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THE ROLE OF TRACE MINERALS IN OSTEOPOROSIS

Report of an IAEA Consultants' Meeting

Vienna, Austria

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INTERNATIONAL ATOMIC ENERGY AGENCY

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A report prepared by the

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FOREWORD

A Consultants' Meeting convened by the IAEA in December 1995 made recommendations on trace mineral studies to be conducted within the framework of an on-going Co-ordinated Research Programme (CRP) on Comparative International Studies of Osteoporosis Using Isotope Techniques. Three kinds of studies were recommended: (1) autopsy studies of bone composition (involving the collection of iliac crest samples from healthy accident victims), (2) biopsy studies (involving the collection of iliac crest biopsies from subjects whose bone density has been measured by DEXA in the "core" component of the Agency's CRP), and (3) dietary intervention studies (involving the measurement of bone density in subjects who have received supplemental minerals and trace elements, and in a control group). Bone samples (collected at autopsy or by biopsy) are to be analyzed for several elements, including the major and minor elements: Ca, K, Mg, S, and (2) the trace elements: Cu, F, Mn and Zn. The recommended analytical techniques include neutron activation analysis (NAA) and ICP-MS. In the dietary intervention studies it is proposed that the supplements should be designed individually for each study group to increase the intake of selected minerals, trace elements and vitamins (mainly Ca, K, Mg, P, Cu, F, Mn, and Zn, and vitamins A, C, D, and K) up to the same standard recommended dietary allowances for all subjects. These recommendations are mainly addressed at participants in the Agency's CRP. However, in view of the growing interest in this topic on the part of many other scientists who are concerned with issues relating to bone health, this report has been prepared in order to assist in making these matters better known to a wider audience.

CONTENTS

1 INTRODUCTION

Poor bone health is a major public health problem of worldwide concern. It affects all segments of the population, but is particularly important in older people (and, most of all, in post-menopausal women). In view of the fact that the numbers of older people, both in absolute terms and as a fraction of the total population, are increasing practically everywhere, there is little doubt that poor bone health will become a much more widespread and severe problem in coming decades. Poor skeletal growth in children, which is also a problem of worldwide concern (approximate 40% of the world's populations are stunted in height), may share some of the same aetiological factors.

In 1994 the Agency started a 5-year Co-ordinated Research Programme (CRP) which is addressing one particular measure of bone health - bone mineral density (BMD). One of the concepts underpinning this CRP is the idea that a major determinant of bone health in later years is peak bone mass. The main objective of the Agency's CRP is to determine the age of peak bone mass in various different study groups and countries, and to investigate how BMD varies with the age, sex, ethnicity and geographical origin of the subjects.

The participants in this CRP have also been recommended to do some research on bone composition, particularly with respect to trace elements. However, intensive work on this topic has so far been postponed, namely until such time as appropriate advice could be offered on the purpose, scope and modes of implementation of this work. It was to this end that the Agency convened a consultants' meeting in December 1995, which provided the basis for this report.

The main objectives of the meeting were: (1) to review present knowledge of the role of trace elements in relation to (i) normal bone metabolism, composition and structure, and (ii) bone diseases (particularly osteoporosis); (2) to identify current research priorities in these areas (again with particular reference to osteoporosis) and to suggest specific hypotheses that would be amenable to testing within the framework of the CRP; and (3) to identify suitable techniques for the collection and analysis of bone specimens.

Participants in the meeting are listed in annex 1. The agenda and list of discussion topics are to be found in annex 2.

2 NUTRITION AND BONE HEALTH: A BRIEF OVERVIEW OF SOME CURRENT CONCEPTS WITH PARTICULAR REFERENCE TO TRACE ELEMENT NUTRITION

There is still considerable uncertainty about normal mineral metabolism in bone and, in particular, the genesis of the reported geographic variability in bone health and BMD. On the basis of anecdotal evidence there appear to be major discrepancies between different countries in terms of BMD and habitual dietary intake, particularly of calcium, phosphorus and vitamin D, which are the traditional parameters that have been examined.

The conventional wisdom is that calcium, phosphorus and vitamin D are the major determinants of peak bone mass. However, there is now accumulating evidence that imbalanced diets in general, which may lead to poor nutritional status with respect to several essential nutrients (in particular, Mg, K, Zn, Cu, Mn, F and vitamins C and K) are also crucial determinants of bone health. A further nutrient of great interest is sulphur, the only essential nutrient that is laid down in bulk in cartilage components and other glycosaminoglycans that form the supporting matrix of bone.

Central to the study of trace elements in bone metabolism is the recognition that both the mineral and organic matrices of this tissue are in dynamic equilibrium. The essential role of dietary calcium in bone health is linked to its participation as the major structural cation in hydroxy apatite. The density of the mineral matrix is affected by fluoride. Many metal cations

are adventitiously bound by ion exchange or displacement of calcium. Zinc is a metallo-cofactor for alkaline phosphatase and may influence mineral deposition. Metabolism of the organic matrix, particularly collagen and glycosamine glycans, is dependent upon trace element status. Zinc is required for many of the key enzymes in nucleic acid and protein synthesis. It may also participate by modifying hormones involved in bone metabolism. Copper is a cofactor for the lysine oxidation and pyridinium cross-linking of collagen and elastin. Manganese is required for glycosyltransferases that are essential for the synthesis of glycosaminoglycan chondroitin sulfate, which is required for bone growth and development. Iron, boron and silicon each significantly participate in bone metabolism. The presence of magnesium in bone suggests that this metal may also be essential for skeletal development. Studies in experimental animals demonstrate that deficiencies in Zn, Cu and Mn, lead to teratological bone anomalies and can produce osteoporotic-like bone conditions. Copper and manganese deficient rats have impaired osteoclast and osteoblast activity. Osteoblast activity was more severely inhibited than osteoclast activity. Genetic mutants whose trace element metabolism is disturbed frequently demonstrate bone anomalies.

Prospective nutritional supplementation over a 2-year period in post-menopausal women consisting of calcium (1,000 mg/day) and the trace elements zinc (15 mg/d) copper (2.5 mg/d) and manganese (5.0 mg/d) led to an increase in bone mineral density as measured by DEXA [1]. Trace elements alone had no effect, while calcium alone decreased the rate of bone loss.

Several trace elements are known to be deleterious to bone metabolism. Cadmium affects renal regulation of calcium homeostasis as well as bone synthesis by osteoblast inhibition. Aluminum reduces osteoblast activity. Lead accumulates in bone and has been associated with impaired skeletal development in livestock. Dietary and metabolic interactions among and between essential and toxic trace elements are complex and can be confounding. However, both human and animal experiments clearly demonstrate that both calcium and essential trace elements are required for optimal bone growth and maintenance.

Two recent comprehensive reviews of the subject are available [2,3].

3 ELEMENTAL COMPOSITION OF BONE

Two groups of elements have to be considered:

a) elements which are part of the bone matrix including Ca, F, Sr, Pb, Mn, Ba, Na, Al, P (>95% in the bone matrix and < 5% in the organic matter of the bone);

b) elements such as Co, Fe, Sb and Se which are found to be more evenly distributed (30-70%) between bone matrix and collagen.

Generally, the cortical[1] part of a bone sample contains lower elemental concentrations than the trabecular part. For elements of group (a) the concentrations in trabecular bone are about 2-3 times higher than in the cortical bone.

In support of this consultants' meeting and the related CRP, a literature search has been initiated by one of the contributors (GVI) to collect analytical data on the elemental composition of human bone. Existing sources of compositional data on bone, namely the ICRP-23 publication on Reference Man [4] and the 1978 review by Iyengar et al. [5] have been used to provide data for the period up to 1977. In the updated review that is now being prepared [6], emphasis is being placed on data generated between 1977-1995. Particular attention is being

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[1] The terms ***cortical*** bone (synonyms: compacta, hard tissue, petrous bone) and ***trabecular*** bone (synonyms: spongiosa, cancellous bone, soft bone) are preferred in this report.

paid to analytical quality assurance considerations, including the use of reference materials, in evaluating and selecting the data to be included in this new compilation.

So far, this survey has revealed that the majority of data originate from samples taken at autopsy. Although the range of samples includes temporal, vertebra, humerus, rib, ilium, femur and tibia, the types of samples that appear to have been analyzed more frequently than any others are *rib and iliac crest*. Generally, analyses have been performed on either whole bone or on sub-sections, namely cortical and trabecular segments. The samples cover a wide range of age groups from both sexes.

In view of the wide variability of reported concentrations it is practically impossible to define single *reference values* for most elements and bones. However, sufficient information is already available to suggest *reference ranges* of concentration. Some of these ranges are quoted in table I. More detailed information will be available shortly when the review is completed and published by the Agency [6].

4 TYPES OF STUDY TO BE UNDERTAKEN WITHIN THE AGENCY'S CRP

Three different kinds of study of the role of minerals and trace elements in relation to bone health are recommended to be undertaken within the framework of the Agency's CRP. These are (1) autopsy studies of bone composition, (2) biopsy studies of bone composition, and (3) studies of the effects of dietary supplementation on BMD. It should be noted that the first two of these kinds of study will involve the collection and analysis of bone specimens. The third kind of study could, in principle, be carried out even without any form of chemical analysis. However, ideally, studies 2 and 3 should be combined (i.e. biopsy studies should be conducted on some of the same subjects as are included in the dietary supplementation study).

Two hypotheses underlie all three of these different kinds of study (1) that there are significant differences in mean bone composition between persons living in different countries (probably related to different nutritional habits, and maybe also reflecting different exposure to environmental contaminants), and (2) that some of these differences may be correlated (possibly in a causal manner) with mean BMD (and osteoporosis incidence rates) in the study groups.

As of now, there are no accurate comparative data on the composition of bone across cultures, races and dietary habits. Clearly, there are many confounding variables that could give rise to such differences. In consideration of analytical results from cross cultural studies of bone composition, the important confounding variables will either have to be controlled or measured.

Among the important variables to be considered are:

1. sex
2. age
3. menopausal status and other hormonal factors (i.e. thyroid status in iodine/selenium deficient areas)
4. genetic propensity (COL-1 and IL6 polymorphisms are now known to be a major determinants of bone mass and rate of bone loss)
5. life style factors
   a. anthropometric status; body mass index (BMI)
   b. exercise
   c. education; occupation
   d. fecundity
   e. alcohol and smoking
6. antecedent/habitual diets

4.1 Autopsy studies of bone composition

In order to obtain information on possible cross-national differences in bone composition, all CRP participants are recommended to obtain autopsy samples of bone from healthy accident victims, and to analyse them for the elements of interest (as defined in section 6). However, in contrast to the earlier recommendation made in 1992 [7], it is now proposed that rib bone is not the most suitable sample to use. This is for three main reasons: (1) because rib bone has a highly variable composition, (2) because it is metabolically not very closely related to the parts of the skeleton where measurements of BMD are being made in the Agency's CRP, and (3) because it is not usually available from living subjects with osteoporosis and would not, therefore, provide reference values for biopsy studies.

It is now proposed that the samples of bone to be collected and analyzed should correspond to (a) those areas routinely measured by DEXA (i.e. the vicinity of the upper femur, trochanter/iliac and femoral neck), and (b) those areas which are the focus of the biopsy studies described in section 4.2. In other words, the ***iliac crest*** is the most suitable sample to choose (followed, as second priority, by the femoral neck).

The other main features of the proposed studies are:

- the number of subjects should be chosen so that a difference between geographical sites of ½ SD (standard deviation) of an analyte can be determined with $\beta = 0.9$ and $\alpha = 0.05$; (provisionally - pending more exact calculations - it may be assumed that 35 subjects would be a suitable number in each study group);

- there should be approximately the same number of males and females distributed evenly between the ages of 20 and 50;

- subjects of comparable anthropometric and nutritional status should be selected from each region; this should be done by selecting subjects with BMI in the range 20 to 25 and with height within ± 1 SD of adult NCH[2] standards; (note: some regions may have difficulty measuring weights of corpses – in which case mid-upper-arm-circumference standards can be used instead; height should always be measured);

- as far as possible the accident victims should be drawn from the same population groups, and fairly represent, the groups chosen for the "core component" of the Agency's CRP; they should be apparently healthy (no evidence of chronic diseases);

- other exclusion criteria are chronic infections (e.g. malaria, schistosomiasis) and haemoglobinopathies (e.g. sickle cell anaemia or thalassaemia);

- the time between death and autopsy should be reported (it should be less than 24 hours).

The following measurements need to be made on the bone:

- elemental composition (see sections 5, 7 and 8);

- density of bone (as a sponge) and also density of the material that makes up the bone;

- morphology and immuno-cytochemistry;

- genetic markers (these studies may be done later, for which purpose the samples should be suitably preserved, preferably at $\leq$-70°C if RNA studies are envisaged; otherwise at $\leq$-20°C).

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[2] National Center for Health Statistics (USA)

4.2 Biopsy studies of bone composition

Biopsy studies, although in some ways more difficult than autopsy studies (because of the need to obtain the informed consent of the subjects) are potentially more useful than autopsy studies. If there are constraints on analytical time and resources then the biopsy studies should take absolute precedence. Thereby many problems of *post mortem* migration of elements and sample selection can be avoided, and reliable dietary and other data can be collected simultaneously.

It is recommended to undertake biopsy studies of bone composition of the iliac crest combined with suitable DEXA measures of BMD and the collection of information on lifestyle variables, dietary intakes, cross-link excretion and trace element status.

The main features of the proposed studies are:

- the subjects to be studied will mainly comprise a representative sub-group of the subjects who have already been measured by DEXA in the "core component" of the Agency's CRP; these should be stratified by age, gender and bone density; (in the future it is recommended to include studies on migrants as well as indigenous peoples, taking account of the length of time since migration and changes in dietary habits);

- additional subjects may be included who are undergoing operation (e.g. accident surgery); these subjects should subsequently be measured by DEXA in the same way as the "core component" subjects;

- The same measurements should be made on the biopsy samples of iliac crest as are listed for the autopsy samples;

- dietary intake assessment, including total fat intake, should be made by the 7-day diet diary method (or any other method of comparable reliability), and preferably a subsample of the subjects should also provide duplicate diet samples for analysis;

- lifestyle information should be collected by questionnaire;

- urine samples should be collected for the measurement of cross-link excretion rates (PYD, DPD) and mineral elements (F, Ca, P, K and Mg); ideally one should collect 24 hour urine specimens; a suitable way should be used to store the samples for later analysis [8];

- peripheral blood should be collected for (a) genetic analysis and determination of (b) osteocalcin and (c) bone specific alkaline phosphatase; a suitable way should be used to store the samples for later analysis [8];

- blood plasma or serum specimens should be collected for the determination of trace elements, vitamin C, 25-hydroxy-vitamin-D (and possibly also vitamin K).

4.3 Dietary intervention studies

It is recommended to undertake prospective dietary intervention studies with matched populations to measure sequential changes in BMD in response to dietary supplementation with minerals, trace elements and vitamins. The proposed studies are conceptually similar to those carried out by Strause et al. in the USA, who reported that bone loss in calcium supplemented older postmenopausal women can be arrested by concomitant increases in trace mineral intake [1]. The objective is to reconfirm the findings of Strause et al. in other countries and to extend these studies to other sex and age groups.

The main features of the proposed studies are:

- single placebo, controlled, randomized, double-blind comparisons will be made of the effects on BMD of dietary supplements;

- the supplements should be designed individually for each study group to increase the intake of selected minerals, trace elements and vitamins (mainly Ca, K, Mg, P, Cu, F, Mn, and Zn, and vitamins A, C, D, and K) up to the same standard recommended dietary allowances for all subjects; the philosophy is to correct any imbalances in the habitual diet and thereby to create a balanced and adequate diet that, in principle, is achievable by dietetic means alone in each country;

- the studies should be conducted over a period of a least 2 years with annual (or more frequent) measurements of BMD (by DEXA), as well as collection and analysis of blood and 24 hour urine samples;

- there should be at least 150 subjects per study group including both sexes and covering the whole age range of interest;

- ideally, the same subjects as are included in study 4.2 should be chosen for this study with additional subjects to bring the total number up to at least 150; bone biopsies should be collected from selected subjects both before and after the dietary intervention (preferably annually).

5 ELEMENTS TO BE STUDIED IN BONE SAMPLES

The elements of primary interest in studies 4.1 and 4.2 are: (1) the major and minor elements: Ca, K, Mg, S, and (2) the trace elements: Cu, F, Mn and Zn. Other trace elements of secondary interest are: Sr, Al, Cd, Pb. In addition, CRP participants are encouraged to report results for *any other* trace elements that can be obtained without significant additional effort.

6 SAMPLE COLLECTION OF BONE

The main kind of bone sample to be collected is the iliac crest. Techniques for obtaining these samples are specified below. Three sub-samples of each are required (for elemental analysis, for immediate histological examination, and for possible later genetic analysis). Other sample of possible interest include the upper end of the femur, and the spine.

The iliac crest of the hip bone is recommended as the main sampling site for several reasons:

- local variation in the element concentration along the iliac crest is minimal; 3-4 samples of comparable composition can be taken;

- iliac crest biopsies are commonly taken clinically on patients;

- the cortical part of the sample is small (~ 2 mm) and can easily be separated from the spongy bone;

- the fat- and marrow-free dry spongy part can be clearly defined so that it is possible to compare the results obtained by different CRP participants;

- the use of the spongy part of the iliac crest for trace element analysis has the advantage of reflecting more rapidly changes in the composition of bone due to external parameters, including medication.

Sampling procedures

- in hospitals, bone biopsies from the iliac crest are commonly taken using the instruments developed by Burkhardt (hollow milling cutter, pair of tongs for extraction);

- autopsy specimens can be taken with a hollow milling cutter (e.g. 4 mm internal diameter) and extraction forceps, providing a sample size of 4mm diameter and 20-25 mm length);

- if required, a cylindrical sample can be cut in half down the middle using a saw blade with embedded diamonds.

7 PREPARATION OF BONE SPECIMENS FOR ANALYSIS

Bone biopsy and autopsy samples should be placed immediately in pre-cleaned polyethylene bags. Further handling of the bone samples should be done within 5-6 hours.

7.1 Initial preparation procedures

The following steps are involved:

- the compact cortical and trabecular parts of the bone should be separated with an osteotome[3] or a blade and then divided into smaller parts;

- soft tissues, muscle and fat should be removed mechanically as far as possible; the specimen should then be weighed;

- possible surface contamination should be removed with 5% citric acid (isotonic solution); both cortical and trabecular parts of the same bone should be placed in 50 mL 5% citric acid for about 30 seconds in an ultrasonic shaker;

- blood should be removed by placing the entire biopsy sample or 2-5 g of the autopsied bone in a high quality polyethylene bag containing 50 mL 5% glucose solution (isotonic solution); place in an ultrasonic shaker for about 60 minutes; remove the solution and repeat the procedure six times; (the solution should be clear after the last operation; it is helpful to check the last solution under a microscope to see whether it still contains blood cells);

- fat and muscle should be removed using the same ultrasonic shaker; use 50 mL ether and shake for 15 minutes; remove the ether and repeat the experiment twice; (alternatively, a Soxhlet extraction may be used);

- the samples should be dried at 80°C for three hours; weigh both cancellous and cortical parts; label them and store in polyethylene bags in a refrigerator prior to trace element analysis.

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[3] Preferably an osteotome made of titanium should be used for this purpose (otherwise, stainless steel is probably acceptable, but this needs to be confirmed - see section 8.3)

7.2 Preparation of samples for Instrumental Neutron Activation Analysis (INAA)

After separation of blood, fat and muscle, part of the bone sample should be frozen in a polyethylene bag with liquid nitrogen. Then grind in a Teflon ball mill to produce a homogeneous sample. Alternatively, the sample, after cooling with liquid nitrogen in a polyethylene bag, can be broken into small fragments simply using a hammer. Then dry at 80°C for three hours (or until a reasonable constancy of weight has been achieved). Yet another procedure is to dissolve the whole sample in nitric acid as described in the next section; however in this case the resulting solution should be taken to dryness before irradiation in the reactor.

Bone samples (cortical and trabecular parts separately) weighing approximately 200-300 mg (depending on the power of reactor) will be subjected to both short and long irradiation.

7.3 Preparation of samples for methods requiring sample introduction as a solution

The common approach in the literature for preparation of bone samples for AAS and ICP is either dry ashing or wet digestion with strong acids. Wet digestion with 1:1 HNO3:H2O in a closed vessel (e.g. a Teflon "bomb") gives the best results. If a Teflon bomb is used, the temperature should normally not exceed 240°C and if a quartz vessel is used it should not exceed 320°C. Dry ashing should not be used if any volatile elements (e.g. Cd and Pb) are to be determined.

8 ELEMENTAL ANALYSIS

8.1 Analytical techniques

The main techniques that are available to participants in this CRP for the analysis of bone samples are atomic absorption spectrometry, instrumental neutron activation analysis, inductively coupled plasma optical emission spectrometry, and inductively coupled plasma mass spectrometry. Table I gives information on their potential suitability for determining the elements of interest in the studies described in this report.

All CRP participants are strongly recommended to make local arrangements for the analysis of their samples by whatever techniques they have available to them. It is essential that all elements in groups I and II of table I be determined in every sample. Aliquots of approximately 10% of the samples should also be sent to the Agency for independent cross-checking of the results.

The analysis of "real" samples should not be started until the analytical techniques have been properly validated by the use of a certified reference material (see next section).

In cases where CRP participants are completely unable to make arrangements for the samples to be analyzed locally, the Agency will try to make arrangements for them to be analyzed elsewhere (either in the Agency's own laboratory, or in the laboratory of one of the other CRP participants).

8.2 Quality assurance (QA)

Reference materials for bone are rather scarce. The only two materials currently available are NIST SRM 1486 (bone meal) and SRM 1400 (bone ash). Table II gives information on their composition.

Bone meal is the more appropriate CRM (certified reference material) for the studies proposed in this report, although it lacks certification for three of the elements of primary

interest in these studies (i.e. Cu, F and Mn). Nevertheless, it is recommended that the Agency make efforts to acquire a suitable stock of this material for use as a QA standard in this CRP. This is the primary material that should be used for validation of the analytical methods prior to the analysis of "real" samples.

In the longer term there is also a need for two additional kinds of QA sample representing cortical and trabecular bone respectively. It is recommended that the Agency make efforts to obtain or prepare such samples. This does not need to be done at the level of a *primary* CRM, but they should at least be sufficient to meet the needs of this CRP for proficiency testing and in-house QA .

8.3 Role of the Agency's Laboratory

The main activities foreseen for the Agency's Laboratory are:

- to distribute a suitable certified reference material (CRM) to all participants in the CRP, and assist with the evaluation of the results reported;
- to organize, as appropriate, additional analytical proficiency tests, including the preparation and characterization of new bone reference materials (see section 8.2);
- to analyse a representative fraction (e.g. 10%) of the samples collected by the CRP participants, for cross-checking purposes;
- to test some of the procedures that still need to be properly validated; in the first place, there is a need for two kinds of studies: (1) to check that there is no significant contamination of bone autopsy and biopsy specimens resulting from the use of stainless steel tools, and (2) to develop simpler methods for wet-ashing the bone specimens, e.g. in open digestion vessels;
- on request, to provide advice, training and support on any other aspects relating to the analysis of the bone samples.

9 DATA EVALUATION AND INTERPRETATION

All CRP participants who are undertaking studies of the kinds specified in section 4 are encouraged to evaluate and interpret their own data as far as possible. In addition, however, it is recommended that the same data be reported for "central evaluation" since this is the only way to make meaningful comparisons between different study groups and countries.

To this end, the Agency is recommended to appoint a single "data monitor" responsible for (1) defining the format of the data to be reported centrally, (2) collecting the data from all CRP participants, (3) checking the data for completeness and for conformity with QA specifications, (4) creating a database of a kind that can be made available to all CRP participants, and (5) with the assistance of a sub-committee of advisors, performing statistical evaluation and interpretation of the data.

10 PUBLICATIONS POLICY

The Agency strongly encourages and supports the publication of scientific data arising out of these studies. All CRP participants are therefore strongly recommended to use whatever means may be available to them to publish their data as soon as possible, preferably in a reputable peer-reviewed international scientific journal.

This recommendation refers specifically to publications describing the work carried out by *individual* CRP participants. The only constraints are that the Agency's support of this work should be acknowledged, and that a copy of the submitted manuscript should be sent to the Agency's technical officer for information and comments.

A different policy applies to publications describing the results of projects that have been evaluated centrally. The preparation of such publications will be the responsibility of the "data monitor" described in section 9, and of his/her subcommittee of advisors, including the Agency's technical officer. Such publications will be co-authored by *all* CRP participants who have contributed in any meaningful way to the outcome of the study.

11 SUMMARY OF MAIN RECOMMENDATIONS

All participants in the Agency's Co-ordinated Research Programme (CRP) on "Comparative International Studies of Osteoporosis Using Isotope Techniques" are recommended to ***extend*** the scope of their research by the inclusion of studies of the role of minerals and trace elements in relation to bone health. Specifically, three types of study are described in section 4. All CRP participants are recommended to undertake one or more of these projects according to their individual interests and possibilities.

The Agency is recommended to support this research in various ways including the provision of quality control services and backup analyses, and by appointing a data monitor.

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TABLES

Table I Suitability of various analytical techniques for the elemental analysis of bone (x = suitable, but with constraints; xx = suitable; xxx = highly suitable). The elements are listed in 3 groups (I = high priority major and minor elements; II = high priority trace elements; III = other trace elements of secondary interest in this CRP). The techniques listed are as follows: AAS = atomic absorption spectrometry; ICP-OES = inductively coupled plasma optical emission spectrometry; ICP-MS = inductively coupled plasma mass spectrometry; INAA = instrumental neutron activation analysis; RNAA = radiochemical neutron activation analysis; EC = electrochemical techniques (e.g. voltammetry or ion-specific electrode); col = colorimetry. The typical concentrations reported in this table have the units mg/kg of fresh bone unless otherwise noted; the values are for bone in general (i.e. all types) unless otherwise noted (when mentioned, c and t refer to cortical and trabecular bone respectively and ? refers to paucity of data).

Group	Element	Typical concentration in bone	AAS	ICP-OES	ICP-MS	INAA	Other
I	Ca	13-27 % (c,t)	xx	xxx	x	x	
	K	1300 - 1500 ?	xxx		xxx	x	
	Mg	1700 - 3400 (c,t)	xxx	xxx	xxx	x	
	S	500 ?			x		EC col
II	Cu	0.2 - 4.0 (c,t)	xxx	xx	xx		RNAA
	F	500 - 3000 (c,t)				xx	EC
	Mn	0.3 - 3.0 (c,t)	xx	xx	xx	xxx	
	Zn	50 - 200 (c,t)	xx	xx	xx	xxx	
III	Al	1 - 20	x	x	x	xx	
	Cd	0.5 - 2.0	xx		xx	xx	EC
	Pb	2 - 50	xxx		xxx		
	Sr	25 - 100	xx	xx	xx	xx	

Table II Elemental composition (mg/kg) of two certified reference materials with a bony matrix, NIST SRM 1486 (bone meal) and NIST SRM 1400 (bone ash).

Element	Bone Ash		Bone Meal	
	Conc.	T*	Conc.	T*
Al	530	N	1	N
As	0.4	N	0.006	N
Ca	381 800	C	265 800	C
Cd	0.03	N	0.003	N
Cu	2.3	N	0.8	N
F	1 250	N	800	N
Fe	660	C	99	C
K	186	C	412	C
Mg	6 840	C	4 660	C
Mn	17	N	1	N
Na	6 000	N	5 000	N
P	179 100	C	123 000	C
Pb	9.07	C	1.335	C
Se	0.08	N	0.13	N
Si	1 300	N	200	N
Sr	249	C	264	C
Zn	181	C	147	C

* Type of value (C = certifed; N = not certified)

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AGENDA

SESSION 1: MONDAY, 11 DECEMBER 1995, 13:30 - 17:00

Opening and introductions

Background and purpose of the meeting (R.M. Parr)

Overview of research conducted in the participants' own institutes.

Each participant is kindly request to make a short (20-30 minute) presentation describing recent and current research activities conducted in his institute on any topics that are relevant to the subject of the meeting

N.K. Aras
P. Braetter
M.H.N. Golden
G.V. Iyengar
P.D. Saltman

General discussion (see list of discussion topics)

SESSION 2: TUESDAY, 12 DECEMBER 1995, 09:00 - 12:30

General discussion (continuation)

SESSION 3: TUESDAY, 12 DECEMBER 1995, 14:00 - 17: 30

Preparation of recommendations to the Agency

SESSION 4: WEDNESDAY, 13 DECEMBER 1995, 09:00 - 12:30

Preparation of the report of the meeting

SESSION 5: WEDNESDAY, 13 DECEMBER 1995, 14:00 - open end

Final discussions

Approval of the report of the meeting

CLOSING

DISCUSSION TOPICS

Persons named within brackets are kindly requested to be prepared to OPEN the discussion on the named topic

GENERAL DISCUSSION ON TRACE ELEMENTS IN RELATION TO BONE METABOLISM AND COMPOSITION

Overview of present knowledge of the ***role of trace elements*** in relation to:
(i) normal bone metabolism, composition and structure (Saltman), and
(ii) bone diseases (particularly osteoporosis) (Golden)

Overview of present knowledge of the ***elemental composition*** of:
(i) normal and diseased bone (Iyengar), and
(ii) normal variations within the skeleton and within a bone sample (Braetter)

Overview of current research priorities in these areas (with particular reference to osteoporosis)

DISCUSSION ON MATTERS RELATING TO THE AGENCY'S CO-ORDINATED RESEARCH PROGRAMME (CRP) ON COMPARATIVE INTERNATIONAL STUDIES OF OSTEOPOROSIS USING ISOTOPE TECHNIQUES

Objective and Scope

What should be the objective and scope of trace element studies within the Agency's CRP? What elements are of priority interest?

Are there any specific hypotheses that are amenable to testing within the framework of the CRP?

Sample Collection

What kinds of samples should be collected? Note: the following kinds of samples have already been identified as being of interest in the CRP-- autopsy specimens of rib and biopsy specimens of ileac crest (together with some samples of tooth). However, this decision still needs to be confirmed or modified.

What should be the sampling design (how many samples, which subjects, and what information should be collected on the subjects)? Is there a need for a questionnaire?

What techniques and tools should be used for sample collection?

How should the samples be stored before analysis?

Sample Analysis

How should the samples be prepared for analysis (e.g. by NAA and AAS)? How should fat and blood be removed? (Aras)

What are the elements of primary and secondary interest to be determined in these samples?

How should be samples be analyzed (by which techniques, and by which analysts)?

What techniques and reference materials are needed to ensure adequate analytical quality assurance? Is there a need for new certified reference materials? (Iyengar)

What should be the role of the Agency's Laboratory, Seibersdorf, in this CRP?

Data Evaluation and Interpretation

How should the data be collected, collated and evaluated, and by whom?

REPORT OF THE MEETING

Scope and structure

Drafting assignments

OTHER MATTERS