONS IN THE DEVELOPING OFFSPRING

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Epidemiological studies have suggested that skeletal growth is programmed during intrauterine and early postnatal life. Maternal nutrition appears to be important in determining skeletal size through fetal adaptation of endocrine and metabolic systems. We have used a rat model of maternal protein insufficiency to investigate the cellular mechanisms involved in the programming of bone development.

The aims of this study were to determine whether colony formation (colony forming unit-fibroblastic, CFU-F), proliferation and differentiation of bone marrow stromal cells from offspring of female rats maintained on normal (18% casein) or low (9% casein) was altered and their response to key endocrine factors, Growth Hormone (GH), 1,25(OH)2D3 and IGF-1. Dams were fed an 18% casein control diet or 9% casein low protein diet from conception until the end of pregnancy. Offspring (n=40) were fed a normal protein diet until harvest at 4, 8 and 12 weeks after birth. At four weeks, total CFU-F and alkaline phosphatase-positive CFU-F were significantly reduced (21.9% and 41.6%, $P<0.01$) in the low protein group. At 8 weeks, total CFU-F was 38.0% lower ($P<0.01$) and alkaline phosphatase-positive CFU-F 90.6% lower ($P<0.01$). At 12 weeks, no significant differences were observed in colony formation. However, colony size was significantly reduced in the 9% group, indicating reduced cell proliferation. The results indicate that normal proliferation and differentiation of mesenchymal stem cells was delayed by restricted maternal nutrition during early life. Modulation of osteoblast proliferation and differentiation was observed in the 18% group by 1,25(OH)2D3, IGF-1 and GH at 8 weeks and the low protein group at 12 weeks. Alkaline phosphatase specific activity was also significantly decreased at 4 and 8 weeks ($P<0.001$) in the low protein group. At 12 weeks this was reversed with significantly increased specific activity in the low protein group. Bone mineral measurements by DXA confirmed decreased bone mineral content in the low protein offspring. With skeletal maturity, "catch-up" or a physiological shift in bone cell activity was observed. These data provide further evidence that growth trajectory and bone growth are programmed in early life and indicate the importance of maternal nutrition on programming of skeletal development.

OC17

OSTEOBLASTIC EXPRESSION OF ADAMTS FAMILY MEMBERS

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Proteoglycans play a fundamental role in regulating bone formation. Not only can they influence cell:growth factor and cell:extracellular matrix (ECM) interactions, but through specific associations with type I collagen they modulate the mineralisation process itself. Consequently the mechanisms that underlie the temporal and spatial distribution of proteoglycans in bone are of considerable importance to our understanding of bone cell biology. A sub-family of the ADAMs (a disintegrin and metalloproteinase) has been identified, that contains thrombospondin-like motifs (ADAMTS), and ADAMTS4 and 5 have recently been shown to cleave the major proteoglycan of cartilage, aggrecan. We propose that ADAMTS family members play a novel role in regulating osteoblast function by determining the distribution of proteoglycan in bone.

We used RT-PCR and Northern blotting to show expression of ADAMTS1, 3 and 5 in primary rat osteoblasts and in the osteosarcoma cell lines, MG63, TE85 and SaOS-2. Treatment of MG63 and SaOS-2 cells with the potent bone resorption factor interleukin-1beta enhanced the expression of mRNA for ADAMTS1. Furthermore, ADAMTS1 transcript levels increased with time in primary rat osteoblasts driven by dexamethasone, beta-glycerophosphate and ascorbic acid phosphate to produce bone-like nodules in vitro.

Since we are interested in the relationship between the osteoblast and matrix molecules, we plated TE85 cells onto an ECM synthesised by MG63 cells and isolated RNA at 1, 24 and 48 hours. Northern analysis showed a transient upregulation of mRNA for both ADAMTS1 and 5 at 1 hour that was reduced to control levels at 24 and 48 hours. Transcripts for ADAMTS1 and 3 were also upregulated in primary rat osteoblasts when seeded on ECM molecules like fibronectin and type I collagen for 48 hours.

These data suggests that cells of the osteoblast lineage express ADAMTS1, 3 and 5 and that transcript levels can be regulated by ECM components. The focalised production of ADAMTS family members in response to matrix-derived and other cues may be an important part of bone formation.

OC18

THE ROLES OF THE OSTEOBLAST-STIMULATING FACTOR-1 DURING ENDOCHONDRAL OSSIFICATION

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Osteoblast-stimulating factor-1 (osf-1) enhanced bone mineral content in transgenic mice over-expressing the human osf-1 gene[1,2]. To elucidate further the roles of osf-1 in osteogenesis, this study i) immunolocalized the osf-1 protein in bone and cartilage of transgenic and control mice and ii) investigated the effects of exogenous osf-1 on bone formation in a bone organ culture system.

In transgenic and control mice, osf-1 was localized in the periosteum, osteoblasts and the bone matrix of the primary spongiosa. Expression of osf-1 occurred early during osteoblast differentiation, together with alkaline phosphatase, but prior to the expression of bone sialoprotein and before osteoid mineralization. In transgenic mice, positive immunostaining for osf-1 was found in the chondrocytes and cartilage matrix of the growth plate, including the mineralized cartilaginous struts onto which osteoblasts are recruited. In controls, this staining was weak or absent, possibly because levels were below immunocytochemical detection limits.

Our previous findings2 had identified synthesis of type I collagen in articular chondrocytes of transgenic mice, but never in controls. Similarly, the present study demonstrated osf-1 in articular chondrocytes of transgenic mice, but not in controls. When cartilaginous bones were cultured with exogenous osf-1, ~20% of chondrocytes synthesized type I collagen, and both osf-1 and type I collagen could be co-localized in the same chondrocytes.

Conclusions: 1. Osf-1 is synthesized by osteoblasts and, to a lesser extent, by chondrocytes and stored in the bone or cartilage matrix. During resorption, osf-1 will presumably become available and may assist in recruitment of osteoblasts/ precursors either to bone formation sites or to the struts of mineralized cartilage. 2. Since expression of osf-1 preceded mineralization and coincided with alkaline phosphatase expression, it may play a role in matrix mineralization. This may explain the enhanced bone mineral content of osf-1 transgenic mice. 3. Synthesis of osf-1 and type I collagen by articular chondrocytes was only found in transgenics or following addition of osf-1 to organ-cultured cartilaginous bone, suggesting that osf-1 provided an

increased osteogenic stimulus, which caused chondrocytes to transdifferentiate along an osteogenic lineage.

1Biochem. Biophys. Res. Comm. (1997) 238:528-533. 2Calcif. Tissue Int. (2000) 66, S59.

OC19

OSTEOCYTES EXPRESSING ENOS ARE PERIPHERALLY LOCATED IN CORTICAL BMU'S: A ROLE IN MAINTAINING CORTICAL INTEGRITY?

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Nitric oxide release from bone cells is increased by both the application of mechanical strain and estrogen, and the endothelial nitric oxide synthase (eNOS) isoform is present in bone cells, particularly osteocytes. We have investigated the location and density of eNOS positive osteocytes in cortical BMUs in the aged human femoral neck from fracture cases and controls.

Expression of eNOS in osteocytes was determined by immunohistochemistry in sections from 7 cases (female, 70-96y) and 7 controls (female, 68-96y). Their number and location in the superior and inferior regions was measured by image analysis. Similar analyses of all osteocytes used propidium iodide (PI) stained sections. Median, minimum and maximum distances from eNOS+ve and PI+ve osteocytes to the nearest canal surface was calculated for each BMU and averaged for both regions of each biopsy.

The median distance of eNOS+ve osteocytes from the canal surface was higher than that for PI+ve osteocytes (eNOS: 66.6±2.0um; PI: 54.7±1.2um, $p<0.0001$); this was independent of region and biopsy type (case vs control). The nearest eNOS+ve osteocyte was further away from the canal surface than PI+ve osteocytes (eNOS: 40.2±1.7um; PI: 25.5±1.1um; $p<0.0001$). This difference was dependent on biopsy type (controls: +12.0±2.2um, cases: +17.5±2.9um; $p<0.0001$) but not region. The maximum distance from the canal surface (mean 96.2±2um) was unaffected by osteocyte category, region or biopsy type.

The density (mm-2) of PI+ve osteocytes was independent of region or biopsy type. However, the density of eNOS+ve osteocytes was dependent on region (72% higher inferiorly, $p=0.0001$), and biopsy type. Cases had a 53% lower density inferiorly ($p=0.0004$) but not superiorly.

In conclusion eNOS+ve osteocytes are located towards the periphery of cortical BMUs, and in hip fracture their density is reduced in the usually more heavily loaded inferior region. As NO reportedly inhibits osteoclast activity, eNOS+ve osteocytes may act as sentinels to confine resorption within cortical BMUs. Such a role might explain the increased presence of "giant" composite canals in the inferior half of the femoral neck in fracture cases. We showed previously that these canals have an appearance consistent with the merging of osteonal BMUs. and are the principal contributors to cortical porosity.

OC20

CELL CYCLE CONTROL DURING OSTEOBLAST DIFFERENTIATION AND POST-CONFLUENCE GROWTH REGULATION BY C-FOS

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The proto-oncogene c-fos plays an important role in osteoblast biology as demonstrated by the formation of osteosarcomas in c-Fos transgenic mice. We have shown previously that cell cycle control genes exhibit altered expression patterns in osteoblasts of c-fos transgenic mice relative to normal osteoblasts. We have additionally demonstrated that c-Fos overexpression in primary murine osteoblasts and AT9.2 cells, an MC3T3-E1 clone expressing tetracycline (Tc)-regulatable c-fos, causes increased expression of cyclin A resulting in accelerated growth. However, the relationship between c-Fos and cell cycle control and differentiation is unclear.

We show that expression of exogenous c-fos in primary osteoblasts or in Tc inducible clones show reduced differentiation, demonstrating that c-Fos can inhibit osteoblast differentiation. In order to determine whether changes in cell cycle regulation are related to differentiation or long term culture, we measured the expression of cell cycle regulators in primary osteoblasts cultured with or without ascorbate. Early differentiation specific changes were prolonged expression of cyclins A and D3, CDK1, CDK2 and p27, but reduced p57 expression, suggesting that there is a population of cells that continue to proliferate during differentiation. At later time points there was an increase in p16 expression in differentiated cultures. Expression of p16 is also associated with senescence, and staining for senescence-associated beta-galactosidase activity revealed increased staining in cells cultured in ascorbate, suggesting an interrelationship between differentiation, cell cycle and induction of senescence.

In AT9.2 cells, there was no effect of ectopic c-Fos on growth rate in exponential cultures. However, at confluence, exogenous c-Fos expression correlated with persistence of cyclin E expression, increased cell number and a higher number of cells in S-phase as determined by FACS analysis.

Together, these results suggest that there are specific changes in cell cycle regulation during osteoblast differentiation which differ from those observed in non-differentiating cultures, and that c-Fos can regulate post-confluent growth regulation in osteoblasts.

OC21

MALE OESTROGEN RECEPTOR-BETA KNOCKOUT MICE SHOW INCREASED SENSITIVITY TO OESTROGEN-INDUCED BONE FORMATION

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Previous studies suggest that oestrogen stimulates bone formation if relatively high doses are administered. Following the report that the beta isoform of the oestrogen receptor (ER beta) inhibits ER alpha-dependent transcriptional activity, we examined whether the requirement for relatively high doses of oestrogen to stimulate bone formation reflects a negative influence of ER beta on this response. We compared the dose-responsiveness of oestrogen-induced bone formation between twelve-week-old mice homozygous for a targeted gene deletion in ER beta (BERKO mice) (Krege et al., Proc Natl Acad Sci USA 1998, 95:15677) and age-matched wild-type controls (WT). Animals were administered vehicle or 17beta-oestradiol (E2) 4, 40, 400, 4000 microg/kg/day for 28 days (4-7 animals per group). The subsequent response was quantified by measurement of bone mineral density (BMD) at the femur and tibia using a Lunar PIXI scanner with dedicated mouse software. We have previously found that this technique provides an accurate estimate for new cancellous bone formation as measured by histomorphometry. Since bone modelling has previously been found to be altered in female BERKO mice, which may complicate interpretation of DXA-derived data, the present analysis was confined to males. No significant difference in BMD was observed between vehicle-treated WT and BERKO mice at any measurement site. The maximal response to oestrogen was also similar in WT and BERKO mice at all regions. However, the pattern of this response differed, since E2 40microg/kg/day was associated with maximal response in BERKO animals, compared to E2 400microg/kg/day in WT mice. Thus, BMD was significantly greater in BERKO compared to WT mice at E2 40microg/kg/day at the whole femur (65 +/- 2 vs 58 +/- 2, $p = 0.01$), distal femoral metaphysis (140 +/- 2 vs 122 +/- 6, $p = 0.02$), whole tibia (57 +/- 2 vs 51 +/- 1, $p = 0.02$), and proximal tibial metaphysis (119 +/- 4 vs 101 +/- 4, $p = 0.01$) (mean +/- SEM mg/cm2; one-way ANOVA with Fisher's test for least significant difference). Our results indicate that BERKO mice show a greater sensitivity to oestrogen-induced bone formation, suggesting that ER beta is a negative regulator of this response at sub-maximal oestrogen doses.

OC22

INDUCTION OF HUMAN OSTEOPROGENITOR CHEMOTAXIS, PROLIFERATION AND DIFFERENTIATION BY A NOVEL BONE FACTOR, OSTEOBLAST STIMULATING FACTOR-1

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The process of bone growth, regeneration and remodelling is mediated, in part, by the immediate cell-matrix environment. Osteoblast stimulating factor-1 (osf-1), also known as pleiotrophin or HB-GAM, is an extracellular matrix-associated protein, which is present in those matrices that act as targets for the deposition of new bone. However, the actions of osf-1 on bone progenitor cells remains unknown. The aims of this study were to examine the effects of osf-1 on primary human bone marrow stromal cell adhesion, chemotaxis, proliferation, differentiation and colony formation (colony forming unit-fibroblastic, CFU-F) on tissue culture plastic and, in particular, growth and differentiation on 3-D biodegradable porous biomimetic scaffolds adsorbed with osf-1.

Primary human bone marrow cells were cultured on tissue culture plastic or poly (-lactic acid co-glycolic acid) (PLGA) (75:25) porous scaffolds with or without addition of recombinant human osf-1 (1pg-50ng/ml) in basal and osteogenic conditions. Porous biodegradable scaffolds were generated using a novel supercritical fluid method. Cell adhesion and spreading were examined by confocal microscopy following incorporation of fluorescent labels as well as by scanning electron microscopy and immunocytochemistry. The chemotactic ability of osf-1 was examined on patterned surfaces generated using EM grids on tissue culture plastic coated with osf-1 and irradiated with UV light.

Negligible cellular growth was observed on PLGA scaffold alone. Osf-1 (50ug/ml) was chemotactic to human osteoprogenitors, which migrated to the areas of intact osf-1 after 7 hours. Osf-1 significantly stimulated total colony formation and alkaline phosphatase-positive colonies at concentrations as low as 10pg/ml ($P<0.05$) compared to control cultures. The effects were time-dependent. Alkaline phosphatase specific activity was also significantly stimulated by osf-1 (10pg/ml)($P<0.01$)in basal and osteogenic conditions. On 3-D scaffolds adsorbed with osf-1, alkaline phosphatase activity, type I collagen formation, synthesis of cbfa-1, osteocalcin and osf-1 were observed by immunocytochemistry.

These results demonstrate that osf-1, a novel osteotropic agent, has the ability to promote adhesion, migration, expansion and differentiation of human osteoprogenitor cells. The successful generation of 3-D biomimetic structures incorporating osf-1 indicates the potential for the development of protocols for de novo bone formation that exploit cell-matrix interactions.

OC23

ISOLATION OF OSTEOPROGENITOR CELLS FROM SKELETAL MUSCLE

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Bone diseases such as osteogenesis imperfecta (OI) occur because of the presence of single mutations. One possible treatment for these diseases is the use of transplanted bone marrow-derived mesenchymal stem cells (MSC) stem cells containing a the correct gene. Allogeneic bone marrow transplantation in children with OI achieved a small degree of engraftment (1-2%) but appeared to be accompanied by some clinical improvement. The risk associated with such a procedure is substantial however, autogenic transplantation might offer a safer and more effective therapy. The withdrawal of bone marrow is an invasive procedure and is unsuitable for children with skeletal disorders such as OI. We have therefore been looking for other sources of MSC, one such source being skeletal muscle.

Putative MSC were isolated from soleus muscle from 200g male Wistar rats by 3 methods. 1. The muscle were diced into 0.5mm cubes and allowed to grow as explants in DMEM containing 10% FCS, 10-8M dexamethasone, Pen/Strep, Glutamax and ascorbate. The outgrowing cells were then subcultured into larger culture vessels. 2. The diced muscle was digested in collagenase for 1.5h in 1mg/ml collagenase, the cells washed and then grown in the above medium. 3. The cells obtained from the digests were "preplated" by transferring non-adherent cells daily to new petri dishes leaving the adherent cells to grow further. The cells were then examined for the presence of osteoblast-like cells morphologically and by staining for alkaline phosphatase and calcium deposition.

All 3 methods reproducibly produced cells which morphologically resembled stromal or osteoblast-like cells, stained positive for alkaline phosphatase and produced calcified nodules. By preplating the digests, fibroblastic cells were removed in the first 1-2 preplates and thereafter the cultures were progressively enriched with a population of small round slow-adhering cells which given the appropriate conditions adopted an osteoblastic phenotype. This process of preplating has been maintained for up to 21 d and still maintains the capacity to give rise to osteoprogenitor cells.

These data show that there exists in skeletal muscle a population of primitive slow adhering cells with osteogenic potential. Whether these are true MSC requires further work.

OC25

CALCIUM DEPENDENT PROTEIN KINASE II ACTIVITY IS OBLIGATE FOR BONE FORMATION IN VITRO

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At central glutamatergic synapses long-term potentiation and synaptic plasticity are initiated and regulated by the activity of NMDA-type glutamate receptor associated calcium-dependent protein kinase II (CAMKII). We have previously demonstrated that osteoblasts express many of the synaptic components contributing to the formation of the neuronal post-synaptic density - an electron dense signalling specialisation enriched with receptors, clustering molecules and down stream signalling apparatus. Experiments were performed to investigate the role of CAMKII in glutamate signalling and cell differentiation in osteoblasts. Using a combination of immunolocalisations, nested RT-PCR and western blot analysis we demonstrate that osteoblasts express all of the CAMKII subunits including the putatively neurospecific isoforms CAMKII-alpha and beta found downstream of NMDA receptors at central synapses. We compared the variable domains of osteoblastic CAMKII subunits with isoforms expressed in the CNS and found that CAMKII alpha and beta are alternatively spliced, resulting in an 11 amino acid insertion and a 23 amino acid deletion respectively in the region responsible for subcellular targeting. Studies demonstrate that CAMKII-alpha is constitutively expressed during primary rat osteoblast differentiation while the relative abundance of CAMKII-beta increases ten-fold as the cells differentiate to the osteoblast phenotype. This expression pattern may reflect an increasing importance of CAMKII during differentiation and is in contrast to developing neurones where alpha subunit abundance increases and beta subunit remains constant or decreases during development. In primary rat osteoblasts CAMKII activity is specifically increased by the application of depolarising concentrations of KCl (60mM) or glutamate (100microM) and glycine (1microM) demonstrating the direct association between glutamate receptor induced increases in intracellular calcium and CAMKII activity. We recently reported that osteoblastic CAMKII activity is increased by brief periods of mechanical loading in vitro, to further these studies the effects of CAMKII inhibition on osteogenesis were determined. The CAMKII inhibitor KN-62 (50nM-10microM) caused a time- and dose-dependent reduction in alkaline phosphatase activity and bone nodule formation without affecting cell proliferation suggesting distinct phases of CAMKII activity are necessary for normal osteoblast function.

OC24

PHOSPHORYLATION OF PROTEIN KINASE B BY MECHANICAL STRAIN IN OSTEOBLASTS - A MECHANISM FOR ACTIVATION OF NITRIC OXIDE SYNTHASE IN THE ADAPTIVE RESPONSE.

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In endothelial cells, endothelial Nitric Oxide Synthase (eNOS) is activated by the Protein Kinase B (PKB/Akt) intracellular signalling pathway that is also a mediator of cell survival. PKB signalling is initiated by activation of membrane-associated phosphatidylinositol-3-kinase (PI-3-K) which in turn phosphorylates adjacent phosphatidylinositol lipids to generate PIP(sub3). This event leads to the recruitment of PIP(sub3)-dependent kinases (PDK-1 & -2) and PKB from the cytoplasm to the membrane. Assembly of these proteins in the same microenvironment facilitates phosphorylation of PKB at residues Ser473 and Thr308 and activation. We investigated the ability of mechanical strain to regulate PKB activity in osteoblasts in vitro.

Clonal and primary rat calvarial cells were grown on type I collagen coated wells, maintained in medium alone or supplemented with dexamethasone and beta glycerol phosphate to induce osteoblast differentiation for 7 days before mechanical stimulation. Cells were subjected to cyclical strains (peak 0.005, 1 Hz) for 10 minutes and total protein extracted after loading. Lysates were analysed by immunoblotting using specific phospho-PKB (Ser473) antibody. Immunoblots were subsequently stripped and analysed with a PKBalpha antibody to control for protein loading.

In clonal cells, a significant 2.5-fold increase in phosphorylation of PKB at Ser473 was evident within 2 minutes of the end of the loading regimen and peaked at 20-40 minutes. This response to strain was completely blocked by pre-treatment of cells for 1 hour with specific PI-3-K inhibitors (wortmannin, 500 nM or LY294002, 10 microM). In primary osteoblasts, PKB phosphorylation was significantly increased (3-fold) in differentiated osteoblasts subjected to mechanical strain, but not undifferentiated calvarial cells subject to the same stimulation.

These findings demonstrate that in osteoblasts, PKB is phosphorylated rapidly in response to mechanical stimulation. Additionally, differentiating osteoblasts show a much greater mechanical induction of PKB activity than less differentiated cells. These finding suggest 1) a mechanism for regulation of the eNOS activity known to be an obligate step in the adaptive mechanism, and 2) that even early commitment towards the osteoblastic lineage sensitises cells to the effects of strain.

P1

INVOLVEMENT OF RANKL-DEPENDENT/INDEPENDENT MECHANISMS IN OSTEOCLAST FORMATION IN ASEPTIC LOOSENING

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The current paradigm to explain the osteolysis of aseptic loosening is that wear debris generated from prostheses are phagocytosed and stimulate the activation and secretion of pro-inflammatory cytokines (e.g. TNF-alpha) by macrophages. TNF-alpha and other cytokines are known to induce RANK ligand (RANKL) expression on osteoblasts and bone stromal cells. Interaction of RANKL with RANK, expressed on osteoclast precursors, is now known to promote osteoclast development and activation. We have shown that particle-associated macrophages isolated from failed joint arthroplasties can differentiate into mature functionally active osteoclastic cells in the presence of soluble RANKL and M-CSF. We have also shown that RANKL-induced osteoclast differentiation in these tissues can be significantly inhibited by osteoprotegerin (OPG). Recently it has been reported that TNF-alpha (in the presence of M-CSF) is capable of substituting for RANKL in supporting osteoclast differentiation from mouse marrow precursors and human mononuclear cells. The aim of the current investigation was therefore to determine whether TNF-alpha (in the presence of M-CSF) could support macrophage-osteoclast differentiation in periprosthetic tissues in a manner independent of RANKL/RANK mechanism.

Following collagenase digestion of periprosthetic tissues, obtained from 6 patients undergoing hip revision due to aseptic loosening, CD14-positive macrophages were separated using the MiniMACS magnetic cell sorting system. CD14-positive populations were cultured on coverslips and dentine slices the presence of: (i) M-CSF and soluble RANKL or (ii) M-CSF, TNF-alpha and IL-1-alpha ± OPG. All cultures were maintained for 1-14 days after which the extent of osteoclast differentiation was determined by the expression of specific osteoclast markers such as TRAP and VNR on coverslips and evidence of lacunar resorption on dentine slices.

Extensive osteoclast formation and lacunar resorption was evident in macrophage cultures in the presence of soluble RANKL and M-CSF. In the absence of soluble RANKL, but in the presence of TNF-alpha and IL-1-alpha, macrophages isolated from periprosthetic tissues were capable of differentiating into active bone resorbing osteoclasts. Addition of OPG did not reduce the extent of TNFa-mediated osteoclast differentiation by periprosthetic macrophages. These results indicate the existence of two different cellular mechanisms of osteoclast formation associated with aseptic loosening.

P2

COOPERATIVITY BETWEEN C-FOS AND FGF RECEPTOR SIGNALLING IN CHONDROCYTE DIFFERENTIATION AND TRANSFORMATION

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The c-fos proto-oncogene has important roles in both the differentiation and transformation of chondrocytes in vivo in knock-out and ES-cell chimera mouse models. Moreover, using inducible c-Fos expression in clones of the ATDC5 chondroprogenitor cell line, we have shown that exogenous c-Fos inhibits chondrocyte differentiation in vitro. In one such clone (DT12.4) inhibition is most significant when c-Fos is expressed early during differentiation, and this time frame coincides with a c-Fos-dependent morphological change from polygonal to more spindle-shaped cells.

Recently, we have demonstrated that ectopic c-Fos upregulates the expression of the fibroblast-growth factor receptor 1 (FGFR1) at both the mRNA and protein levels from these early stages, thus implicating FGFRs as potential mediators of c-Fos effects. We show here that c-Fos?dependent upregulation of FGFR1 can be seen in additional ATDC5 clones (DT7.1, DT8.6) and in an osteoblastic clone (AT9.2). Moreover, high level expression of FGFR1 is seen in a cell line (wT2) isolated from c-Fos-induced chondrosarcomas, possibly implying a co-operation between c-Fos and FGFR1 in oncogenic transformation. FGFR1 expression can also be stimulated following activation of c-Fos after treatment with FGF-2 or serum, and this expression is enhanced in the presence of exogenous c-Fos. We have further investigated FGF-2-dependent signalling and demonstrated receptor activation in ATDC5 cells and subsequent Erk phosphorylation. Data detailing the activation of FGF signalling pathways +/- c-Fos in response to FGF-1 as well as FGF-2 will be presented.

Finally, we have demonstrated that FGF-2 inhibits chondrocyte differentiation. This inhibition is reduced in the presence of exogenous c-Fos, suggesting that c-Fos may lie downstream of FGF-signalling in the inhibition of differentiation. FGF-2 also inhibits the proliferation of DT12.4 cells, and this appears enhanced by exogenous c-Fos. Additionally, like c-Fos, FGF-2 can induce a morphological transformation of DT12.4 cells and long-term FGF-2 treatment leads to the formation of foci in a c-Fos-dependent manner. Moreover, c-Fos transformed wT2 cells, which express elevated FGFR1 levels, form foci spontaneously without exogenous FGF-2. These data demonstrate that there is cross-talk between c-Fos and FGF signalling pathways in the control of chondrocyte differentiation, proliferation and transformation.

P3

OSTEOBLASTIC 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1: INDUCTION BY GLUCOCORTICOIDS AND METABOLISM OF PREDNISONE/PREDNISOLONE

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Glucocorticoids have profound effects on bone due to direct effects on osteoblasts. In many tissues glucocorticoid action is regulated by 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) which interconverts inactive cortisone with active cortisol. We have previously demonstrated that human osteoblasts express 11beta-HSD1 and that this enzyme regulates intracellular glucocorticoid levels. We have now demonstrated that glucocorticoids induce osteoblastic 11beta-HSD1 activity and that this enzyme metabolises prednisone and prednisolone. Human osteoblast cells were generated from collagenase treated trabecular bone chips obtained from orthopaedic specimens. Confluent cultures were treated with various steroids for 48hrs and assayed for 11beta-HSD1 activity using 250nM cortisone with tritiated tracer. 11beta-HSD1 activity increased significantly with either 1microM dexamethasone (DEX) (165+/-15% control; $p<0.01$, n=9) or 100nM cortisol (163+/-17% control; $p<0.01$). 11beta-HSD1 mRNA levels, quantified by 'real-time' RT-PCR, increased 276+/-62% with DEX ($p<0.05$). This induction of activity increased progressively over 96hrs. In contrast, there was no induction of 11beta-HSD1 activity with 10nM 1,25VitaminD (134+/-17% control, NS). Induction of alkaline phosphatase activity was similar with DEX and 1,25VitaminD (3.1+/-1.4 vs 2.7+/-1.3 fold control, difference NS), suggesting that increased 11beta-HSD1 expression with glucocorticoids is a direct effect rather than via altered differentiation status. The capacity of 11beta-HSD1 to metabolise the therapeutic glucocorticoids prednisolone and prednisone was investigated in 293 cells stably transfected with 11beta-HSD1 cDNA. These cells interconverted prednisolone/prednisone with reaction kinetics indistinguishable from cortisol/cortisone. Human osteoblasts also efficiently converted prednisone to prednisolone but converted prednisolone to prednisone poorly. To assess in vivo availability of substrates for osteoblastic 11beta-HSD1 plasma cortisone and prednisone were measured by HPLC before and after 5mg oral prednisolone. Baseline cortisone levels were 110+/-5nmol/l and prednisone levels peaked at 78+/-23nmol/l 120min after prednisolone.

These data indicate that commonly used therapeutic glucocorticoids are substrates for osteoblastic 11beta-HSD1 and that glucocorticoids themselves increase 11beta-HSD1 expression. Significant amounts of active glucocorticoid can be generated within osteoblasts via 11beta-HSD1 and this effect may be an important determinant of glucocorticoid action on bone.

P4

POLYMORPHISMS IN THE 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 GENE ASSOCIATE WITH LOW BONE MINERAL DENSITY: A ROLE FOR AUTOCRINE GLUCOCORTICOID METABOLISM IN BONE PHYSIOLOGY

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Glucocorticoids are important for osteoblastic differentiation in vitro but in excess in vivo cause osteoporosis, manifested particularly in the spine. We recently described expression and activity of 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) in human adult osteoblasts. 11beta-HSD1 interconverts hormonally active cortisol with inactive cortisone. We have additionally described association of two intronic microsatellite polymorphisms (designated CA15 and CA19) within the HSD11B1 gene with urinary measures of corticosteroid metabolism; long CA15 alleles and short CA19 alleles predicting increased 11beta-HSD1 activity. We have now examined the association of these markers with bone mineral density (BMD) in a cohort of 102 individuals with osteoporosis (OP). Both markers were independently amplified by PCR of genomic DNA and genotyped using an ABI Prism 377. Results were compared to a control group comprising over 400 individuals (CON) with no evidence of bone disease. There were significant differences between allele lengths for both CA15 and CA19 repeats in the OP and CON populations. For CA15 there was overrepresentation of short alleles (68/102 OP vs 222/411 CON with 2 short alleles, $p<0.05$ by Chi squared test) and for CA19 there was a trend towards excess long alleles (74/102 OP compared with 261/411 CON with 2 long, $p<0.09$). This genotypic pattern of markers has been associated with decreased 11b-HSD1 activity. On subanalysis this association was stronger for females than males and significant for females with long CA19 alleles (53/68 OP compared with 122/194 CON with 2 long alleles, $p<0.05$). Within the OP group, individuals with short CA19 alleles had a reduced mean spinal BMD Z-score (for short vs long CA19 -2.58+/-0.13 vs -2.26+/-0.08, $p<0.05$). However there was no difference in femoral neck BMD Z-scores. There was no association of allele length with BMI or age.

These data demonstrate genetic association of markers in the HSD11B1 gene with BMD, and in individuals with low BMD, low spinal BMD Z-scores. These findings suggest that osteoblastic corticosteroid metabolism is important in bone physiology and that local glucocorticoid production is important in determining bone mass.

P5

DIFFERENTIATION-DEPENDENT REGULATION OF 'PRESYNAPTIC' SNARE PROTEINS IMPLICATED IN GLUTAMATE SIGNALLING IN OSTEOBLASTS

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Previous studies have shown that osteoblasts take up glutamate in a regulated transporter-dependent manner and use molecular mechanisms similar to those in the CNS to regulate glutamate release in response to alterations in intracellular calcium. Specifically, we have identified in osteoblasts, the vesicular vSNARE (soluble NSF attachment protein receptor) VAMP and target tSNAREs (syntaxins and SNAP-25) as well as multiple accessory proteins responsible for regulated vesicular glutamate exocyotsis in the CNS. Here we show that the expression of a number of 'presynaptic' SNARE and related accessory proteins is regulated during differentiation of primary osteoblasts in vitro.

Using western blot analysis of whole cell lysates extracted from primary human osteoblasts grown under osteogenic conditions, we identified a differentiation-dependent decrease in expression of the core exocytotic target membrane protein SNAP-25 but not Syntaxin 4 (days 0-30). Syntaxin 1 levels demonstrated a phasic expression profile with expression levels decreasing between days 8 and 12 of culture and increasing thereafter (days 12-20). Levels of Munc-18, a signalling protein that binds syntaxins in steady-state intracellular environments, disabling the formation of the core exocytotic SNARE complex, appeared to remain constant. However using an antibody specific for the Munc18-1 isoform, which is primarily expressed in neurons, we detected a decrease in levels with differentiation, indicating a change in co-expression of Munc-18 isoforms during osteoblast differentiation. Additionally, Rab3a, which is implicated in the regulation of intracellular vesicle traffic was upregulated in rat calvarial osteoblasts undergoing differentiation in osteogenic conditions. These data demonstrate that specific alterations in the expression profiles of fundamental vesicular signalling proteins occur during osteoblast differentiation and suggest that glutamate signalling is important at some but not all stages of osteoblast development.

P6

EFFECTS OF INSULIN LIKE GROWTH FACTORS (IGF-I AND -II) DURING OSTEOINDUCTION

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IGF-I and -II are endogenously present in bone and expressed at sites of new bone formation. Hydroxyapatite has been reported as a biocompatible and osteoinductive biomaterial. It is used clinically as a bone replacement material or for coating of implants.

We hypothesise that the addition of IGFs will enhance osteoinduction, and osseointegration of hydroxyapatite in vivo.

The aim of this study was to investigate the effects of IGF-I and -II, known mitogens of osteoblasts(1), on the serum levels of IGFs and in osteoinduction.

IGF-I/-II (0.5microg/implant) was adsorbed on to the surfaces of open macro (400-600micron) and closed micro (2-15micron) pores of cylindrical porous hydroxyapatite (PHA) (4.5+/-0.4mm x 6.6+/-1.2mm) implants. PHA was implanted bilaterally into the cancellous bone of the femoral intercondylar area of female NZW rabbits (2.75+/-0.25kg) under general anaesthesia. Fluorochrome labels (Tetracycline and Calcein Green) were administered alternatively at 7day intervals. New bone formation and serum levels of IGFs were compared among treatment groups (basal, sham, PHA, PHA+IGF-I, and PHA+IGF-II) at 1week and 3weeks postimplantation.

Stained sections (H&E, toluidine blue, Goldner and von Kossa), fluorochrome labelling, and electron microscopy demonstrated increased new bone formation at the interface, surrounding trabecular bone and within the pores of the implants at 3weeks in PHA, compared to sham. It was further enhanced in IGFs-coated PHA. ELISA for IGF-I immunoreactivity indicated a corresponding increase of IGF-I peptide (2-fold for PHA+IGF-I; 2.5-fold for PHA+IGF-II) in serum at 3weeks. IGF-II was undetectable. These results indicate that IGFs could be paracrine or autocrine factors in the bone-induction process. During this process, IGF-I peptide is increased in serum at an early stage, 1week, in correlation with the recruitment and proliferation of mesenchymal cells seen histologically and correlating to the beginning of the calcifying process, at 3weeks.

Our findings provide a basis for further studies on the future clinical usage of IGFs to enhance bone formation in fracture healing and the response to prosthetic-bone implants. These results suggest that PHA might provide a delivery system for bioactive agents to accelerate bone healing.

(1) Damien et al., 2000 J Bone Miner Res 15; 2169-2177.

P7

IDENTIFICATION OF A BISPHOSPHONATE WHICH INHIBITS FPP SYNTHASE AND IPP ISOMERASE

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We and others have recently demonstrated that nitrogen-containing bisphosphonates (N-BPs) inhibit osteoclastic bone resorption by inhibiting farnesyl diphosphate (FPP) synthase, an enzyme in the mevalonate pathway. Heterocycle-containing N-BPs such as zoledronic acid and risedronate are extremely potent inhibitors of FPP synthase (IC50 3nM and 10nM respectively). Inhibition of FPP synthase in osteoclasts causes loss of FPP and geranylgeranyl diphosphate (GGPP), which are required for the isoprenylation of small GTP-binding proteins. Some N-BPs (ibandronate and incadronate) also inhibit squalene synthase, a distal enzyme in the mevalonate pathway. To date, no other enzymes in the pathway have been shown to be inhibited by N-BPs.

We have screened a large number of N-BPs for the ability to inhibit recombinant human FPP synthase and isopentenyl diphosphate (IPP) isomerase (the proximal enzyme to FPP synthase in the mevalonate pathway). One compound, NE21650 (containing an ortho-amino group attached to an aromatic ring) was found to be a potent inhibitor of FPP synthase (IC50 ~0.03microM) and also a weak inhibitor of IPP isomerase (IC50 ~70microM). By contrast, an isomer of this compound, NE10571 (containing a para-amino group) was a poor inhibitor of FPP synthase (IC50 ~40microM) and, like all other bisphosphonates tested, had no effect on IPP isomerase activity at concentrations up to 300microM. In an MTT cytotoxicity assay with J774 macrophages, NE21650 decreased the number of viable cells (IC50 ~40microM), whilst the isomer NE10571 had no effect at concentrations up to 100microM. NE21650 was also a potent inhibitor of bone resorption by rabbit osteoclasts in vitro, whereas NE10571 had no effect. Furthermore, NE21650 (which inhibits FPP synthase and IPP isomerase) was a more potent inhibitor of bone resorption in vitro that ALN (which only inhibits FPP synthase, with a similar IC50 as NE21650).

These data illustrate that slight modifications to the structure of the nitrogen-containing side-chain of N-BPs determine the potency for inhibition of FPP synthase. Furthermore, certain side-chain structures (as in NE21650) also appear to allow inhibition of other enzymes in the mevalonate pathway, such as IPP isomerase. Inhibition of more than one enzyme may lead to a slight increase in anti-resorptive potency.

P8

HIP AXIS LENGTH, A PREDICTOR OF HIP FRACTURE IN ELDERLY WOMEN IN ENGLAND

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Longer hip axis length (HAL) has been shown to be associated with the risk of hip fracture. We examined whether HAL is associated with hip fracture risk among elderly community dwelling women in England, unselected for osteoporosis.

Method

We undertook a case-controlled study of elderly women aged 75 years and above, enrolled in a large, randomised controlled study looking at the effect of a bisphosphonate, clodronate, in the prevention of hip fractures. Cases comprised those women who sustained a hip fracture during the follow-up period (median of 3.1 years). All hip fractures were radiologically verified from the GP and the hospital records. Two age, height and weight-matched controls were selected for each case. At baseline, hip bone mineral density (BMD) was measured using a Hologic 4500 QDR and the HAL was measured using the automated computer software program in the same machine.

Results

Of the total of 4347 women, 92 (2.1%) sustained a hip fracture, but two women had not received a baseline BMD assessment. Thus, 90 women with hip fracture and 180 matched controls were studied.

The mean age of the cases and controls was 81.9±4.9 years. The mean total hip BMD (gm/cm2) was significantly lower in the hip fracture women than the controls (0.65±0.31 vs 0.72±0.13, p<0.001). The mean HAL (cm) was significantly longer in the hip fracture women than the controls (11.1±0.6 vs 10.9±0.6, p= 0.03). The increase in the HAL was significantly associated with the risk of hip fracture (OR per 1 standard deviation increase was 1.33, 95% CI 1.02-1.72; p=0.03) and it remained significant after the adjustment for hip BMD (OR 1.32, 95% CI 1.01-1.71; p=0.04).

Conclusion

We conclude that an increase in hip axis length is associated with an increased risk of hip fracture in elderly English women independent of their hip BMD. The odds ratio appears somewhat lower than that reported in other studies.

P10

EFFECTS OF RISEDRONATE, ALENDRONATE AND ETIDRONATE ON VIABILITY AND ACTIVITY OF RAT STROMAL CELLS IN VITRO

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Bisphosphonates (BP) suppress bone resorption by directly inhibiting osteoclast (OC) activity. The effects of BPs on osteoclastic cells have been extensively studied in vitro and current evidence suggests that the BP mediated inhibition of OC activity can be achieved by (i) inhibiting OC differentiation, (ii) inhibiting the OC activity, (iii) stimulating the release of an osteoblast-derived OC inhibitory activity or (iv) by inducing OC apoptosis

The effect of BPs on osteoblastic activity has been relatively little studied. Long-term treatment with BPs leads to an increase in wall thickness which indicates a localised increase in osteoblastic activity i.e. an anabolic effect. Consistent with this, it has been found that BPs can prevent the induction of apoptosis in murine osteoblasts and osteocytes and stimulate fibroblastic-colony formation by murine and human bone marrow.

The purpose of this study was to investigate the effects of risedronate, alendronate and etidronate (representing three generations of BP) in two in vitro rat-based models of bone formation; high-density bone marrow cultures and calcifying fibroblastic colony forming unit (CFU-f) cultures. Biphasic effects on bone formation were observed. In CFU-f cultures, high concentrations (10-5-10-4M) of alendronate and risedronate caused total inhibition of colony formation whereas etidronate had relatively little effect. At lower doses (10-9-10-7M) all three drugs increased colony numbers suggesting an anabolic effect. These results were not entirely paralleled in the high-density cultures where all three drugs inhibited colony numbers and cell activity. Etidronate, however, was considerably less potent than the other two drugs and had no anabolic effect at the lower concentrations. The inhibitory effects of alendronate and risedronate could be partially reversed by co-incubation with geranylgeraniolpyrophosphate suggesting a similar mechanism to the induction of apoptosis in OCs by BPs. In contrast, the anabolic effects of alendronate and risedronate were augmented by co-incubation with geranylgeraniolpyrophosphate. Indeed, geranylgeraniolpyrophosphate, geranylgeraniol and farnesol all stimulated colony formation.

These data suggest that high dose BPs can induce cells death in bone marrow derived osteoprogenitor cells by a mechanism similar to that seen in OCs. At lower doses an anabolic activity is present which is not obviously mediated via inhibition of the cholesterol biosynthetic pathway.

P9

AGE-DEPENDENT ANDROGEN RECEPTOR EXPRESSION IN HUMAN FRACTURE CALLUS

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It is now recognised that oestrogen is important in maintaining bone mineral density in men and women, but it is still unclear if testosterone has direct or indirect effects on the skeleton. Previous observations demonstrate that responses of cultured bone cells to testosterone, as denoted by expression of androgen receptors (AR) depend on skeletal site and age of donor. We therefore wished to ascertain if there is a similar control of AR expression in skeletal cells in vivo and present here an investigation of in vivo expression of AR protein in fracture callus, which had both chondrogenesis and osteogenesis, from patients aged 3-86 years (n=34; M=19, F=15) using indirect immunoperoxidase and Western blotting. We used an affinity purified polyclonal antibody (sc-816, Santa Cruz) directed against the N-terminal of human recombinant AR. Fracture callus was demineralised in 20% EDTA until radiologically decalcified. Biopsies were formalin-fixed and wax embedded. Retrieval in glycine/EDTA buffer (pH 3.5) using heat and pressure was essential for receptor detection. Benign prostate hyperplasia was used as positive control throughout. Fracture callus and prostate tissue sections were incubated with antibody overnight at 4oC at dilutions of 1 in 10 and 1 in 500 respectively. AR expression was localised to nuclei of glandular epithelial cells in prostate sections. Pre-incubation of antibody with immunising peptide blocked all immunoreactivity in prostate and fracture callus. There was nuclear AR immunoreactivity in osteoblasts and osteocytes and, occasionally, in osteoclasts, of both males and females, aged 3-30 years. AR was also clearly expressed in mesenchymal cells. In areas of endochondral ossification, nuclear AR expression was intense in small chondrocytes, but not in immediately adjacent hypertrophic chondrocytes. AR was also present in nuclei of vascular endothelial cells and pericytes surrounding larger blood vessels in fracture callus. In the men and women over 30 years of age, AR expression was markedly reduced in all skeletal cells, particularly chondrocytes, osteoblasts and mesenchymal cells. We conclude that testosterone may have a direct effect on cells involved in human skeletal growth, specifically from birth to young adulthood, but its action on bones of older men and women are indirect.

P11

COLLAGEN TYPE I ALPHA 1 GENE POLYMORPHISM AT THE SPI BINDING SITE AND THE RISK OF OSTEOPOROSIS IN MEN

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Recent studies have shown that a polymorphic Sp1 binding site in the collagen type I alpha 1 (COLIA1) gene is associated with bone density and vertebral fracture in postmenopausal women. However few studies have considered the importance of this polymorphism to the risk of osteoporosis in men. In this study we examine the association between the COLIA1 polymorphism and bone density and bone turnover in a population of healthy Caucasian men.

Subjects were 178 men, ages 20 to 79 years (approximately 20 per decade, mean 50 years). Bone mineral density was measured at total body (TBBMD), greater trochanter (TROCHBMD), femoral neck (FNBMD) and lumbar spine (LSBMD) by DXA (Lunar DPX). Procollagen propeptide of type I collagen (PINP) was measured by RIA (Orion Diagnostica) and the cross-linked N-telopeptide of type I collagen (NTX) was measured by ELISA (Osteomark). COLIA1 genotypes were determined using an ABI Prism 7200 Sequence Detection System. BMD and Bone marker measurements were adjusted for age and expressed as z scores. Comparisons between genotypes (SS vs Ss and ss) were made using a 2-sample t test.

The frequency of genotypes was SS 65%, Ss 32%, ss 3%, and was in Hardy-Weinberg equilibrium. FNBMD, TROCHBMD and LSBMD was between 4 and 5% higher in the SS genotype compared to Ss and ss. This reached significance at the femoral neck (p=0.05) and the greater trochanter (p=0.02). TBBMD was 2% higher in the SS genotype compared to Ss and ss (p=NS). There were no differences in PINP and NTX levels between genotypes.

We conclude that there is an association between bone density and COLIA1 genotype in men, and that this is greater in the region of the hip. The underlying molecular and cellular events leading to this association remain unclear since we found no association between type I collagen markers of bone turnover and genotype. We suggest that the relationship between bone density and COLIA1 genotype may arise from differences in peak bone mass.

P12

IS PAGET'S DISEASE SEVERITY DECREASING IN NORTH WEST ENGLAND?

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Previous studies have suggested that the prevalence of Paget's disease of bone is decreasing in the United Kingdom and also that, in New Zealand, patients with Paget's disease are less severely affected at presentation. The aim of this study was to investigate whether there is also a decline in the severity of Paget's disease in Britain, and if so whether this was a true time related effect or due to the birth cohort of the patient.

Retrospective data were collected from 233 patients (117 females and 116 males) attending the nuclear medicine department for scintigraphy to assess Paget's disease before treatment. These were divided into two groups; group I comprised 119 patients attending between 1985 and 1994, and group II 114 presenting between 1995 and 1999. The mean age of group I was 68.7 (SD 10.3), while for group II it was 72.0 (SD 9.4). This increase in age at presentation was significant ($p=0.01$).

Alkaline phosphatase was not normally distributed, and so a logarithmic transformation was used. Mean alkaline phosphatase was significantly higher in group I than group II (705 vs 559 IU/L (normal range 70-330), $p=0.03$). Severity and distribution of the disease were also assessed from the radionuclide bone scan using a semi-quantitative scale. A high summated score indicates both more lesions and higher severity. The median number of lesions per patient and the median summated score were both significantly lower in patients presenting after 1994 (number of lesions, group I = 4, group II = 3, $p=0.04$; summated score, group I = 9, group II = 6, $p<0.001$)

Re-examination of the results using year of birth rather than year of presentation revealed no evidence for a birth cohort effect

These results suggest that patients with Paget's disease of bone have less severe disease, as indicated by scintigraphy and biochemical activity than previously. We therefore conclude that the severity of Paget's disease of bone in this part of the UK is declining in parallel with the previously observed decline in prevalence.

P13

DIAGNOSIS OF OSTEOPOROSIS IN CLINICXAL PRACTICE: A COMPARISON OF SPINE AND PROXIMAL FEMUR T SCORES

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As spine bone mineral density (BMD) may be overestimated because of degenerative changes, it has been suggested that proximal femur BMD should be used for the diagnosis of osteoporosis (OP). In addition, hip BMD is the best indicator of the risk of hip fracture. However, vertebral OP may occur in the absence of OP in the proximal femur, and the diagnosis may therefore be missed if lumbar spine density is not assessed. The OFELY study found that fewer than 10% of postmenopausal women had a diagnosis of OP at all sites (spine, neck of femur, distal radius), while a retrospective database review found 56% concordance between WHO definitions at hip and spine.

We prospectively evaluated a cohort routinely referred to the Metabolic Bone Unit for dual X-ray absorptiometry (DXA). Over 700 consecutive patients had spine, total hip (TH) and neck of femur (NOF) DXA. Scan images were reviewed to exclude those with significant degenerative changes (n=72). Data from 111 men and 525 women were analysed in age decades. Using WHO definitions, patients were classified as having OP in the spine only (SOP), NOF only, TH only, or in a combination of sites.

The proportion diagnosed as osteoporotic at any of the three sites was compared with the proportion diagnosed on hip measurements only (chi-square test). Differences were statistically significant for women aged 40-79 and for men in the 50-59 decade. Sensitivity for OP at any site was calculated for NOF and/or TH. For example, among women aged 60-69 years (37% OP at any site), 52.5% of these had only SOP; sensitivity for NOF BMD was 42.5% whereas TH measurement would have wrongly classified 82.5% of these women as non-osteoporotic. This pattern was similar across all age-groups with the exception of 80-89 year-old men and women.

We conclude that a significant proportion of patients referred for bone densitometry in clinical practice have predominantly vertebral involvement, and that over half of these patients would not be diagnosed if only proximal femur BMD was measured.

P14

FURTHER CHARACTERISATION AND MOLECULAR CLONING OF THE ANTIBODY H8G

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We reported previously on the antibody H8G which was generated against STRO-1+/AP- human bone-derived cells (HBDC). Preliminary in vitro data suggested that the antigen may be developmentally regulated. In this study we have attempted to further characterise H8G and to identify the epitope recognised by this antibody.

Localisation of H8G in cryosections of human neonatal rib and adult osteophyte was performed using an indirect immunoperoxidase method. Expression of STRO-1, H8G and alkaline phosphatase (AP) was studied in HBDC cultures using three-colour analysis by flow cytometry. Intracellular staining with H8G was assessed by flow cytometry following saponin permeabilisation of HBDC or osteosarcoma cell lines. Molecular characterisation of the H8G was carried out using the murine cell line BAF-3 infected with a human bone marrow stromal cell (BMSC) cDNA expression library. This identified H8G as being homologous to CD68. Subsequent studies were performed to compare the expression of H8G to that of CD68 in cultures of HBDC by flow cytometry, and in selected cell lines by immunohistochemistry.

In osteophyte and/or rib sections, H8G showed immunoreactivity in chondrocytes, with a reduction in expression at the mineralising zone. Osteoblasts within the primary spongiosa demonstrated intense staining as did osteoclasts at resorptive sites. A proportion of marrow cells were positive and osteocytes were generally negative. H8G was found to bind to a cell surface antigen present on a minor population (5-20% +ve) of HBDC which were STRO-1+ but AP-. However, in HBDC and cell lines tested there were significantly higher numbers of H8G binding sites intracellularly. In support of the cloning studies, cell surface staining of HBDC cultures with H8G and an antibody to CD68 showed identical patterns of fluorescence in terms of the percentage of positively labelled cells and the number of binding sites. However, immunohistochemical analysis of acetone-fixed cells using a different CD68 antibody revealed that SaOS-2 and MG-63 were strongly positive for H8G expression, as expected, but negative for CD68.

Further studies are required to address the conflicting data obtained for the identity of H8G antigen. However, histological data suggests that H8G expression may have significance in the process of bone development and remodelling.

P15

A COMPARISON OF STRO-1, HOP-26, CD166 AND CD49A AS DEVELOPMENTAL MARKERS FOR HUMAN CELLS OF THE OSTEOGENIC LINEAGE

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Osteogenic precursors and/or colony forming units fibroblastic (CFU-F) can be identified and isolated from human marrow using one of a range of markers. We have compared the ability of antibodies directed against STRO-1, HOP-26, CD166 (ALCAM) and CD49a (alpha 1 integrin subunit) to select for CFU-F in human marrow. In addition, the effect of ex vivo expansion on the expression of these antigens was assessed in cultures of human bone marrow stromal cells (BMSC) or trabecular explant-derived cells (HBDC).

The proportion of cells expressing these antigens, alone or in combination, in suspensions of freshly isolated bone marrow mononuclear cells (BMMNC) and cultures of BMSC or HBDC was determined by flow cytometry. CFU-F were assayed in antigen-positive fractions of BMMNC isolated by magnetic-activated cell sorting (MACS), after 14-17 days in culture, in the absence or presence of 10nM dexamethasone (Dx) where possible. The expression of alkaline phosphatase (AP) was used as an early index of osteogenic differentiation.

In suspensions of BMMNC the proportion of antigen-positive cells was markedly donor-dependent: STRO-1, 15-40%; HOP-26, 25-50%; CD166, 5-25%; CD49a, 2-10%. CFU-F were recovered in the STRO-1+, HOP-26+, CD166+ and CD49a+ fractions, with the CD49a+ fraction demonstrating the greatest enrichment in CFU-F. The corresponding antigen-negative fractions were devoid of colonies. The proportion of AP+ colonies that formed, in the presence or absence of Dx, where tested, was independent of the antigen used for selection. After 5-7 weeks in culture, 85-99% of BMSC and HBDC expressed CD166 and CD49a. The expression of the remaining antigens was variable and donor-dependent: STRO-1, 30-98%; HOP-26, 20-99%. AP was co-expressed by a subset of STRO-1+ cells and of HOP-26+ cells.

The results are consistent with the antigens investigated being expressed by overlapping subsets of BMMNC. When studied in isolation, CD49a appeared to be the most specific marker for CFU-F in human marrow. Ex vivo expansion of bone and marrow cells was associated with an upregulation in the expression of all antigens, although STRO-1 and HOP-26 may continue to be differentially expressed. The significance of the expression of these antigens in vitro and remains unknown.

P16

SYSTEM AND ORGAN CONSERVATION OF BONE MASS

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Aim of the Study: To demonstrate the efficient conservation of bone minerals (calcium, phosphorus) as a biological mechanism for effective conserving of bone mass. The objectives are: a) to quantify and compare the metabolic turnover and mass among 13 different whole bones for Ca and Pi conservation, and b) to measure which bones have the most resorption (loss of 3H-tetracycline) and are the most susceptible to losing bone mass. Two different deficient states (calcium or phosphorus) are compared to the normal dietary state.

Methods: Male SD rat pups (70) were prelabeled with 45Ca and 3H-T on alternate days (9-21 days of age). At 28 days of age rats were placed on control diet (1.0% Ca and 0.03% Pi). Bloods, urines, feces, and 13 different whole bones (7 axial and 6 appendicular) were collected initially at sacrifice on 28 days, and at 35, 42, and 49 days. Whole bones were removed intact for dry weight, chemical, and isotopic analyses. Bone formation was measured by the change in Ca mass, and Ca or Pi conservation by comparing the percent retention of 45Ca, Ca, and Pi, versus percent loss of 3H-T. All bones appeared osteopenic and osteoporotic.

Results: Ca-deficient and Pi-deficient rats showed a large increase in the rate of 3H-T loss in all bones and a large excretion in the urine as compared to small rates of 3H-T loss in normals. In Ca-deficient rats only very small amounts of Ca and 45Ca, and large amount of Pi were lost in the urine. Of the 13 bones, 10 showed 100% efficiency in Ca conservation. Three bones(all axial) showed a lower efficiency(68-78%) for Ca conservation and absolute loss of bone mass (22-32%). In Ca-deficiency, Ca is more highly conserved in more bones than in Pi-deficiency (low conservation of Ca).

Conclusions: Since little excretion of Ca and 45Ca occurred in urine or feces, little loss of total bone mass occurred. In contrast, axial bones lost mass and appendicular bones gained mass, indicating an efficient redistribution of bone mass (Ca and 45Ca) from axial bones to appendicular bones.

P18

USE OF THE OESTROGEN-TREATED MOUSE AS AN ANIMAL MODEL FOR STEROID-INDUCED OSTEOPOROSIS

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Steroid-induced osteoporosis is largely mediated by an inhibitory effect of glucocorticoid (GC) therapy on osteoblast function. However, progress in understanding the mechanisms involved has been hampered since, under in vitro conditions, GC's generally stimulate rather than inhibit osteoblast function. Though previous in vivo studies suggest that GC's reduce osteoblast survival, whether osteoblast supply is also reduced, reflecting inhibition of osteoprogenitor formation, is unclear. Following recent findings that oestrogen-induced osteogenesis in female mice largely reflects increased osteoprogenitor formation, we analysed whether this model can be used to further characterise how GC's influence bone formation in vivo. Ten-week-old intact female CBA mice were administered vehicle, 17beta-oestradiol (E2) and/or Dexamethasone (DEX) 40 and 300 microg/kg/day respectively for 28 days (8-9 animals per group). Femoral bone mineral density (BMD) was measured by Lunar PIXI with dedicated small animal software. The osteogenic response was further quantified by histomophometric analysis performed on longitudinal sections of the proximal tibial metaphysis. Statistical analysis was by one-way analysis of variance. As expected, when given alone, E2 led to a significant osteogenic response, as reflected by an increase in femoral BMD and absolute extent of mineralising cancellous bone surfaces within the proximal tibial metaphysis (dLS/TV) ($p<0.005$ versus vehicle-treated animals). Though DEX had no significant effects on these parameters when given alone, mineral apposition rate, which may be related to cell survival more specifically, was significantly reduced compared to animals receiving vehicle ($p = 0.01$). In animals receiving E2 in combination with DEX, the increase in BMD and dLS/TV induced by oestrogen was almost completely abolished ($p = 0.03$ and $p < 0.005$ respectively, for E2 versus E2 + DEX). Based on our observation that oestrogen-induced cancellous bone formation in long bones of female mice is prevented by administering DEX, we conclude that oestrogen-treated mice represent a useful in vivo model for analysing inhibitory effects of GC's on osteoblast function, particularly those involved in osteoprogenitor formation.

P17

A RAPID METHOD FOR DETECTING THE COL1A1 SP1 POLYMORPHISM

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Examination of transcription control regions in the gene for the alpha 1 chain of type I collagen (COL1A1) revealed a common polymorphism associated with osteoporotic fracture (1). A G to T transition was found at the first base of the Sp1 transcription factor binding site in the first intron in the gene (COL1A1_2046/GenBank X65120). Double replacement techniques used to introduce a mutated COL1A1 allele into mice showed that this intron does play a tissue-specific and developmentally-regulated role in control of gene expression (2). Interest in the polymorphism has increased with several demonstrations of its association with fractures in both men and women, largely independent of bone mineral density. The elevation of urinary pyridinoline in subjects with the T allele suggests that this allele also affects tissues other than bone. (3).

The original PCR-based screening method for the Sp1 polymorphism included a mis-matched primer which introduced a Bal1 restriction site in amplicons from polymorphic alleles with the T substitution. The reaction products were analysed by agarose gel electrophoresis after overnight enzyme digestion (1). We have developed a quicker PCR-based assay that, while it does require two amplification reactions, does not require restriction enzyme digestion and can be analysed either by agarose gel electrophoresis or by examination of dissociation curves of products containing SYBR Green dye. Primers were designed using Primer Express software (Applied Biosystems) with default parameters. Two forward PCR primers with either COL1A1_2046-G or -T at their 3' ends are used in separate reactions. Specificity is attained by the addition of 6% formamide (4) and a hot-start enzyme to the reactions to overcome the effects of the high GC content of the amplicon. Assays run in a GeneAmp SDS 5700 (Applied Biosystems) using SYBR Green I with a dissociation step are completed in less than three hours.

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P19

OPTIMISATION OF SELECTION PROCEDURES FOR THE ISOLATION OF PHAGE ANTIBODIES FROM STRO-1+ CELL POPULATIONS

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The STRO-1+ /Glycophorin A- fraction of human bone marrow mononuclear cells (BMMNC) contains rare clonogenic precursor cells (CFU-F) which form colonies in vitro. CFU-F have a high capacity for self renewal and have the potential to produce cells of different stromal lineages including osteoblasts. We propose to generate monoclonal antibodies against STRO-1+/Glycophorin A- BMMNC using phage antibody display, to identify the osteogenic subset of CFU-F present in human marrow. We will use the Griffin.1 human synthetic single chain phagemid library, which contains a highly diverse repertoire of both heavy and light chain antibody fragments generated from a bank of human V gene segments, and a simultaneous positive/negative selection procedure to reduce selection of irrelevant and non specific antibodies.

We have used the STRO-1 expressing osteosarcoma cell line MG-63 in order to optimise our selection procedures. Antibody phage were isolated by incubation of library phage with MG-63 cells. Selected phage were then amplified by infection in E.coli, and enriched over multiple rounds of selection. By optimising blocking, binding, washing and elution conditions, we have shown that after 5 rounds of panning, we can obtain more than a 10,000 fold enrichment of library phage. STRO-1+ cells were then mixed with a STRO-1- cell line in proportions which are representative of the numbers of STRO-1+ and CFU-F present in the Glycophorin A- fraction of BMMNC (5-0.01% respectively). Following incubation with phage, STRO-1+ cells were separated by magnetic activated cell sorting, and bound phage were eluted and amplified in E.coli. We have shown that when STRO-1+ cells constitute 2% of the starting population, we can achieve more than a 400 fold enrichment of library phage after just 2 rounds of panning. In conclusion, we have optimised selection procedures for the isolation of phage antibodies and show that such antibodies can be isolated from rare target cell populations.

P20

EXPRESSION OF ESTROGEN RECEPTORS IN CULTURED CELLS OF OSTEOBLAST LINEAGE

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Estrogen is important in maintaining bone mineral density; bone loss after menopause is a major cause of osteoporosis. It is possible that estrogen may suppress osteoclast activity and/or recruitment and also enhance osteoblastic activity and differentiation. The hormone interacts with at least two isoforms of the estrogen receptor (ER) in its target cells; ER alpha, which is also expressed in uterus and breast cancer cells, and the more recently described ER beta, the major form in prostate and ovary. Although human osteoblasts express ER alpha and beta, in vivo, it is still unclear when, in osteoblast differentiation, the two isoforms are expressed. We have therefore investigated ER alpha and beta expression, using indirect immunoperoxidase, in cultured cells, which represent stages of osteoblast differentiation. We used a recently developed polyclonal antibody, (ER beta 40), raised to a sequence from the hinge domain of human recombinant ER beta, and an affinity purified monoclonal antibody, (F-10) raised to a peptide mapping to the carboxy terminus of the human ER alpha, (Santa Cruz Biotechnology). All cells were cultured without phenol red. MCF 7 cells were positive controls for ER alpha, and the human prostate line PC-3 for ER beta. With these antibodies, ER alpha was clearly localised to nuclei of MCF7 cells, and ER beta to nuclei of the PC-3 cells. Three cell populations were compared for receptor expression; human mesenchymal stem cell line (HMSC), which are positive for the STRO 1 marker of early osteoblast differentiation, but do not express alkaline phosphatase activity, cells derived from primary cultures of trabecular cells, which contain a variety of phenotypes, including some expressing alkaline phosphatase activity and SaOS2 cells, well-known to exemplify a more differentiated phenotype. There was little expression of ER beta in HMSC and trabecular cell cultures, although, this isoform was expressed in a minority cells, scattered throughout the HMSC cultures. In most SaOS2 cells, however, there was clear nuclear expression of both ER alpha and ER beta. We conclude that, in culture, expression of ER beta increases during osteoblast differentiation and that both isoforms are expressed in more mature osteoblasts.

P21

2-METHOXYESTRADIOL INDUCES APOPTOSIS IN CELLS OF THE OSTEOBLASTIC LINEAGE

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2-Methoxyestradiol (2-ME) is the major endogenous metabolite of 17 beta-estradiol and the contraceptive agent 17-ethylestradiol and is present in human blood and urine. It is not known whether endogenous 2-ME plays any particular physiological role however, it has been shown to inhibit proliferation and induce apoptosis in a number of cell lines including CHO, MCF-7 and HeLa cells and to inhibit endothelial cell proliferation and angiogenesis in vitro. Because of this 2-ME has been suggested as a novel antiangiogenic therapeutic agent. As the formation of new bone is associated with angiogenesis it seems likely that 2-ME may interfere with new bone formation. We have therefore investigated the effects of 2-ME osteoblastic activity.

MG-63 cells and rat bone marrow stromal cells (BMSC) were plated out in DMEM containing 10% FCS, Pen/Strep and glutamax at a density of 10000 cells per square centimetre. They were challenged with 17 beta-estradiol, 17 alpha-estradiol or 2-ME. Cell number was then assessed using Alamar blue after 18 h to determine cell death or 96 h to determine proliferation. Fibroblastic-colony forming unit cultures were performed as previously described and stained for alkaline phosphatase, calcium, collagen and total colonies. Apoptosis was determined by flow cytometry after staining for Annexin V.

Treatment of either MG-63 or BMSC with 10-5 or 10-6 M 2-ME gave rise to more or less total cell killing within 18h. 17 beta-estradiol had no effect over the dose range 10-9 to 10-5 M but somewhat surprisingly high doses of 17 alpha-estradiol induced cell death in MG-63 cells but not BMSC. Exposure to 2-ME for 96 h produced identical results with lower doses of 2-ME having no significant effect on proliferation. A similar pattern was also seen in the CFU-f cultures with 10-5 or 10-6 M 2-ME completely inhibiting colony formation whilst alpha and beta estradiol had no effect. Flow cytometric analysis of BMSC after annexin V staining demonstrated that the majority of the cells had entered apoptosis after treatment with 2-ME.

These data demonstrate that 2-ME can induce cell death in osteoblastic cells in vitro. Whether this activity plays any role in vivo requires further work.

P22

AGONISTS FOR PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR INCREASE OSTEOGENESIS IN VITRO

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A number of substances, in particular prostaglandin E2 (PGE2), are known which stimulate bone formation when administered to adult animals. However, its use in the clinic is prevented by its profound side effects. There are currently no bone anabolic agents on the market except for fluoride, which increases bone mineral density by an unknown mechanism but paradoxically is also associated with an increase in fracture rate. Hence there is an urgent need for new bone anabolic drugs.

The half-life of PGE2 in vivo is known to be less than 2 seconds. It is also known that PGE series prostaglandins undergo a dehydration reaction in the presence of serum albumin to form prostanoids of the A series. Therefore, we considered the possibility that some of the effects of PGE2 may be caused by its degradation product, PGA2. We have subsequently shown that PGA2 shows positive activity in a number of in vitro assays normally indicative of bone anabolic activity, namely the CFU-f assay, non-adherent stromal precursor assay and the calvarial collagen synthesis assay.

PGA1 & A2 are known to bind to the nuclear receptors PPAR alpha, delta and gamma. We investigated the effects of a number of PPAR agonists with known specificity on colony formation using the CFU-f assay. Initial studies show that these drugs are indeed anabolic. In particular, agonists that preferentially activate PPARalpha and PPAR delta cause an anabolic response similar to that of PGE2, suggesting a role for PPARs alpha and delta in bone formation. Agonists that preferentially bind PPARgamma had no effect on bone formation.

P23

THE IMPACT OF ATMOSPHERIC POLLUTION RELATED HAZE ON VITAMIN D STATUS OF TWO-YEAR-OLDS IN DELHI, INDIA

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Atmospheric pollution may reduce the solar ultraviolet B radiation reaching ground level, and this in turn may reduce the cutaneous synthesis of vitamin D.

The aim of this study was to compare the vitamin D status of 9 to 24 month old children from a community in central Delhi, an area with high levels of atmospheric pollution (HP), with a comparable group of children from a relatively less polluted (LP) community on the outskirts of the city. Children in both the areas received community healthcare from St. Stephen's Hospital Community Outreach Department. Haze scores (HS) in the two areas were measured at 9, 12 & 16 hours by the modified Haze Sensor (S Carlson, Scientific America, May 1997).Plasma concentrations of calcium (Ca), 25-hydroxyvitamin D (25(OH)D, a measure of an individual's vitamin D status), 1,25-dihydroxyvitamin D (1,25(OH)2D), parathyroid hormone (PTH) and alkaline phosphatase (ALP) from 26 children from HP and 30 children from LP communities were measured. No subjects received vitamin D supplements. There were no differences in the socio-economic characteristics between the two groups.

The mean 25(OH)D of children in the HP area was 12.6+7 ng/ml, compared with 28.2+7 ng/ml in children living in LP area ($p<0.001$).The mean haze score in HP area (2.1+0.5) was significantly lower ($p<0.05$) than LP area (2.7+0.4). The median ALP ($P< 0.05$) and mean PTH ($p<0.001$) concentrations were significantly higher in children living in HP area than in LP area.

Children living in areas of high atmospheric pollution are at risk of developing vitamin D deficiency rickets and should be offered vitamin D supplements. Atmospheric pollution control measures need to be implemented in order to maintain skeletal and general health of children growing up in cities such as Delhi.

P24

EFFECT OF CHRONIC METHOTREXATE TREATMENT ONBONE METABOLISM IN STEROID-DEPENDENT ASTHMATICS

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The skeletal effects of methotrexate(MTX) as it is used in steroid-dependent asthmatics (SDA) are not known. This study was undertaken to examine the effect of low dose MTX on biochemical markers of bone metabolism in these patients.

Nine SDA patients, mean age 54 y completed 28 weeksof MTX treatment (15 mg IM / week). Mean baseline daily prednisolone was 15.8 mg (12 -20) in addition to inhaled steroids and bronchodilators. During the first 12 weeks of MTX treatment there was no alterations in their asthma therapy. Prednisolone dosage was reduced by 78 (54 -94)% of baseline between 12-28 weeks of MTX treatment but following cessation of MTX prednisolone dose returned close to baseline by 40 weeks. Blood and urine samples were obtained at baseline, 12, 28 and 40 weeks for measurement of bone markers. Serum osteocalcin (OC) and bone specific alkae phosphatase (BSALP) were measured as formation markers while urinary free deoxypiridinoline cros-link (DPD) and cross-linked N-telopeptide of type I collagen (NTX) were measured as bone resorptin markers.

The mean serum OC levels were 4.73, 5.09, 8.12 and 6.63 ng/ml at baseline, 12, 28 and 40 weeks respectively. There was a significant increase in serum OC at 28 weeks ($p<0.008$) and 40 weeks ($p<0.012$) compared to baseline. No significant changes were observed in the other markers between baseline and the other time intervals.

The results suggest that the benificial effect of steroid reduction on bone metabolism does not appear to be impaired by concomitant MTX treatment in these patients.

P26

OESTROGEN INDUCED SUPPRESSION OF MATRIX METALLOPROTEINASE-2 IN MURINE LONG BONES

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It is well recognized that mice administered with supra-physiological levels of oestrogen display marked bone formation. Conversely, loss of oestrogen following ovariectomy (OVX) in rats precipitates an increase in bone resorption that appears to be mediated, in part, by matrix metalloproteinases (MMPs). Indeed, elevated levels of active MMP-2 have been reported in the proximal tibia of OVX rats (Mansell et al. 1997: Bone 20;533-8). However, the influence of oestrogen on MMP-2 expression within the bone compartment of mice has received scant attention. We therefore sought to investigate how exogenous oestrogen administration in mice affected MMP-2 levels within bone.

Male and female twelve-week old C57Bl/6 mice were administered with 17beta-oestrdiol at 0, 4, 40, 400 or 4000 micrograms/kg/day for 28 days. The metaphyses from the proximal tibia and distal femur were removed from each animal, snap frozen in liquid nitrogen and milled prior to enzyme extraction. MMP-2 levels were analyzed using gelatin gel zymography and subsequent scanning densitometry. Our results indicate that estrogen inhibits the expression of both pro- and active-MMP-2 in a dose dependent manner ($p<0.0001$) at the sites examined. Of particular significance was the observation that low doses of oestrogen (40 micrograms/kg/day) suppressed the levels of both pro ansd active MMP-2. This finding is in concurrence with data reporting elevated MMP-2 in bone of OVX rats. Our data support the hypothesis that oestrogen has a modulatory effect on MMP-2 expression by osteoblasts in vivo.

P25

DIFFERENT RESPONSE OF SERUM BETA-CROSSLAPS AND SERUM NTX TO SUBCUTANEOUS OESTRADIOL IMPLANT

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Following oral or transdermal oestradiol (E2) replacement, bone resorption typically decreases to a new steady state within 3 months. The aim of this study was to investigate changes in new serum markers of bone collagen resorption (beta-CTX and serum NTX) in response to low-dose subcutaneous E2 implant. 21 postmenopausal women (hysterectomy 2-38 yrs previously; age range 49-76) were assigned to a treated group (T-group, n=10; 25mg subcutaneous E2 implant at 0,25, 53 and 78 wks) or sham implant followed by E2 implant (S/T-group, n=11; sham implant at 0 and 25 wks; 25mg E2 implant at 53 and 78 wks). Morning fasting blood and second morning void urine samples were collected at 12 time points up to 104 wks.

Serum oestradiol increased progressively with successive implants (mean 64.5, 87.5, 90.4 and 114.6 pg/ml at 25, 53, 78 and 104 weeks). There was a progressive decrease in bCTX level (57.9% 5.3%SEM decrease at 24 wks; 83.7% 6.8%SEM decrease at 104 wks). In contrast serum NTX showed a relatively small response to therapy (25.4% 3.4%SEM decrease at 24 wks; 39.7% 3.7%SEM decrease at 104 wks). The S/T-group showed no response to sham implant, but a similar decline in bCTX following E2 implant.

Within subject variability, CV(a+i), off treatment was 14.9% for bCTX and 15.4% for serum NTX at a repeat-test interval of 1 wk. A signal to noise ratio (SN; % change at 24wks/CVa+i) was calculated for bCTX and compared to SN for other markers measured. bCTX had the highest SN (4.3) compared to serum NTX (1.7), serum markers of bone formation (BAP,1.3; OC 1.4; PINP 3.8) or urinary markers of resorption (ifDpd, 1.9; NTX 2.3). The progressive response to E2 implant may be related to escalating serum E2. Reliable serum markers of bone resorption are likely to provide a better indication of therapeutic response than urinary markers.

P27

THE EFFECTS OF DIET ON HIP BONE LOSS: A PROSPECTIVE STUDY.

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Healthy diet may contribute to bone health. We investigated dietary and nutritional determinants of rates of hip bone loss in an EPOS/EPIC cohort prospectively followed for 2-5 years (mean=2.6). 714 subjects (353 women) aged 67-76yrs were studied. Those treated with bone active medication were excluded. Data on 31 nutrients and 29 food types were derived from 7-day food diaries. Initially, stepwise multiple regression was used for model selection. Since there were some rather high correlations among the nutrients and foods, co-linearity diagnostics were used to identify and remove redundant variables. Regression on principal components as a strategy to reduce dimensionality was unsuccessful. Partial least squares (PLS) regression was used as a complementary approach, since it allows for imprecision in the response. Anthropometry, physical activity, smoking and lung function (FEV1) were considered as possible confounders.

Univariately, weight gain was protective for both men and women ($P<.002$), but none of the nutrients had a significant effect in either sex. In multivariate modelling, nutrients and foods together accounted for 15-17% of the variance in the bone loss data. After removing redundant variables and adjusting for the positive effects of weight gain and measures of physical activity, the stepwise and PLS models agreed on which variables were most significant. In men, magnesium ($P=0.0059$) and nuts ($P=0.0061$) were protective while alcohol ($P=0.0115$), calcium ($P=0.0340$) and one group of cereals ($P=0.0092$) had adverse effects. In women fibre (associated with phytoestrogens,$P=0.0013$) was protective, while niacin ($P=0.0042$) and the same group of cereals ($P=0.0024$) had adverse effects. The PLS model agreed with the effects of niacin and cereals but it alone suggested that carotene and starch are protective.

The results confirm that that weight gain protects against bone loss. Diet composition appears to account statistically for an appreciable proportion of bone loss, but the food groups/nutrients responsible are hard to identify with certainty. Apparently implausible results, e.g. the negative contribution from calcium suggest we may not have removed all statistical redundancy. More subjects or more precise outcome data, such as can be achieved with the longer study we have in progress, are needed to measure small dietary effects.

P28

EVIDENCE THAT P1-PURINOCEPTORS OF THE A1- AND A2-SUBCLASSES MEDIATE OPPOSING EFFECTS ON THE PROLIFERATION OF MG-63 CELLS

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There is increasing evidence for the involvement of receptors for extracellular nucleosides and nucleotides in the modulation of osteoblast proliferation and differentiation. We are studying the effect of purinergic ligands on the proliferation of MG-63 cells, a human osteosarcoma line with the characteristics of pre-osteoblasts.

For experiments MG-63 cells were subcultured into DMEM plus 10% v/v FCS and maintained for 3 days after which they were switched to DMEM plus 1% v/v FCS for 24 hours. Cells were then exposed to test agents for 72 hours, and were subsequently detached using trypsin/EDTA and counted in a haemocytometer.

Initial work showed that exposure of the cells to relatively high concentrations of adenosine (100 - 500 micromolar) for 72 hours decreased cell number dose-dependently, with no apparent loss of cell viability (assessed using trypan blue). Attempts to characterise the subtype of P1-purinoceptor responsible revealed that the response was not reduced by the A1 receptor-specific antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DCPCX). Moreover, in some experiments DPCPX evoked a small decrease in cell numbers in control cultures and slightly enhanced the inhibitory effect of adenosine. This raised the possibility that adenosine might also be capable of enhancing proliferation of MG-63 cells via an action at A1 receptors. Subsequent work has demonstrated that the A1 receptor-specific agonist N6-Cyclopentyladenosine (CPA) enhances proliferation over the range 100 - 200 nM, and this response is selectively inhibited by DPCPX. We have also shown that the inhibitory effect of adenosine is mimicked by 20 micromolar 5'-(N-Ethylcarboxamido)adenosine (NECA), an agonist whose potency exceeds that of adenosine at A2-receptors.

Taken together, the data suggest that MG-63 cells possess P1-purinoceptors of both the A1- and the A2-subclass, and that these receptors mediate stimulatory and inhibitory effects on cell proliferation, respectively. As A1-receptors have higher affinity for adenosine than A2 receptors, the precise effect of adenosine will depend on its concentration. Given that proliferation of osteoblast precursors is an essential element in bone formation, and that purinergic ligands may be released to a different extent under physiological and pathological conditions, this biphasic effect of adenosine could be of significance in both health and disease.

P29

THE APPLICATION OF DIGITAL X-RAY RADIOGRAMMETRY TO THE DIAGNOSIS OF OSTEOPOROSIS

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Radiogrammetry and peripheral cortical assessment of bone mineral density (BMD) have been established techniques for many years. However, until recently, these methods have been limited by poor precision due to technical aspects and operator subjectivity in manual measurements. A newly developed system (Pronosco X-posure (TM) System) overcomes these problems and provides a measure of the average BMD, striation and porosity of the 2nd-4th metacarpals, distal radius and ulna.

215 patients attending for bone densitometry were recruited (48 males (M), 167 females (F)); aged 22-76 years, mean M 52.9+/-13.6 years: F 54.4 +/- 12.1 years. A systematic difference was found between BMD on duplitised and mammographic film, duplitised film giving consistently higher values (mean difference 0.017g/sqcm). Precision was good, CV=0.92% (n=20).

Various correlations were found between DXR-BMD and DXA spine ($r=0.58$, $p<0.01$), femoral neck ($r=0.59$, $p<0.01$) and SXA they were extremely high ($r=0.92$, $p<0.01$).

DXR is quick and simple to use, having potential for application in a variety of settings, as analysis can be made in a central unit, with radiographs being performed in other centres in a wide geographic area.

P30

BONE MINERAL DENSITOMETRY OF THE TIBIA AND LUMBAR SPINE USING THREE-DIMENSIONAL QUANTITATIVE COMPUTED TOMOGRAPHY IN CHILDREN WITH DISABLING CONDITIONS

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QCT is unique in giving separate volumetric density measurements of cortical and trabecular bone. Volumetric density measurements are important in children, as they are not influenced by growth and bone size. The main limitations have been the inability to scan different anatomical sites, patient movement due to long examination times and radiation dose. Spiral CT enables a volume of image data to be acquired in approximately 60 seconds. Recent developments in QCT software permit both conventional 2D and 3D QCT techniques.

3D QCT was applied to children (n=46, aged 5-19 years) with disabling conditions, ranging from hemiplegia, athetosis to severe epilepsy who were part of two studies investigating the effects of weight-bearing exercise on the skeleton. The tibial site was chosen because this is where the effect of the interventions would be presumed to be the greatest. Scanning with conventional DXA and pQCT proved impossible due to long scan times, involuntary movement and limb contractures. Therefore 3D QCT (Philips SR-400) was applied to the lumbar spine (L1-L3) and to the tibia. Scan analysis was performed using QCT-Pro (Mindways Software Inc).

Scan protocol

The tibia were placed over the calibration phantom (Mindways), the limb to be scanned was wrapped in a gel bag. This simulates soft tissue and reduces streak artefacts, which would give false results. A PA scan projection radiograph was taken from the knee joint to the upper third of the tibia and sections prescribed from the tibial plateau and 87mm distally (maximum number of slices = 30). Scans were also performed on 2 or 3 lumbar vertebrae.

CT measurements were obtained at 120kV, 50mA, 2s scan time, 3mm slice width, 3mm table increment, spiral ct mode pitch =1, scan field of view = 420. The radiation dose was 85 microSv for the two sites.

As sedation was not permitted, movements of the lower limb was restricted by holding at the ankle joint. A parent/ researcher stood with the child to allay anxieties.

Standardised precision for repeat analysis of scans was 0.88% tibia; 0.9% spine.

44/46 scans were successful; 2 scans had to be excluded due to excessive movement artefact.

3D-QCT has been applied successfully to a novel site in a difficult group of patients.

P31

EXPRESSION OF MINERALOCORTICOID RECEPTORS BY HUMAN OSTEOBLASTS IN RESPONSE TO STEROID TREATMENT

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Osteoporosis is a common complication of glucocorticoid therapy but understanding of steroid-induced bone loss is incomplete. The actions of glucocorticoids are largely mediated via glucocorticoid receptors (GR), of which there are two isoforms (GR alpha and GR beta). Mineralocorticoid receptors (MR) show considerable homology to GR: MR and GR interact with identical response element sequences and glucocorticoids can also bind to MR to induce gene expression. In addition to immunolocalisation of GR protein isoforms in human bone, we recently demonstrated expression of MR protein by osteoblasts and osteoclasts in vivo. Consequently, the effects of glucocorticoids on bone may be mediated via MR as well as GR.

To investigate the effect of steroid treatment on MR expression by human osteoblasts, a primary cell-line was cultured for 3 and 10 days in the presence of low (approximate physiological concentration) and high doses of hydrocortisone (4x10^-7 M, 6x10^-6 M), and corresponding low and high doses of prednisolone (1.8x10^-7 M, 2.7x10^-6 M) and dexamethasone (5.6x10^-8 M, 8.5x10^-7 M). Immunohistochemistry revealed nuclear and cytosolic staining for MR protein in most osteoblasts during each treatment. Levels of MR mRNA in the cultured cells were examined by fluoresence-based real-time RT-PCR. Five replicate measurements were performed on 2 culture experiments, and values were normalised to levels of 28s ribosomal RNA. MR mRNA was expressed by the cultured osteoblasts during each steroid treatment and both time-points. Steroid treatment did not influence expression of MR mRNA. Compared to low dose treatments, higher doses of hydrocortisone, prednisolone and dexamethasone resulted in similar levels of MR mRNA at 3 and 10 days of culture. In addition, there were no distinct differences in MR mRNA levels in response to any of the three glucocorticoids.

These preliminary data reveal the presence of MR protein in cultured human osteoblasts during low and high dose steroid treatments. Levels of MR mRNA were not altered by steroid treatment. This suggests alternative mechanisms may operate to influence the response of osteoblasts to physiological and high doses of steroids.

P32

DETERMINANTS OF VERTEBRAL FRACTURE IN MEN ATTENDING AN OSTEOPOROSIS CLINIC

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The factors which determine the presence of fractures in men with osteoporosis are not well understood. In order to try and determine risk factors for vertebral fracture in men with osteoporosis we examined the records of 188 men attending clinic with osteoporosis defined as a T score at either spine or proximal femur of less than –2.5.

79 patients had at least one vertebral fracture. Patients with fracture were older than those without (57 plus or minus 13 vs 48 plus or minus 16 y: $p<0.05$). There was no difference in weight or height between the groups. 36 (46%) of the patients with vertebral fracture had an identified secondary cause of osteoporosis compared with 59 (55%) of those without fracture ($p=0.3$).

There was no significant difference in sBMD of the lumbar spine (fracture 831 plus or minus 17 mg/cm superscript 2 , vs no fracture 792 plus or minus 16 mg/cm superscript 2) or femoral neck (704 plus or minus 9 vs 666 plus or minus16 mg/cm superscript 2) between the two groups. In the subset who had had quantitative computed tomography (QCT) of the spine performed there was a significant reduction in those with fractures (56.2 plus or minus 5.0 g/cm superscript 2 vs 93.0 plus or minus8.7 g/ cm superscript 2 ; $p<0.05$) compared to those with no fractures

Histomorphometry of bone biopsy was available in 39 patients; this did not reveal any significant differences between the fracture and non-fracture groups.

ROC analysis was used to compare the ability of the different bone density methods to differentiate between fracture and non-fracture case. QCT was most efficient with an area under the curve of 0.76, sBMD of the spine was comparable (AUC 0.74) whilst femoral neck appeared less efficient (AUC 0.67).

We conclude that osteoporotic men with vertebral fracture are older and have lower spinal bone density than those without such fractures.

P34

STABILITY OF URINARY NTX

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Urinary NTX may be used in clinical practice to monitor response to treatment and to provide additional information for diagnosis. In clinical practice urine samples may not be analysed for several hours or may have to be sent by mail to a central laboratory for analysis. The aim of this study was to investigate the stability of urinary NTX under theses conditions. Spot urines were collected from 22 healthy subjecst (2M,20F) mean age 34 years (range 20 to 65 years). Each sample was divided into five 1ml aliquots and one 5ml aliquot. One 1ml aliquot was stored immediately at -20degC. The other four aliquots were kept at room temperature (20-25degC) for different lengths of time (24,48,72 and 96 hours) and then stored at -20degC until analysis. The 5 ml aliquot was sent to another laboratory and returned to our laboratory by mail (72 hours) and stored at -20degC. Urinary NTX was measured by the automated Vitros ECi assay and creatinine (Cr) by Vitros drya slide chemistry(Ortho-Clinical Diagnostics). NTX, Cr and NTX/Cr for the different conditions, expressed as a percentage of baseline, were analysed by repeated measures ANOVA followed by Dunnett's multiple comparison test.

There was no significant change in NTX, Cr or NTX/Cr at 24 or 48 hours. By 72 hours NTX increased by 8.7% ($p<0.05$), Cr decreased by 1.4% ($p<0.05$) and NTX/Cr increased by 10.3% ($p<0.05$). There was no significant change in NTX, Cr or NTX/Cr in the mailed samples.

We conclude that storage at room temperature for 48 hours or mailing of urine samples does not affect the stability of NTX or creatinine.

P33

WHO SHOULD TREAT PAGET'S DISEASE OF BONE?

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Paget's disease is managed by physicians from a variety of different specialities. It is unknown whether this has any influence on the type of care offered to patients. To investigate this, questionnaires were sent to physicians treating patients with Paget's disease of bone. In addition to speciality, these recorded the number of patients seen, the types of treatment offered, and the perceived indications for treatment.

Of the 247 replies received 215 (87%) said that they treated patients with Paget's disease. These comprised 173 (80%) rheumatologists, 32 (15%) endocrinologists, and 10 (5%) other specialists. The median number of new patients seen annually was 6 and the median number of follow up patients was 12, this did not differ between specialities. Of the various treatments pamidronate was offered by 77% rheumatologists and 75% endocrinologists; tiludronate by 41% rheumatologists and 63% endocrinologists ($p=0.03$); etidronate by 43% endocrinologists and 57% endocrinologists ($p=0.18$); and calcitonin by 23% rheumatologists and 25% endocrinologists ($p=0.82$). No physician used plicamycin. Treatment choice did not differ between those physicians who saw more than 10 new patients per year and those who saw fewer. The main indication for treatment was pain (mean score 4.8 plus or minus 0.6) followed by fracture (3.9 plus or minus 1.6), deafness (3.4 plus or minus 1.8), and heart failure (3.4 plus or minus 1.9). There was no difference in treatment indications between speciality or between those who saw more or less patients.

Endocrinologists may be more prepared to offer oral treatment for Paget's disease. Otherwise, there are no substantial differences between rheumatologists and endocrinologists in their approach to treating the condition. Therefore, the speciality of the physician treating Paget's disease appears to be of little importance.

P35

STRUCTURAL AND CHEMICAL ANALYSIS OF OSTEOPETROTIC (OP/OP) MOUSE BONE

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Osteopetrotic (op/op) mouse comes out by abnormal autosomal condition and shows a small dome skull with non-erupted teeth. Many studies concerning with op/op mouse have been treated the littermate as normal control model. However, our previous histo-pathological observations revealed that the littermate mouse bone structure was not considered as normal (Sato et al., 1995). This study aimed to investigate the structural and compositional differences among op/op, littermate and normal mice bone. Four week-old B6C3-op/op, littermate and normal mice were used in this study. The maxilla and femur were analyzed using backscatter imaging (BSI, Jeol T-200), and micro-Fourier transform infrared spectroscopy (micro FT-IR, Horiba FT-530) using polished sections, and differential thermal analysis (DTA, Rigaku TAS-100 TG-DTA) using powder samples. BSI showed that the molars of op/op were in dental ankylosis with the maxilla bone, and that calcification pattern around bone lacunae of op/op and littermate mice was differed from that of normal. In micro-FT-IR study, crystallinity of apatite in the op/op bone was differed from that in the littermate and normal. DTA showed that the organic combustion pattern of the op/op was differed from the littermate and normal. These results indicated that properties, formation and architecture of apatite crystal and organic matrix vary among op/op, littermate and normal mouse bone. [This study was expensed by the Grant from MECSST, Japan]

P36

THE EMPLOYMENT OF THE COLLAGEN-APATITE MATERIAL "LITAR" IN PERIODINTOLOGY.

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One of the basic problems of modern-day periodintology is restoring and preserving the bone tissue which in the course of the periodontitis development is diminishing. For advantageous solving this problem it is necessary to provide reparative processes in the bone tissue part after diluting the inflammation in periodontitis. For the purpose of restoring the lost bone tissue the defect around the tooth can be filled with the implant material.

The clinical observation in question was conducted for specifying a possibility of employing the collagen-apatite implant(the material "LitAr") for restoring the bone tissue around the tooth. As a result of the surgical intervention the bone resorbtion region was filled with the implant "LitAr". 17 patients have been treated. In respect of patient K. before the surgical intervention the bone tissue in the region of 25th and 35th teeth was resorbted by 1/2 of the root length. The teeth had the 2nd degree of tooth mobility. After performing the delution the implant was inserted into the defect region, then it was fixed by a mucous-periosteum scrap. The regenesation was checked with the help of the physiograph device "CDR". In the visiogram in the operation zone we can clearly see the bone defect. The defect was visible not clearly because of the material interposition. In 20 days after performing the operation on the visiogram the change was shown. It was connected with the tissue density increase in the apex region (the defect region). In 2 months on the material insertion seat we could observe a sharp outline of the bone tissue. The 25th and 35th have lost their mobility, their percussion was painless. Then these teeth were used for supporting the metal construction.Thus, the shown clinical observation make it possible to assume that for providing the reparative processes in the bone tissue it is advantageous to use the collagen-apatite material "LitAr". Its employment for treating periodontitis not only restores the bone tissue, but also it conserves the chewing efficiency as well as prevents the development of anomalies in the tooth and jaw system.

P37

EFFECT OF SEASON, MEASUREMENT INTERVAL AND RANDOM VARIABILITY ON REPRODUCIBILITY OF NEW SERUM MARKERS OF BONE TURNOVER

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Some studies suggest there might be substantial seasonality of bone turnover which could impair the use of biochemical measurements to monitor therapy. However, this evidence is controversial.

The aim of this study was to address the relative importance of season, interval between measurements (serial correlation) and random factors on the repeat-test reproducibility of new serum markers of bone turnover in young adults. Blood samples were collected at a sampling interval of 4 weeks over 56 weeks from 12 healthy men and premenopausal women (6M 6F mean 32.9yrs, range 24 –44). Samples from each subject were assayed in a single analytical batch. We measured serum PINP (Roche Elecsys 2010), PICP (Orion), immunoreactive bone AP and osteocalcin as markers of bone formation. We measured serum bCTX (Roche Elecsys) as a marker of bone resorption. Seasonality was assessed by ANOVA and cosinor analysis with Hotteling's t-test. Components of variability were determined by nested ANOVA (CVa, CVi, CVg). Effect of interval between sampling on CVi+a was determined by semivariogram analysis.

Semivariograms were constructed for each analyte to study the effect of sampling interval on CVi+a. All analytes showed only slight evidence of serial correlation at an interval of 28 days. For example, based on this analysis, estimated CVi+a for bCTX was 18.7% at a sampling interval of 28 days and 23.8% at an interval of 308 days. Serum 25(OH) Vitamin D showed substantial seasonal change (peak to peak amplitude 60.8%, $P < 0.001$, mean 56.85 nmol/L), but there was no significant effect of season on serum PTH. There was no significant effect of time of year for any marker of bone turnover by ANOVA or by cosinor analysis (Hotteling's T2 at $P<0.05$). The upper 95% confidence limit of the amplitude of any possible wintertime increase was < 4% for serum bCTX and <3% for serum PINP.

In conclusion: 1) within-subject variability of markers is consistent with that reported in previous studies, 2) there is no evidence to suggest that season is an important consideration in this population despite the expected seasonality of 25(OH) Vitamin D, 3) at a test interval of 28 days serial correlation between measurements is not substantial.

P38

DISTRIBUTION OF BMD AND BMC AND HANDEDNESS

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Previous studies have shown a small but significant difference of BMD between the two forearms but no such difference has been demonstrated between the two hips. The distribution of BMD and BMC tend to depend on mechanical loading. This study examined differences in BMD and BMC distribution between left and right sides of a group of right handed women from a developing country where women engage in higher physical activities during their daily activities.

A group of 38 healthy female volunteers (age between 30-68ys), who had not suffered a fracture previously were selected for the study. All women came from a village background. Scanning of both hips and forearms were done using the Norland Eclipse XR machine. To minimise variations caused by positioning the hip, the instructions on hip rotation by the manufacturer were strictly adhered. Both BMD and BMC of equivalent sites in right and left sides were compared using the paired t- test.

The right mean BMD and BMC values were higher than those of left side at all measurement sites. Statistically significant differences in BMD were found in the mid radius and ulna (Mean BMD difference = +0.010, 95%CI = +0.018 to +0.001,p =0.018), femoral neck (Mean BMD difference = +0.016, 95%CI = +0.028 to +0.004, p = 0.007) and Ward's area (Mean BMD difference = +0.029, 95%CI = +0.047 to +0.011, p =0.001). Statistically significant differences in BMC were detected at mid radius and ulna (Mean BMD difference = +0.059, 95%CI = +0.088 to +0.029, p =0.0002) and Ward's area (Mean BMD difference = +0.031, 95%CI = +0.05 to +0.012, p =0.001). The difference in BMC at the femoral neck was not statistically significant. Neither the BMD nor BMC differences at the distal forearm site, the mid-radius alone nor femoral trochanter showed statistically significant differences.

Significantly higher BMD and BMC were detected on the dominant side at sites which are rich in cortical bone. Experimental studies have shown that cortical expansion and thickness is increased by mechanical loading, so these results may reflect the physiological changes occurring as a result of greater use of the dominant limbs during normal life.

P39

DO HOLOGIC AND LUNAR HIP STRENGTH ANALYSIS SOFTWARES GENERATE COMPARABLE DATA ON THE FEMORAL NECK?

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Hip geometry is an independent risk factor for hip fracture. Hip geometry softwares extract geometric and BMC data from BMD images and compute strength-related indices based on Euler beam theory. Factors, which are related either to machine, software design or operator can influence the results. This study examines the extent of correlation between comparable indices of hip geometry generated from conventional DXA hip scans by beta versions of Lunar software and Tom Beck's Hologic-based software. Although they use similar principles, there are differences in selection of the ROI in the neck by the two systems since the Hologic-based software selects the narrowest region of the neck and the Lunar-based software selects the cross-section it judges the weakest.

A group of randomly selected 58 postmenopausal women from a community sample had their right hip scanned on Lunar DPX-L and Hologic QDR 4500 machines on the same day. Each BMD image was further analysed by a different author who was blind to the other's results. The relationships between Hologic and Lunar neck length (NL), neck width (NW), cross-sectional area (CSA), Cross-sectional Moment of Inertia (CSMI), section modulus (CSMI/radius=SM), neck-shaft angle (NSA) and femoral neck BMD, were examined by fitting bivariate regressions.

Good correlations were found between the respective BMD, CSA and CSMI measurements of two systems (r2 = 0.89, 0.86 and 0.72 respectively). The correlations between NW and SM measurements were only moderate (r2 = 0.47, and 0.35 respectively). Neck length and neck-shaft angle measurements showed poor correlation, (r2 = 0.24 and 0.15 respectively).

The Hologic software uses the narrowest section of the neck while Lunar uses the weakest section of the neck for their analysis. Accurate identification of anatomical landmarks by the operator is important when using both softwares. Use of a fan beam by the Hologic 4500 leads to intrinsic magnification of the BMD image. These differences would have contributed to the weak correlation seen in certain parameters. We conclude that these softwares cannot be used interchangeably, but that for hip strength parameters, especially CSMI, they make estimates, which are broadly concordant.

P40

BONE MINERAL DENSITY OF THE HEEL IN WOMEN WITH ACUTE AND CHRONIC STROKE

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Falling and low bone density are risk factors for hip fracture. As many as 10.2% of hip fractures occur in patients with stroke, with about 80% of fractures affecting the affected side ('AS'). In a pilot study we have shown that both heels have equally low bone mineral density(BMD) in females, suggesting that women who suffer a stroke may have low BMD beforehand.

The Lunar PIXI was used to measure BMD (expressed as z-score) in both heels of stroke and control groups, in females over 60yr. Subjects with stroke were acute ('AcuS', within two weeks of episode, n=8, mean age 73.4+/-4.6yr) or chronic ('ChrS', greater than 1 year since event, n=15, mean age 80.9+/-7.9yr). The control groups comprised a healthy population('FC', n=23, mean age 69.4+/-9.0yr) and a 'hospitalised' population('HC', n=36, mean age 81.1+/-6.1yr) recruited from day hospitals and nursing homes.

Mean z-score for AcuS patients was 0.11+/-1.24 on the AS compared with 0.03+/-1.50 in the unaffected heel ('US'), p=0.9. In the ChrS group the values were –0.46+/-1.42(AS) and –0.48+/-1.23(US),p=0.97. In the control groups, the mean z-score for FC, 0.33+/-1.53, was significantly greater than in the HC, -0.61+/-1.48, (p=0.02) but not significantly different from zero (p=0.97). Mean z-score in the ChrS patients was not significantly lower than the AcuS patients on either AS (p=0.33) or US (p=0.43).

BMD in AcuS was not significantly different from either FC or HC (although the number of acute female strokes was small). BMD in ChrS was not significantly different from the hospitalized controls, although the difference between them and the healthy controls almost reached significance at the 5% level (AS, p=0.07; US, p=0.05). ChrS, though not AcuS, patients tend to show lower BMD compared with healthy subjects. Hospitalisation is associated with low BMD. The factors leading to low BMD in ChrS and hospitalization await clarification, but both groups may be at increased risk of fracture.

P41

STABLE ISOTOPES FROM BONE AND TOOTH COLLAGEN REVEAL DIETARY PATTERNS AND THE AGE OF WEANING AT THE MEDIEVAL VILLAGE SITE OF WHARRAM PERCY

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The medieval village site of Wharram Percy in North Yorkshire, was occupied from ca. 1250 to ca. 1500 AD and then deserted. It represents one of the best-preserved archaeological sites from this time period in the U.K. Collagen samples from 80 ribs and 42 teeth were analysed to determine the stable isotope ratios of carbon (13C/12C) and nitrogen (15N/14N). These stable isotopes ratio values permit the reconstruction of general dietary and health patterns in ancient and modern populations. The aims of this project were the elucidation of dietary patterns and the determination of the age of weaning in this medieval archaeological population that encompassed a range of ages from neonate to elderly.

There was no observed difference in the average dietary patterns of adult males and females of Wharram Percy: average delta 13C = -19.7 per mil, average delta 15N = +9.0 per mil. These values reflect the consumption of primarily terresterial protein sources with a slight input of marine protein. Four adult males were found to have elevated delta 15N values (+10 to 10.5 per mil) as well as more positive delta 13C values (-18.5 to -19.2 per mil) indicating a more pronounced consumption of marine meat protein resources such as fish and shellfish.

There is a documented increase of +3 per mil in the ratio of nitrogen isotopes as a result of breast-feeding in animal and human populations. This +3 per mil increase in 15N was observed in both the ribs and teeth of neonates and infants sampled. Collagen samples from neonates at birth were found to have delta 15N values of roughly +9.0 per mil that increased to +12.5 per mil by the third month of life. These elevated 15N values persisted until the 6th to 9th month of life and then decreased rapidly to a value of +9.0 per mil by the age of 1.5 years. We conclude that infants at the medieval village site of Wharram Percy were breast-fed until the age of 6 to 9 months and then weaned to the local food resources by the age of 1.5 years.

P42

IMPORTANCE OF THE ALTERED PHENOTYPE OF "OSTEOARTHRITIC" CHONDROCYTES IN THE AETIOLOGY OF OSTEOARTHRITIS

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Osteoarthritis (OA) is a progressive disease whose aetiology is still unknown. Previous studies have suggested that the chondrocytes of OA cartilage may differ from normal articular chondrocytes. The aims of the present study were to compare the phenotype of OA chondrocytes with that of 'normal' articular chondrocytes. Human articular cartilage was taken from femoral heads after total hip arthroplasty due to OA or following fracture of the neck of femur ('controls'), and processed for histology. Overall morphology was assessed after safranin-O staining. The following proteins were immunolocalized: c-Myc, an indicator of cellular activation; proliferating cell nuclear antigen (PCNA), a marker of cell division; the matrix metalloproteinases MMP-3 and MMP-9, which are known to be involved in cartilage degradation; TIMP-1, an inhibitor of MMPs; and the angiogenic molecule VEGF. In addition, cell viability and cell death were detected with fluorescent markers.

'Control' samples had a relatively smooth cartilage surface and little loss of proteoglycans. Chondrocytes were not activated (c-Myc negative) and rarely divided. There was no synthesis of MMP-9 or VEGF. TIMP-1 was present sporadically, but a few chondrocytes of the superficial zone were immunopositive for MMP-3. Samples from severe OA had reduced cartilage thickness (50% less), extensive fibrillations and proteoglycans had been lost throughout the matrix. The vast majority of chondrocytes were present in clones of 4-32 cells. These cells were highly metabolically active and underwent frequent cell divisions. The numbers of dying chondrocytes was low. All clonal chondrocytes synthesized MMP-3 and MMP-9, but no TIMP-1. Most clonal cells synthesized VEGF.

We propose that a permanent change in phenotype has occurred in "osteoarthritic" chondrocytes, so that these cells now express proteinases that are normally suppressed as well as VEGF. Unlike normal articular chondrocytes, these cells remain active and continue to divide, thus exponentially increasing the numbers of altered cells with the potentially destructive set of degradative enzymes. Hence, once started, OA is self-perpetuating, which may explain the progressive nature of the disease. If the formation of the altered phenotype could be prevented or reversed, then new avenues for the prevention or treatment of OA could be developed.

P43

COMPARISON OF PA AND LATERAL LUMBAR SPINE BONE MINERAL DENSITY MEASUREMENTS FOR THE DISCRIMINATION OF WOMEN WITH VERTEBRAL FRACTURE

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The Hologic Acclaim fan-beam densitometer has the advantage that PA and lateral dual-energy x-ray absorptiometry (DXA) scans of the lumbar spine may be acquired without repositioning of the patient. Lateral bone mineral density (BMD) measurements may have better diagnostic sensitivity compared to postero-anterior (PA) measurements, but this approach has not been evaluated using the Hologic Acclaim. Our aims were to evaluate PA, lateral and lateral volumetric bone density at the lumbar spine for discrimination of women with vertebral fractures using 3 approaches. These were comparison of Z scores, comparison of receiver operating characteristic area under the curve (AUC), and logistic regression analysis.

We acquired paired PA and supine lateral lumbar spine DXA scans (Hologic Acclaim) in 188 postmenopausal women ages 50 to 88 (mean 65, ± 8 years). Of these, 59 (mean age 70 ± 8 years) had radiologically-diagnosed vertebral fractures. Normal BMD values for vertebrae L2-3 were calculated for the remaining 129 women with no fractures (mean age 63 ± 8 years), after adjusting for age and weight.

In women with vertebral fractures, there were no significant differences in mean BMD Z scores for PA (Z = -1.20, SE = 0.10, 95% CI = -1.39, -0.98 g/cm2), lateral (Z = -0.98, SE = 0.09, 95% CI = -1.17, -0.79 g/cm2), or lateral volumetric (Z = -0.94, SE = 0.12, 95% CI = -1.17, -0.71 g/cm3). There were also no significant differences in AUC for PA (AUC = 0.83, SE = 0.03, 95% CI = 0.73, 0.85), lateral (AUC = 0.82, SE = 0.03, 95% CI = 0.76, 0.87), or lateral volumetric (AUC = 0.80, SE = 0.04, 95% CI = 0.73, 0.85). From logistic regression analysis, the increased risk of vertebral fracture per 1 SD decrease in BMD was 4.2 (95% CI = 2.6, 6.8) for PA, 4.6 (95% CI = 2.8, 7.6) for lateral and 3.6 (95% CI = 2.3, 5.6) for lateral volumetric.

Based on our results using these 3 statistical approaches, we found no evidence that lateral or lateral volumetric lumbar spine BMD measurements provide improved diagnostic discrimination.

P44

ANKLE FRACTURE IS ASSOCIATED WITH NORMAL BONE MINERAL DENSITY BUT DISSOCIATION OF ULTRASOUND PROPERTIES OF BONE

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Ankle fracture is a common fracture in postmenopausal women. Few studies have examined the relationship between bone mineral density (BMD) and ultrasound properties of bone in ankle fracture. The aims of this study were to determine if 1) ankle fracture patients have osteopenia at the lumbar spine (LS) and distal tibia and fibula (ankle) 2) differences in bone structure of the heel compared to controls.

We studied 31 healthy post-menopausal women ages 50 to 79 (mean age 63.2 ± 3.3) years from a population based group and 32 postmenopausal women ages 52 to 76 (mean age 61.2 ± 2.2) years with an ankle fracture. LS and ankle BMD were measured using the Hologic QDR 1000/W densitometer. In addition to total ankle BMD, three sub-regions were automatically selected, ultra-distal, middle and one-quarter ankle regions. Speed of sound (SOS) and broadband ultrasound attenuation (BUA) of the heel were measured using the Lunar Achilles+ (LA+) and CUBA Clinical (CC). The non-dominant limb was measured in the population group and the contralateral limb in the ankle group. BMD measurements were adjusted for weight. Differences between the groups were determined using t-tests.

The ankle fracture group was 10 kg heavier than the control group. There were no significant differences in LS or ankle BMD or heel BUA between the groups. However in the ankle fracture group, CC VOS and LA+ SOS were decreased by 19 m/s ($P<0.05$) and 50 m/s ($P<0.001$) respectively and SI was decreased by 14 units ($P<0.0001$) (compared to the control group).

In conclusion, ankle fracture is not a typical osteoporotic fracture. However, there may be structural changes in the bone (unrelated to bone density), which result in increased fragility and susceptibility to fracture.

P45

THE PREVALENCE OF OSTEOPOROSIS AMONG PATIENTS WITH GASTROINTESTINAL DISEASES REFERRED FOR BONE DENSITOMETRY: AN AUDIT

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Patients with inflammatory bowel disease (IBD) and coeliac disease (CD) are at increased risk of osteoporosis and many guidelines recommend routine bone density measurements in such patients. However there is some disagreement about whether all such individuals should be referred or whether densitometry should be limited to those with additional risk factors. This audit was done to assess the prevalence of osteoporosis among patients with either CD or IBD who had been routinely referred for bone densitometry during the year 2000. Bone density was measured using dual energy X-ray absorptiometry and osteoporosis was defined as a T score of < -2.5 at the spine and/or proximal femur.

Among patients with CD (n=28, mean age 50.2 yrs), 32% had osteoporosis in the spine and 11% in the total hip. No osteoporosis was found among premenopausal women (n=9) with CD. In patients with IBD (n= 54, mean age 45.8 yrs) 26% and 12% respectively had osteoporosis in the spine and total hip. Of the 15 males with IBD, 33% had osteoporosis in the lumbar spine but none in the total hip. The corresponding figures in postmenopausal women were 44% and 22% and in premenopausal women 5% and 9.5%.

These results demonstrate an increased prevalence of osteoporosis in patients with CD or IBD routinely referred for bone densitometry. This was most evident in postmenopausal women and in men with IBD; further studies are required to establish whether routine measurements are justified in premenopausal women.

P46

CAVEOLIN-1 EXPRESSION IN HUMAN OSTEOBLASTS, BONE MARROW AND PERIPHERAL BLOOD MONONUCLEAR CELLS

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Caveolae are plasma membrane microdomains that have been identified in most mammalian cell types except lymphocytes and neurons. Functions of caveolae include forming the lipid and protein components of cell membrane that function in transmembrane signaling, biosynthetic transport and endocytosis. Caveolin, a 21-24 kDa integral membrane protein, is the principal structural component of caveolae. The aim of this study is to investigate the expression of caveolin-1 in human bone-derived osteoblastic cells, bone marrow and peripheral blood mononuclear cells.

Human bone derived cells and bone marrow cells were cultured following the collection from the patients underwent hip replacement. Human peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers using the Percoll density-gradient-centrifugation procedure. Total RNAs and proteins were extracted from the osteoblasts, bone marrow cells and PBMCs using the RNeasy Midi kit and a protein isolating kit Qiagen, UK). Semi-quantitative RT-PCR for caveolin-1 was performed at 35 cycles, annealing temperature at 55 degree. Western blotting and immunocytochemistry were performed using a specific monoclonal antibody to caveolin-1 using the standard indirect immunocytochemistry procedures.

Caveolin-1 mRNA and protein were expressed in the human bone-derived osteoblastic cells, bone marrow cells and PBMCs. Immunocytochemistry results demonstrated that caveolin-1 was localized at the plasma membrane of human osteoblasts, bone marrow cells and PBMCs. These findings confirm the gene and protein expressions of caveolin-1 in human bone-derived osteoblasts, bone marrow cells and PBMCs.

Abnormalities in caveolae and/or caveolins have been found to associate with diseases such as cancer, atherosclerosis, muscular dystrophy. The identification of caveolin-1 in osteoblasts and bone marrow cells makes this cellular domain a new focus for further investigation of its role in bone development and function. In particular, we speculate that caveolin-1 may play a role in regulating signal transduction in osteoblasts and bone marrow cells during bone growth, repair and disease.

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P47

THE HISTORY AND THE REALITY OF COLLAGEN-APATITE MATERIALS

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Collagen-apatite implant material was synthesized in the early eighties (Gemany, Japan) for replacing the bone tissue defects, but in view of using the aldehydes as a necessary condition for providing the mechanical strength it was not widely used. Late in the 1980s specialists began to use mechanical collagen-apatite mixtures which were more effective, but they could not provide a high rate of the regeneration process.In early 1990s (Russia) the method for making the apatite-collagen implant (after-wards it was named "an artificial bone" or "LitAr") was patented. It had a high degree of the structural integrity of the components. The change of the salt component distribution nature in the material has provided a considerable time reduction of the bone regeneration in the defective part. The investigation of this commpound by the X-ray-phase analysis, infrared spectroscopy has confirmed the awailiability of hydroxoapatite in it. (The salt component content is equal to 60-80%). We conducted the quality and quantity check of the material by means of computed tomography: with a density change interval of 20-80H the value of 20-40H the was equal to 60%.In the course of the biological tests (laboratorial animals were dogs) for the purpose of replacing the defects through an "artificial bone" in the defferent skeleton parts (flat skull bones, jaws,shin bone, rib) as features we could define only the regenerationtime (from a few weeks to a few months). Morphologically the compound could not be detected in the defective part already in 10-12 days after performing the operation. In clinical practice the material was used for filling the tumour defects. Then it was used for cranioplasty of trepanized holes. At the same time it was used in surgical (with cystectomy) and therapeutic stomatology (filling the tooth duct). The regeneration time is equal to 2-4 mounths. All in all the material was used for more than 200 patients. The result was positive completely. The high biodegradationrate of the collagen-apatite material (short time of being in the body), the lack of immunogenicity ,toxicity, a broad range of application (orthopedics, neurosurgery, stomatology) transform this implant to one of the most promising materials which will be able to regenerate the defective part of the bone in the shortest possible time.

P48

EFFECT OF PRESSURE AND PARTICLE ON OSTEOLYSIS

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Fluid pressure and particles are implicated in macrophage activation in aseptic loosening of joint replacements. Our group previously showed that particles and pressure have synergistic effects on release of cytokines (IL-1b, IL-6, TNFalpha) from human monocyte / macrophages.Macrophage colony stimulating factor (M-CSF) is a required differentiation factor for osteoclasts. Chemokines (chemoattractive cytokines) MIP-1alpha and MCP-1 are known to play a role in recruitment of monocytes into inflammatory sites. Prostaglandin E2 is a known bone resorbing agent and several studies has shown that PGE2 is released by monocytes when challenged with particles. We examined the effect of pressure and particles on the release of these signaling molecules from monocyte / macrophages in vitro.

Monocytes were isolated from human buffy coats and seeded on agarose gel in 24 well plates at the concentration of 100000/ml. Ultra high molecular weight polyethylene (UHMWPE) particles were previously suspended in culture media and centrifuged in agarose gel. The cells were cyclically pressurised for 1 hour at 0.034 Mpa (5Psi) and 0.6Hz at temperature of 37C. Cells from each buffy coat were subjected to four regimes: control, pressure, particles, and particles and pressure. The supernatant was collected after 24 hrs and stored at -20C before analysis. Concentrations of cytokine / chemokine were measured using ELISA. Results were analysed using nonparametric 2 way analysis of variance (Friedmann, $p < 0.01$ with Bonferroni correction).

M-CSF and PGE2 concentrations were increased (relative to control) by both pressure and particles individually, and synergistically increased by pressure and particles simultaneously ($p < 0.001$). MCP concentrations showed a significantly greater increase with pressure alone than with particles alone, or with pressure and particles simultaneously. MIP concentrations were significantly decreased by particles (both alone and with pressure), but were significantly increased by pressure alone.

These findings further indicate that particles and pressure play synergistic role in mediating osteolysis through M-CSF and PGE2. However, expression of chemokines by macrophages in response to pressure and to particles differs, and particles appeared to decrease the production of at least one chemokine (MIP). Pressure and particles may play differing roles in recruitment of macrophages in loosening membranes.

P49

CHARACTERISATION OF THE EFFECTS AND MECHANISM OF ACTION OF DEXAMETHASONE ON MOUSE BONE

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Glucocorticoid administration to human patients can induce significant bone volume loss. We employed bone histomorphometry together with simulation of bone remodelling, to elucidate the mode of action of glucocorticoid on mouse bone. Mice in groups of 5-6 were treated for 3 weeks with a high and low dose of dexamethasone, or vehicle, injected daily. At the end of the study we analysed the effects of dexamethasone on trabecular bone by dynamic and static bone histomorphometry and micro-CT. Three different sites were studied by histomorphometry – distal femur, proximal tibia and lumbar vertebra. Dynamic histomorphometry showed a sharp dose-dependent decrease in bone turnover rate in response to dexamethasone, at all sites, by around an order of magnitude in the high dose group. In the lumbar vertebral body only there was a decrease in osteoid surface and a non-significant decrease in trabecular bone volume and trabecular thickness. Micro-CT analysis of the tibia showed decreased cortical volume due to high dose dexamethasone treatment, but no structural effect on trabecular bone. It is of interest that bone volume remained unchanged or fell slightly, in parallel with a sharp fall in turnover – frequently a reduced turnover rate increases bone volume, as in therapies for osteoporosis. In such cases turnover is decreased by lowering activation rate of new remodelling units. Simulations of bone remodelling (assuming coupling of resorption and formation) suggest that reduced turnover together with trabecular bone loss could would only result from a slowing down and elongation of the remodelling cycle. This could be the mechanism of action of dexamethasone on trabecular bone.

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