

Studies of drugs and other measures to prevent and treat osteoporosis; a guide for non-experts

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About this guide

**Why is this guide necessary?
Why now?**

Bone

Background information

Osteoporosis

Who is affected?

What other factors affect fracture risk?

Research on osteoporosis

What can it offer?

Difficulties of studying bone

Why studies other than human (clinical) trials are needed

Helpful laboratory studies

i) studies of bone breakdown

ii) studies of bone formation

What are preclinical studies?

Animal studies

i) which animals

ii) what type of study?

iii) how many animals?

iv) how is the bone mineral content of animals assessed?

v) how is the microscopic structure of animal bone determined?

vi) how is bone strength tested?

vii) biochemical markers of bone metabolism

viii) fracture healing

ix) what happens to the drug?

x) long-term bone safety issues

Other issues

i) prevention or treatment?

ii) control groups

iii) randomisation

iv) duration of studies

Clinical trials

Trial methods; principles of osteoporosis studies

Assessing non-drug measures

Bone density measurement techniques

Fracture assessment and height

Quality of life and other measurements

Trial methods; general principles

The protocol

The population studied

The treatment approach

Study design

Duration of studies

Numbers of people studied

Frequency of measurement

Safety issues

Good clinical practice

Phases of clinical drug development

i) Phase 1 study

ii) Phase II study

iii) Phase III study

- established osteoporosis

- osteoporosis

- osteopenia

- normal bone mass

iv) Phase IV study

Further information about osteoporosis and clinical trials

Further reading

- on osteoporosis

- on clinical trials

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Prepared by John D Wark and Ann Westmore

About this guide

Why is this guide necessary?

A good deal of information on osteoporosis for non-experts is available currently or is being developed. However in one respect there seems to be a notable absence of information - namely the rationale, performance and interpretation of studies carried out to assess preventive or treatment strategies targeted at osteoporosis.

An excellent opportunity to redress this information gap has arisen from a World Health Organisation project to develop Guidelines for Preclinical Evaluation and Clinical Trials in Osteoporosis.

Using these guidelines as a springboard, we have prepared the following information for non-scientific members of institutional ethics committees, special interest groups, the media, health professionals having little previous contact with osteoporosis, people considering taking part in clinical trials, trial co-ordinators, research nurses, non-experts in the pharmaceutical industry and the broader community.

This guide aims to provide:

- **An improved understanding of important features of osteoporosis and of well-conducted research on it**
- **Relevant information about the laboratory and animal research that ideally underpins human (clinical) trials in osteoporosis**
- **An awareness of the sequence of laboratory, animal and human studies used to provide valid answers to research questions**
- **Background information for considering participation in a clinical trial of a drug or other measure targeted at preventing or treating osteoporosis**

How to use this guide

As you can see, this guide is intended for people with a wide range of interests and backgrounds. Many people will gain all the information they need by reading the main text. If you want to know more, read the fine print sections as well. If you are seeking to learn just some key points, the bold print sections may contain enough information for you.

Why now?

Trends in population aging suggest that these guidelines are timely.

In most countries people are living longer and there are correspondingly more cases of osteoporosis. Even after taking account of this trend in population aging, there is evidence of increasing fracture rates in some parts of the world. This translates into more osteoporosis-related disability, pain, deformity, dependence and even death. Reassuringly it is becoming clear that osteoporosis prevention, early detection and treatment are capable of reducing these negatives.

Bone

Background information

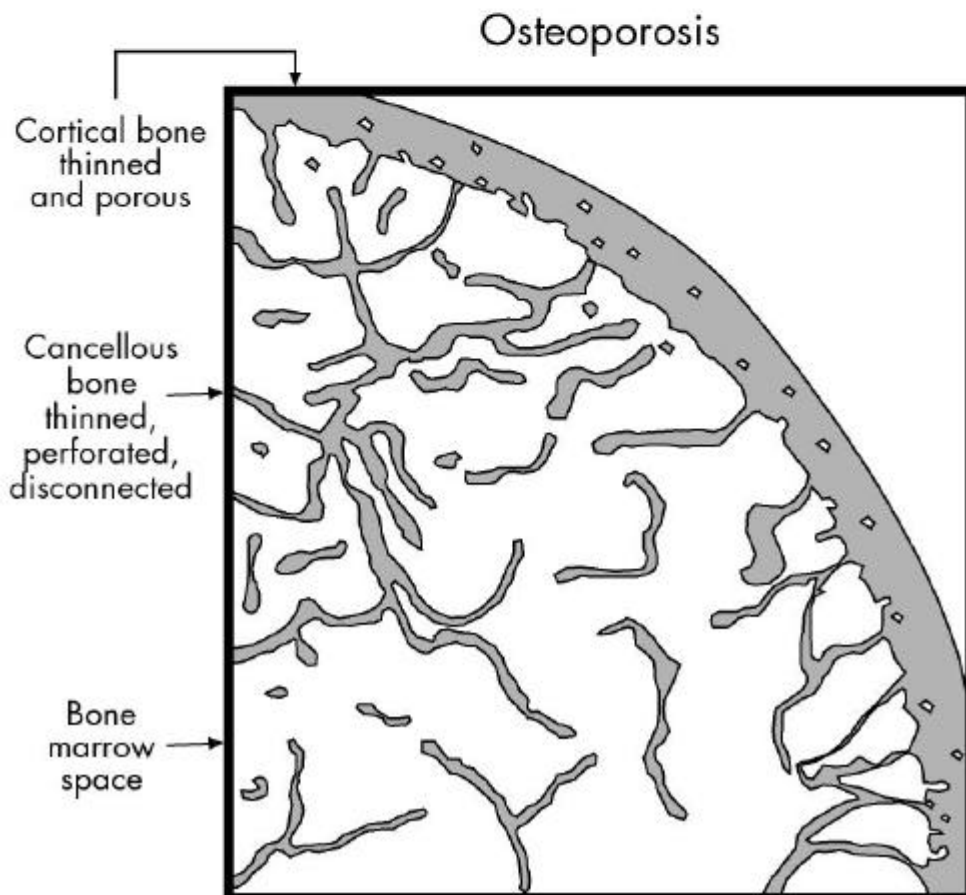
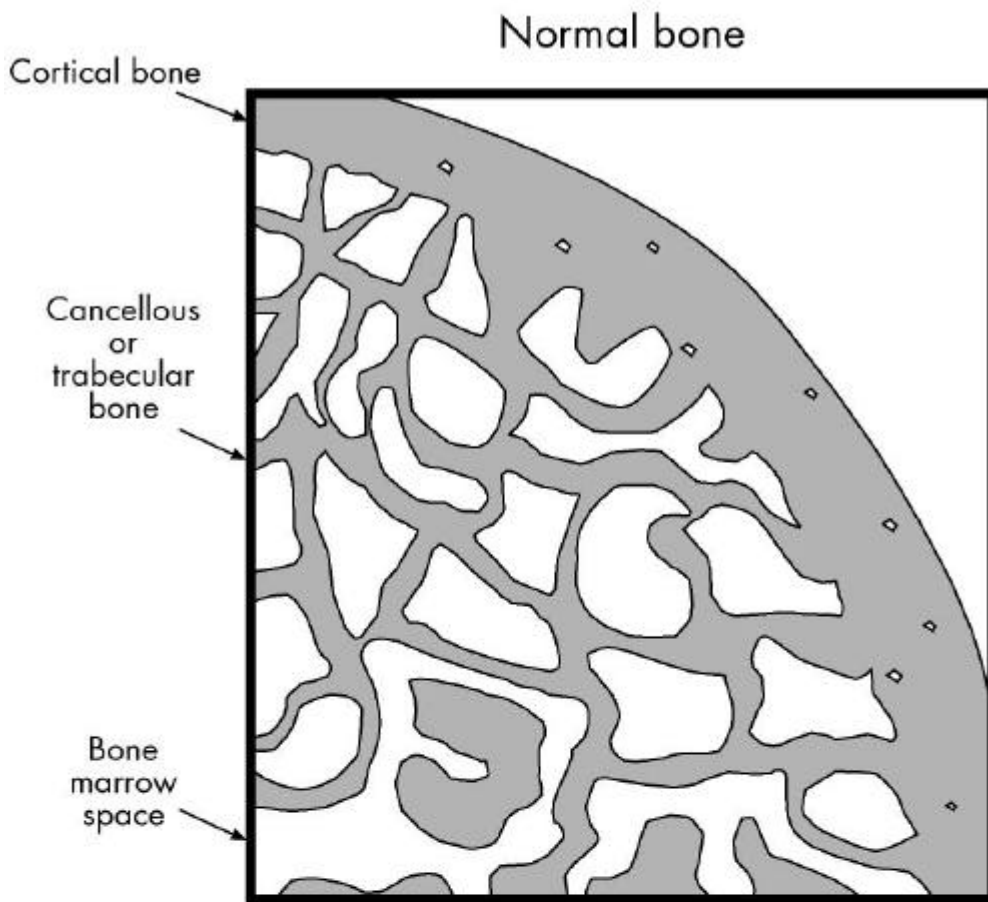
Bone is a hardened mass of living tissue that supports the body and protects internal organs from injury. In adults, there is a continuing process of bone renewal in which small amounts of bone are broken down (bone resorption) and replaced by new bone (bone formation). Within bone, the marrow produces large numbers of blood cells.

Importantly bone is also an essential reservoir of minerals for the body. Minerals like calcium phosphate and calcium carbonate, fluorides and chlorides are constantly shifted from other parts of the body to the bone and back again under the influence of hormones, specialised bone cells and the stresses and strains of weight-bearing activities.

The amount of mineral in bone is largely responsible for its hardness, while substances like the structural protein collagen also contribute to bone's mechanical strength.

The dense outermost bone is known as **cortical** bone while the more spongy internal form is known as **cancellous** or **trabecular** bone. Figure 1 shows the structure of these types of bone.

Figure 1



Osteoporosis

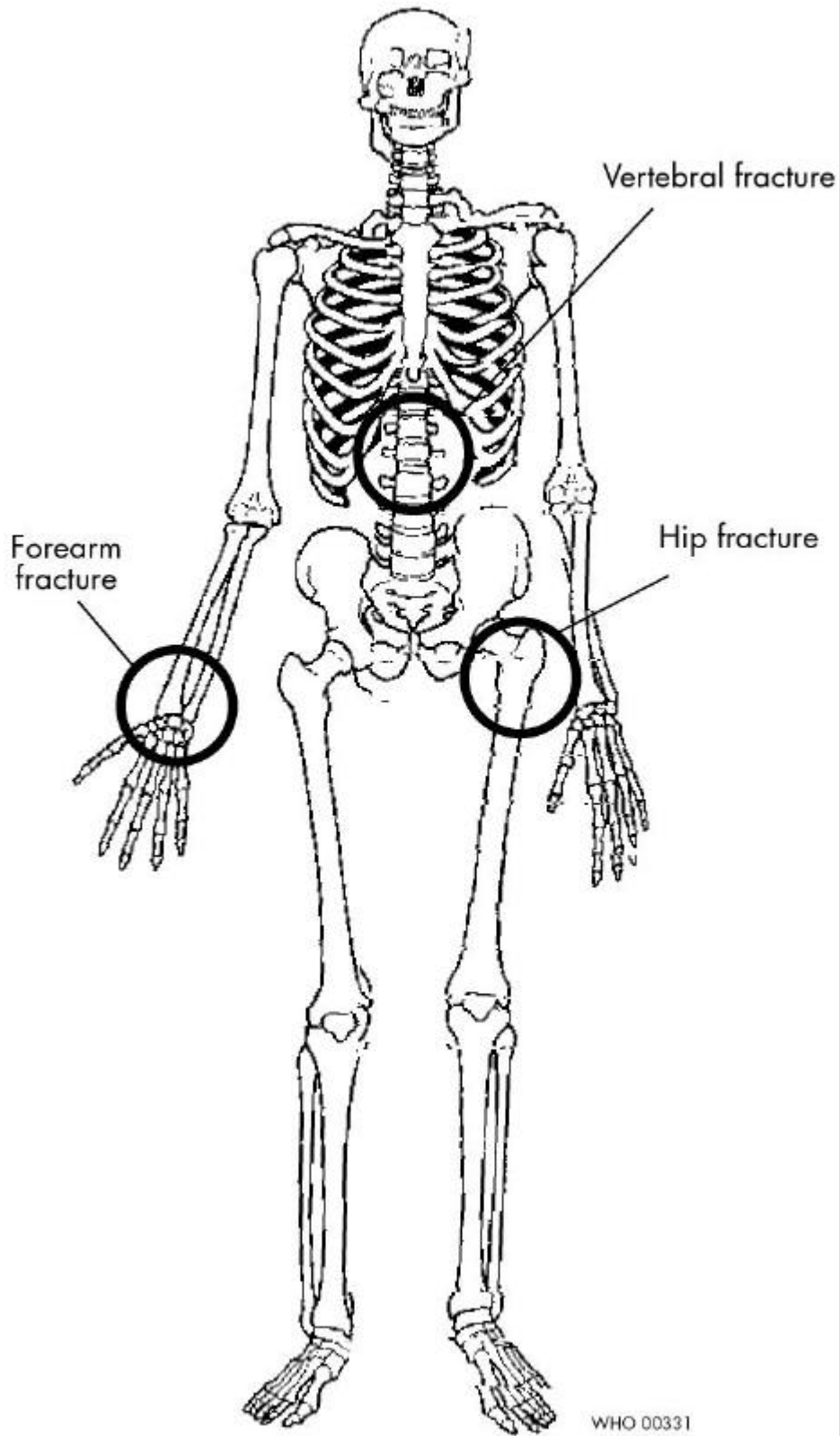
The genetic makeup of individuals, aging, the menopause in women, and other factors affecting both men and women are associated with changes to the structure and function of the body, one of which is a reduction in the strength of bone. These processes are reflected in a decreased bone density or mass and a deterioration in its **architecture**¹ (microscopic structure) and strength. **Osteoporosis is diagnosed when the loss of bone density and strength leads to an unacceptably high tendency for bones to fracture (that is, to break).**

Bone density has been divided by the World Health Organization into four levels:

- **Normal**
- **Low bone mass (osteopenia)**
- **Osteoporosis in which fractures have not yet occurred**
- **Severe or established osteoporosis (when fractures have occurred due to bone fragility)**

¹ Skeletal or bone architecture refers to the microscopic structure of bone, particularly as it relates to the strength of bone.

Figure 2



The bones of the forearm, spine and hip are most prone to osteoporotic fracture (figure 2). Of these, the most serious problems arise following hip fracture due to the high level of disability and loss of independence associated with it as well as complications that increase the risk of death (for example, pneumonia, urinary infection, pressure sores). For individuals who survive a hip fracture, follow-up care tends to be prolonged and costly.

Who is affected?

Although the problem of osteoporosis is shared by aging men, in most western countries the risk of the condition is about twice as high in women, with approximately 40% of women over 60 years of age being affected. The higher incidence of osteoporosis in women reflects, in part, their tendency to live longer than men as well as the occurrence of a period of accelerated bone loss around the time of, and for some years following, the menopause. Indeed, because of women's higher risk and longer lifespan, there are 3-4 times more hip fracture cases in women than in men.

This said, osteoporosis occurs much more often in some parts of the world than in others, particularly affecting white-skinned people from North America, Northern Europe and elsewhere. The risks for osteoporotic fractures are substantially lower in many parts of Asia, Africa and South America, but the gap is closing with improvements in life expectancy and increasing rates of fracture. Between 1990 and 2025, the estimated size of the population aged over 50 years will increase 130-150% in Europe and about 200% or more in all other regions, with the most marked increase in Asia. These sorts of changes suggest that the number of hip fractures annually will rise from around 1.5 million worldwide in 1990 to between 4 and 6 million in the year 2025.

What other factors affect fracture risk?

While sex, age, race and menopausal status can help predict the likelihood of developing osteoporosis, a number of other risk factors have also been identified. These may include a family history of osteoporosis, thin body build, prolonged amenorrhoea (absence of periods in pre-menopausal women who are not pregnant or breast-feeding), smoking, excessive alcohol intake, a lifestyle with little physical activity and inadequate calcium intake or poor vitamin D nutrition. Poor vitamin D nutrition is usually due to a low level of sunlight exposure secondary to factors such as lifestyle (especially indoor living) and climate. Menopause occurring at an unusually early age (whether naturally or induced by medical treatment) carries a well-recognised risk of osteoporosis. The long-term use of cortisone-like medications such as prednisolone to treat, for example, asthma or rheumatic conditions, carries a very significant risk for osteoporosis. Oral contraceptive use appears not to increase osteoporosis risk and may be protective.

There are also a number of factors which influence fracture risk in other ways. These include factors which increase the likelihood of falling, for example, poor vision, and the use of sedative medications.

Research on osteoporosis

What can it offer?

Researchers studying osteoporosis have concentrated their efforts in several main areas:

- **Understanding the basic biology of bone**
- **Studying the relationship of bone quantity and quality to fracture risk**
- **Examining other factors that predict a high risk of developing osteoporosis.**
- **Studying measures that decrease the risk of fractures and associated ill-health and death**

Difficulties in studying bone

Bone does not lend itself easily to direct study because it is less accessible than blood, skin and some other body tissues. However, numerous useful non-invasive techniques have been developed to assess bone characteristics in humans without removing a sample of bone (i.e. many tests can be performed non-invasively). Unfortunately, bone strength, the property of most interest, cannot be measured non-invasively and is estimated instead by “surrogate” (substitute) measurements such as bone density.

Another difficulty in studying factors that affect bone is its slow rate of turnover. That is, most body tissues are built up and broken down continually throughout life and some undergo the process at a much faster rate than others. The rate at which this process occurs is referred to as turnover. Bone turnover is very slow; only about half the skeleton is broken down and rebuilt over 10 to 12 years. In comparison, the lining of the intestine is replaced every two to three days. An important consequence of the slow turnover of bone is that it may take several years following the start of drug therapy or other interventions for a significant change in bone composition or characteristics to become evident. In the same way, effects on bone may build up over many years.

Why studies other than human (clinical) trials are needed

In view of these and other factors, many researchers have focussed their efforts on studying bone cells or bone extracts in the laboratory, or they have studied animals as models for what may occur in humans. Both approaches have been found helpful in predicting the potential usefulness of drugs or other measures in preventing or treating human osteoporosis. Collectively, these sorts of investigations are described as basic and preclinical studies.

Basic (laboratory) studies

Studies performed in tissue culture laboratories can provide useful insights into how many test agents work. However, with current knowledge, these experiments cannot replace animal studies because bone as an organ, together with the complex mechanisms controlling it, cannot be mimicked in the laboratory.

There are two main types of osteoporosis-related laboratory investigation;

i) studies of bone resorption (breakdown)

Bone tissue, for example from fetal mice, can be maintained in a culture system (a special mix of nutrient fluids in life-sustaining conditions) to provide valuable information about the effect

of particular agents on bone loss. Sometimes the individual cells that resorb bone, called osteoclasts, are studied directly.

ii) studies of bone formation

These usually involve an assessment of the way a proposed measure affects bone- forming cells (osteoblasts) in culture.

Preclinical studies

What are preclinical studies?

Before embarking on human testing, research is usually conducted in animals to assess the safety and potential usefulness of a proposed intervention (that is, a means of prevention or treatment of the disorder)². Such studies are described as preclinical.

Why we need animal studies:

- **to gain initial evidence that an agent is safe to administer to humans**
- **to establish how a proposed intervention affects the mineral content, mass or strength of bone, thus providing grounds for its use in humans**
- **to show that the proposed intervention can be effective in animals that show features of osteoporosis as it occurs in humans**
- **to determine whether any new bone tissue produced as a result of the intervention is normal**
- **to clarify whether long-term exposure to the intervention results in the formation of good quality bone**
- **to shorten the time required to screen for potentially useful treatment agents**
- **to gain insights into the way in which an intervention influences bone.**

Ideally, preclinical studies should always examine the effect of the proposed intervention on bone mass and quality and on bone breakdown and formation. The above information helps researchers decide whether human studies should be conducted and, if so, guides their design. Another important aim of preclinical studies is to assess the general properties, metabolism (chemical processing in the body) and excretion of new drugs in animals before human exposure.

It is important to appreciate that appropriate use of animal studies reduces the exposure of human participants to experimental agents, speeds up access to useful new treatments and keeps the number of animals needed to a minimum. The use of live animals to study measures for preventing or treating osteoporosis must comply with the regulations of local ethics committees.

² An intervention is any form of drug or action (e.g. a change in diet or physical activity, or the use of some type of device) designed to prevent the onset of, or to treat a disease such as osteoporosis.

Which animals?

The choice of which type of animal is most suitable for a particular study should take account of the following:

- the extent to which there is a match between a particular animal species and humans in respect of **bone remodelling** characteristics³.
- the appropriateness of the animal model for the intended clinical application. For example, certain animal models mimic more or less all the characteristics of oestrogen deficiency osteoporosis due to lack of the female hormone, oestrogen. Others more faithfully simulate other types of osteoporosis such as those due to cortisone-like medications and immobilisation.
- the capacity of the animal to respond to a particular intervention
- the extent to which a particular animal species can predict a human response.
- animal availability and
- the financial cost of obtaining and caring for particular types of animal
- the duration of studies needed to draw conclusions in particular types of animal. This will vary according to the biological properties of animals, including the rapidity of bone loss at various skeletal sites in the model compared with the loss in humans
- the presence in some types of animal of changes in non-bone systems that do not occur in humans, thereby introducing confounding factors into the study
- ease and safety in handling of the animals
- cultural sensitivities.

Animals selected for studying the prevention or treatment of osteoporosis may include mice, rats, rabbits, mini-pigs (specially bred small strains of pigs), sheep, dogs and non-human primates.

ii) what type of study?

In order to simulate clinical situations in humans, scientists may choose experimental designs that induce bone mass changes in experimental animals. For example they may alter the calcium intake of lactating and non-lactating animals, treat animals with cortisone-like drugs or immobilise them with plaster-casts. Many of these animal experimental models predict with a high degree of reliability the effect of interventions in humans.

These experimental systems can be used to select agents, treatment schedules, dosages of potential interest, and to study fracture healing. Findings in these systems may decrease the requirements in a subsequent program of testing in humans. For instance, adult rats whose ovaries have been removed (ovariectomized) are useful models for assessing drugs intended for use in preventing and/or treating postmenopausal osteoporosis. As occurs in women, female rats whose ovaries have been removed show increased bone turnover and a persisting loss of bone mass in several parts of the skeleton, as well as similar changes in aspects of bone structure. Furthermore, for many measures designed to counter osteoporosis, there are striking similarities in bone mass and bone strength changes between these oophorectomised rats and the bone changes in postmenopausal women.

Nevertheless the adult oophorectomised rat model has some limitations that should be considered in the design and interpretation of experimental studies. For instance, oophorectomised rats increase their food intake; the formation and breakdown of bone also differ in some respects between the two species; and the parts of the rat and human skeleton that carry load are quite different, with much greater weight-bearing by the human spine and hip.

³ Remodelling (of bone). This term refers to the process occurring in the adult skeleton whereby microscopic amounts of bone are resorbed (broken down) and then new bone is formed at the same site.

Rodent models are widely used in osteoporosis research. However it is also highly desirable to conduct experiments using non-rodent species such as monkeys, mini-pigs, sheep, ferrets or dogs because their bones and other body systems behave in some respects more like those of humans. For example, rabbits, dogs and sheep experience an osteoporosis induced by cortisone-like medications that is similar to that occurring in humans. Non-human primates share many characteristics relevant to studies of osteoporosis in humans, including menstrual cycles, reproductive hormone patterns similar to those of premenopausal women, and osteoporosis due to rapid bone loss, particularly at the spine following removal of the ovaries. In choosing to do experiments in non-human primates, careful consideration should be given to ethical and cultural issues, availability, safety and cost factors.

iii) how many animals?

Another important choice in animal studies concerns the number of animals to be included in experimental groups. The number of animals studied should be no more than needed to adequately test the proposed treatment.

iv) how is the amount of bone mineral assessed in animals?

Techniques for assessing bone include single and dual photon absorptiometry (SPA and DPA), single or dual x-ray absorptiometry (SXA, DXA) and peripheral quantitative computed tomography (pQCT)⁴. These methods can be used in living animals without causing any injury. It is essential that whatever technique is chosen, an assessment is made of both its **precision** and **accuracy**⁵ for the animal model to be used in the experiment. The parts of the skeleton in which bone mineral is measured need to be chosen carefully if the measurements are to be useful in improving understanding of the disease and of proposed interventions.

v) why and how is the microscopic structure of animal bone determined?

Assessing the structure of bone microscopically in animal studies can provide essential information about how treatments work and about their safety in the short- and long-term. Microscopic analysis can provide useful measurements related to bone remodelling (see page 7). Several components of bone quality can be assessed including microscopic architecture, bone texture and the adequacy of mineral deposition. Abnormalities in any of these can make bone susceptible to fracture. The type of microscopic assessment selected should be appropriate for cancellous or cortical bone (see figure 1).

vi) how is bone strength tested?

When assessing the structural properties of bone removed from large and small animals, researchers should ensure standardised conditions for the preservation and testing of specimens. Researchers should always perform tests on several parts of the skeleton, such as the spine, hip and mid-shaft regions of the long bones. This is because the relative contributions of cancellous and cortical tissue to overall bone strength vary markedly according to the skeletal site. In addition, these types of bone tissue can be influenced differently by the experimental manoeuvre used to induce osteoporosis and by the treatment

⁴ These techniques determine the amount of mineral in bone by measuring the absorption of a beam of radiation by the presence of mineral-containing tissue in its path. Small amounts of radiation are used, either gamma rays from a radioactive source, or x-rays.

⁵ Precision is the ability to obtain the same result from repeated measurements, while accuracy refers to the technique's ability to produce a result that agrees with direct measurements (for example, of the amount of calcium in bone samples).

being tested. Usually, long bones are tested using bending and torsion (twisting) tests; vertebral bodies and the neck of the femur (thigh bone) can be assessed using compression tests.

vii) biochemical markers of bone turnover⁶

In short-term studies to determine the range of dosages over which a test agent works, markers of bone turnover can be helpful by predicting how bone density and strength may change with longer exposure to the intervention. They can also provide supporting evidence of how a test agent might work in long-term studies. In choosing markers of bone turnover, preference should be given to those for which there is experimental evidence to support the validity of their use. Markers of both bone formation and resorption should be measured.

Other approaches to gaining information about calcium deposition in, and release from, bone involve the use of radioactive calcium, tetracycline or strontium. These substances are deposited in bone as it is being built, and released as the bone is being broken down.

viii) fracture healing

Interventions that affect the working of living cells in bone may influence bone repair following fracture. For this reason, researchers should conduct appropriate tests on the healing of broken bones, particularly when clinical studies of fractures have not been planned. Standardised experimental testing of fracture healing has been devised for rats, dogs and ewes. To determine how well fractures have healed, both X-ray and microscopic examination should be undertaken and mechanical tests performed.

ix) what happens to the drug?

In order to obtain appropriate information about the fate of a drug in an animal, researchers should conduct experiments in at least two species. It is important to discover how well the drug is absorbed when given by various routes, where it goes in body fluids and tissues, and how it is chemically processed and eliminated from the animal's body.

For an agent that is deposited in the skeleton, the long- and short-term build-up of low and high doses of the agent at various skeletal sites should be explored. It is also essential to examine how the substance is later released from bone.

x) long-term bone safety issues

Animal studies also should be used to seek any evidence that potential treatments might cause damage to the bones in the long term, for example, impaired bone growth, defects in the incorporation of minerals into bone, reduced bone mass and changes in bone fragility.

Other issues: How animal studies are designed

i) prevention or treatment?

⁶ Biochemical markers of bone turnover are molecules produced in humans and experimental animals as part of the processes of bone formation or resorption (break-down). These substances can be measured in blood or urine samples to gain information about how actively bone is being formed or broken down.

Preventive interventions may be introduced in a situation where the amount of bone is normal, to prevent an unwanted reduction in bone mass and associated problems. When bone mass is a little below normal (osteopenia) or much below normal (osteoporosis), a potential treatment may be studied with the aim of increasing bone mass or otherwise reducing the risk of future fractures. To test preventive interventions they should be started immediately after an osteoporosis-inducing measure is instituted in an animal model. Interventions being tested for their ability to treat osteoporosis should be started once the osteoporotic state is evident in an animal model.

ii) control groups

A control group is a comparison group similar to the treatment group in all respects except in not receiving the treatment under study. Control groups are essential for well-designed preclinical studies.

iii) randomisation

This is the random assignment (that is, like picking numbers out of a hat) of animals to treatment and control groups, or to different treatment groups. It helps the statistician to separate the effect of a treatment from other differences found among animals in any group. Randomisation helps in calculating the likelihood that the difference seen in an experiment is due to an effect of the treatment rather than to chance. When we randomise, we are more likely to end up with groups that are similar in terms of all these chance effects and so bias (recognised or unrecognised) is minimised. Randomisation and bias are discussed more on page 16.

iv) duration of studies

The duration of the study should be sufficient to take account of the relatively long time it takes for bone to be replaced. In small animals such as rats, this turnover time is shorter than in larger species, like man, where full bone replacement takes many years. There is some uncertainty among scientists about how to decide the ideal duration of experiments in various animal studies. One suggestion is that trial measures in rats should continue for a quarter the time required for humans while non-human primates should be exposed to interventions for half the time required for humans.

Clinical trials

A clinical trial is any systematic study of a drug or other intervention (see page 6) in patients or non-patient volunteers.

Usually the aim is to discover or confirm the effects of and/or identify any adverse reaction to the intervention under investigation. The ultimate aim of drug and non-drug interventions - whether their focus is prevention, minimisation or treatment of osteoporosis - is to reduce the risk of fragility fractures and associated ill-health. Clinical trials may also attempt to determine the absorption, distribution, metabolism (chemical processing in the body) and excretion of test drugs.

This guide refers mainly to pharmaceutical (drug) interventions for preventing, stabilising or treating osteoporosis. However, much of the discussion is also appropriate for the assessment of non-drug measures, such as the use of hip-protecting devices in the elderly and physical activity in all age groups.

Ideally, non-drug interventions should be assessed with the same rigour as drugs. This requires;

- measurement of starting conditions, such as the nutritional state
- careful trial design and analysis, including monitoring of **adverse events**⁷, **compliance**⁸ and drop-out rates (the number of subjects who leave a study before completing it)
- random allocation of participants to intervention or control groups where feasible, and monitoring for use of the intervention by control subjects, and other biases (such as changes in dietary habits induced by exercise programs).

In some non-drug interventions such as regular physical activity it is impossible not to reveal to participants and investigators who is, and is not, receiving the intervention, and its nature. Such difficulties should be taken into account when judging claims for effectiveness.

What follows is an outline of particular aspects of bone-related research in humans and a brief description of the individual phases of clinical drug development.

Use of bone density as a “surrogate” (substitute) for fracture risk.

Measurement of bone mineral content (bone density or bone mass) has a central place in osteoporosis research and the assessment of preventive or treatment interventions.

In long-term population studies, bone density has been found to be a reliable predictor of fracture risk, at least in Caucasian women. In other races and in men, the validity of using bone density to indicate fracture risk is less strong. Determining overall fracture risk involves more than measuring bone density, because of the influence of a number of other factors which are involved, such as age, ethnicity, family history of fracture, and the tendency to fall.

In studies where bone density measurements are used to estimate fracture risk, it is important to understand that a low bone density does not mean a fracture is inevitable - only that the risk is higher than normal. On the other hand, a high bone density does not guarantee a person will never experience a fracture.

Bone density is most reliable in predicting fracture risk in the early stages of osteoporosis when the microscopic structure of bone is near normal. With advancing age and progressive bone loss, “other skeletal factors” and factors beyond the skeleton have an increasingly evident influence on fracture risk. Examples of these other skeletal influences include the shape

⁷ Adverse events are symptoms, diseases or other deterioration in the state of health of trial subjects, whether or not the occurrence is thought to be related to the study intervention.

⁸ Compliance is the extent to which study subjects comply with the requirements of a clinical trial. It refers in particular to their taking of the intervention being tested.

of bone and microscopic abnormalities within it. The contribution of factors other than bone density to fracture risk increases with age and varies with ethnicity. Therefore, bone density measurements provide less information about the risk of fractures in elderly non-Caucasian groups.

Before embarking on a clinical trial in which bone density measurements are to be used to estimate fracture risk, researchers first need to perform animal studies including appropriate studies of the fine structure of bone and its response to a potential therapy to ensure that any proposed treatment leads to the production of healthy bone with adequate strength.

Bone density measurement techniques

Non-invasive techniques for measuring bone density⁹ include single and dual photon absorptiometry (SPA and DPA), single or dual energy x-ray absorptiometry (SXA, DXA), quantitative computed tomography (QCT), neutron activation analysis, quantitative radiography and ultrasound. Standard x-rays are a very unreliable way to assess bone mineral levels. The widely-used dual energy x-ray absorptiometry (DXA) technique has many advantages but does not measure the true density of bone, instead giving a value for mineral content within a particular area of bone.

In principle, any of the well-established bone density measurement techniques is usable in clinical trials of osteoporosis provided that characteristics like precision, accuracy (see page 8) and biological interpretation are taken into account. In general, bone density should be measured at two or more skeletal sites, one of which should be the spine or hip.

Fracture assessment and height

All fractures that a subject has suffered before entering a clinical trial should be recorded. Once a trial has started, details of any other fractures occurring should be documented with information on when and how they happened.

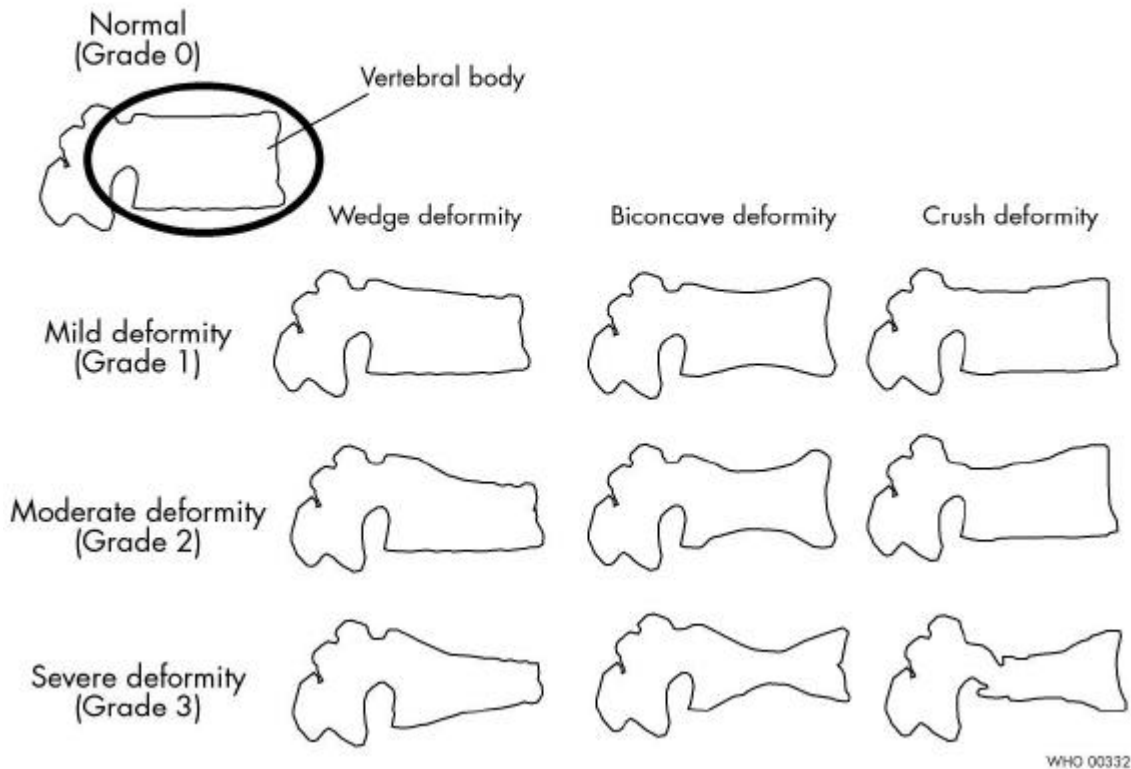
In assessing the occurrence of spinal fractures, measurements of vertebral bone dimensions are needed. This is important because obtaining confirmation of suspected vertebral fractures can pose problems due to the lack of uniformity among researchers in defining the features of spinal fractures seen on x-ray.

See Figure 3.

⁹Explained on page 10. Most of these techniques determine the amount of mineral in bone by measuring the absorption of a beam of radiation by the presence of mineral-containing tissue in the beam's path. Small amounts of radiation are used, most often in the form of x-rays.

Figure 3. Lateral (side) view of vertebral bodies.

Source: van Kuijk and Genant, 1995



In interpreting vertebral bone dimensions the following issues should be considered;

- not all vertebral deformities are due to osteoporosis (for example, they may result from an abnormality a person is born with)
- variations in “normal” vertebral dimensions occur at different vertebral levels. An overestimate of spinal fractures may occur when a large number of vertebral measurements are made in individual participants (this is the problem of “false positives”).

A high false-positive rate for spinal fractures can have a profound impact on the interpretation of clinical trials, tending to decrease the perceived efficacy of an intervention or to distort the trial’s results in other ways.

Studies of spinal fracture should;

- provide detailed information about the criteria (that is, the rules) used to define fracture occurrence before or during trials
- distinguish between fractures occurring in previously undeformed vertebrae and progressive deformities in previously fractured or deformed vertebrae
- clearly state numbers and severity of fractures and numbers of affected individuals
- in multicentre studies, ensure that x-rays are read at a central location from which quality control is overseen.

An additional informative way of assessing an intervention’s effect on spinal fracture occurrence in trials of established osteoporosis is to monitor differences in the height of individuals in treatment and control groups.

Quality of life and other measurements

Decreased illness and improvement in quality of life are what anti-fracture interventions ultimately aim to achieve. Improved ways to measure quality of life are under development and should provide researchers with useful tools in future intervention studies.

Other types of measurement are used not to test the effectiveness of an agent, but to assess how it affects bone and its safety in clinical use.

Bone biopsy measurements (measurements on small samples of bone usually removed with a needle) may be useful in assessing how an intervention works, its impact on bone structure and, very importantly, its safety. Measurements on bone specimens are helpful particularly in determining the rate at which bone is formed and resorbed (broken down) and the adequacy of incorporation of minerals into the bone. However, because biopsies are necessarily taken from a restricted skeletal site they are not helpful in obtaining general bone density information. Biopsies of bone should usually be performed at the end of the period of exposure to an intervention.

Indicators of bone turnover can sometimes help in assessing treatments. They include calcium levels in the blood and urine, indices of bone collagen turnover and other markers of the activity of bone cells, e.g. alkaline phosphatase (an enzyme produced by bone-forming cells). When kidney or liver disease is present, interpretation of findings demands great care since these organs are involved in the chemical processing and excretion of these indicators.

Clinical trial methods; general principles

When studying the effectiveness of preventive or treatment interventions, a number of general design principles should be considered.

The protocol

Every clinical trial should be carried out in accordance with a protocol, that is a document stating the background, rationale and objectives of the trial and that describes its design, methodology and organisation, including statistical considerations, and the conditions under which it is to be performed and managed.

The protocol should state the reasons why the trial should be undertaken in humans; the nature and degree of any known risks; the groups from which it is proposed that trial subjects will be selected; and the means for ensuring that they are adequately informed before they give their consent to participate. It should receive scientific and ethical appraisal by one or more review bodies (such as an institutional review board, ethics committee, drug regulatory authority), constituted appropriately and independent of the investigators and the source of funding.

The population studied

The trial being undertaken should have relevance for participants. For example, it would be inappropriate to study the effectiveness of an osteoporosis prevention measure in individuals with established osteoporosis. Further, the trial should be performed in a population sample of the same ethnic composition as the population to be targeted for the intervention.

Before the trial starts, the target population should be defined according to gender, age, ethnic composition, nutritional status, state of health, development and maturity, and, where appropriate, the cause of osteoporosis or osteopenia (low bone mass). Other factors implicated in osteoporosis should be documented, for example, smoking and family history of fractures. In postmenopausal women, the date of menopause should be documented and whether it occurred naturally or was induced, e.g. by gynaecological surgery. If a known bone-depleting therapy such as a cortisone-like medication has been used, its dose and duration should be noted. In future, as clinically useful genetic markers of low bone density or fracture risk are identified, these may have a role in determining responsiveness to a particular preventive or treatment measure. Therefore, these markers may also influence the selection of subjects for clinical trials.

The characteristics of individuals suitable and unsuitable for inclusion in the trial need to be specified. Factors to be considered include the cause of bone loss, the presence of other diseases which might affect bone and treatment with other bone-active medications. Careful use of these and other criteria can strengthen a study, but it is important that entry in a trial is not so restricted that the results can be applied in only a few patients.

Since men may also be affected by osteoporosis it is important that clinical trials are conducted in both sexes, a situation that applies equally in studies of all diseases where gender-specific risks have not been identified. Men may be studied in separate trials or included with women if the study design method called stratification is used (see page 17).

The treatment approach

When the trial design includes a combination of treatments, or treatments given one after the other, each individual treatment should be tested unless previous studies provide sound scientific reasons for combined or sequential therapies.

Study design

The protocol should include a description of the end-points, that is, criteria that can be measured to assess whether an intervention has been effective. End-points for effectiveness differ from measurements used to assess an intervention's safety or intended to provide information about the way a treatment affects bone. The choice of end-points depends on the stage of drug development and associated aims.

For long-term studies, end-points may include the occurrence of fractures, bone mass measurements, skeletal architecture (studies of fine structure of bone and of those factors determining bone strength), and improvement in symptoms related to osteoporosis.

Many trials are **randomised** (see page 12) **and placebo-controlled**.

A **control group** is a comparison group intended to be similar to the treatment except in not receiving the treatment under study.

A **placebo** is a harmless inactive substance which is given to the control group instead of a biologically active agent to help separate the effects of the agent from other influences related to participation in the trial. The use of a placebo means that the involvement of the control

group in the trial can mimic the actively-treated group in all respects except that the control group does not receive the treatment being tested.

In many clinical trials, all participants are given an active agent (usually calcium tablets) in addition to the placebo or test agent.

Stratification - the separation of the study population according to relevant characteristics so that groups can be matched - may be appropriate when mixed populations are studied such as men and women, or patients with different causes of osteoporosis.

Cross-over studies - that is, when the intervention is applied to one group of participants then to another during the progress of the clinical trial - are not usually practical in bone studies due to the long-term effects of interventions on bone turnover. Exceptions occur in studies that are looking specifically at what happens to bone when a particular intervention is ceased.

It is important that clinical trial participants are randomly assigned to a placebo group or to treatment groups. This helps the statistician to separate the effect of the treatment from that of the differences found among patients within different groups. Randomisation is one of the ways to minimise the chances of bias in a trial. Without randomisation, someone has to make a decision about whether a patient gets the test intervention or something else. If we do not randomise we are likely to end up with subtle differences between groups. For example, we might think that a particular person is more likely to be suited to the test therapy than to its alternative. Unfortunately, these subtle selection differences tend to happen without awareness that they are occurring. It is also important that, where possible, researchers and others dealing with the different patient groups in a trial are not aware which patients are receiving an active intervention and which are receiving a placebo. This is referred to as **blinding** of the research team. Blinding is important to ensure that the researchers do not (subconsciously) deal with some subjects or their results differently because of the treatment the subjects are receiving. Where possible, the trial subjects also should be blinded, that is, unaware during the trial of the intervention or placebo they are receiving. When the researchers and the trial subjects both are unaware of who is receiving active and placebo agents, the trial is described as **double-blind**.

Direct evidence for effectiveness of therapies (rather than supporting or circumstantial evidence) is best obtained from prospective studies, that is, trials that monitor the responses of subjects for a fixed period of time from the start of treatment. Retrospective studies which look back on previous use of the intervention are more likely to produce misleading findings. They are limited due to factors such as lack of randomisation, lack of blinding and, generally, an inferior quality of information gathering.

While clinical studies of efficacy generally should be randomised, controlled and double blind, researchers should recognise that in long-term randomised studies the rate at which subjects leave the study before completing it (i.e. “drop out”) may be appreciable, which also complicates interpretation of the findings. To help detect biased results due to drop-outs, reasons for withdrawal should be reported together with an analysis of the way withdrawing individuals differ from those completing the trial.

In most study designs, the duration of exposure of controls (to a proven active agent or to a placebo) should be identical to the exposure of participants receiving the test intervention. The timing and dose of the therapy should duplicate that intended for use in the health care setting.

#Duration of studies

The ability to detect changes in bone mass or density in a clinical study depends on;

- the rate of bone gain or loss
- the duration of the study
- the precision of the techniques used to measure end-points (see page 8).

Virtually all the interventions investigated to date to prevent, minimise or reverse human bone loss induce initial changes in bone mass that take around two years to reach a steady state, that is, a new balance between bone gain and loss. This is an important reason for continuing clinical trials of bone therapies for several years at least. In addition, at least several years of observation are needed to detect meaningful changes in the rate of fracture.

Numbers of people studied

Factors that influence the number of participants in a study are;

- the nature of the hypothesis and end-point(s) to be examined
- clinically relevant differences in effects between the treated and control groups
- the precision of the techniques selected for measuring end-points in the population under study
- the duration of the study
- the anticipated rate and probable timing of drop-outs.
- the range of observed responses to the test and control agents during the study.

Studies should include sufficient participants to detect clinically important differences where they exist, and not to falsely detect an apparent difference that may have occurred by chance. The study should be designed so that there is at least an 80% chance of detecting a clinically significant effect of the intervention and a less than 5% chance that an apparent difference is untrue, having occurred by chance.

Frequency of measurement

Bone mineral measurements should be undertaken at least every six months where studies of two to three years' duration are envisaged, and at least once a year for studies continuing beyond three years. X-rays of the spine, where taken, should be yearly.

This measuring schedule should be undertaken to;

- safeguard against measurement problems
- document short-term changes and new steady states
- compare rates of change in bone density

Safety issues

Researchers should take account of potential hazards of techniques. For example, bone density tests such as SPA, DPA, SXA and DXA (see page 14) use very small amounts of radiation and may be acceptable for children but computed tomography (CT) has a higher radiation dose and may not be suitable in a research setting.

Tools for investigating safety aspects include studies of how drugs are processed in the body, evaluation of blood counts, tests of kidney and liver function, blood calcium and phosphate levels and, in some circumstances, bone biopsy (see page 16).

There should be provision for analysing safety and efficacy end-points at least annually during a trial without revealing to participants and investigators details of the response to intervention. Rules should be established for every trial to ensure that this monitoring procedure will detect an unacceptable hazard to participants and that the trial will be stopped in such an event.

The size of a trial should be sufficient to adequately assess the safety of an agent in relation to its likely benefit to a target population. The lower the risk of fractures in a population, the greater is the need to demonstrate the safety of an intervention by studying large numbers of subjects (that is, to show that there is a favourable benefit-to-risk ratio).

Good clinical practice

Researchers should consult the guidelines for good clinical practice for trials on pharmaceutical products (World Health Organisation, 1995).

Phases of clinical drug development

The clinical development of a drug progresses through several phases allowing the short- term safety, chemical processing and excretion by the body, the way the drug works, its efficacy, long-term safety and overall therapeutic usefulness to be evaluated in an appropriate sequence.

i) Phase I studies

The first study of a new pharmaceutical agent (drug) or new drug formulation in humans is known as a Phase I study.

Its principal aim is to ensure the agent or formulation is safe and well-tolerated. Other aims are to gain an outline of how the agent or formulation acts in the body and the way the body processes and excretes it. This type of information helps in predicting whether people in ill-health (with conditions such as kidney or liver disease) will experience adverse effects as their bodies attempt to handle the agent. Preclinical (animal) findings should be used as a guide in designing these studies.

Phase I studies on bone-active agents are generally carried out in small numbers of healthy volunteers. However, it may be appropriate to go beyond the healthy population and include patients with osteoporosis, for example, where the agent is known to persist in the body for a very long time.

ii) Phase II studies

The purpose of a Phase II study is to demonstrate that the agent being tested has a beneficial effect and is safe for short-term use in patients suffering from a disease or condition which the agent is intended to counter.

A further aim of phase II studies is to explore a range of possible dosages and their effects. In so doing, it is hoped to identify a dose which provides a beneficial treatment effect and which is also well-tolerated by patients.

Phase II studies involve a limited number of participants, should include double blind, randomised, controlled trials and should be of a sufficient size and of a duration long enough to demonstrate the effect of the drug on a defined end-point. Whether the study is placebo-controlled or not depends on whether an established treatment or placebo is to be used in the control group.

Possible end-points for phase II studies include:

- biochemical indicators of bone formation and resorption.
- bone mineral measurements at clinically relevant sites.
- in some circumstances (depending on preclinical findings), bone biopsies to assess bone architecture and quality.
- additional endpoints need to be considered where an intervention causes changes in bone size, body fat or lean body mass, or affects the accuracy of bone density measurements.
- measurements related to the body's handling of calcium also may be performed to improve understanding of how the agent works.

iii) Phase III studies

These are trials in large numbers of subjects with the purpose of determining the short- and long-term safety and effectiveness of a drug preparation and of assessing its usefulness as a treatment.

Confirmation of effectiveness requires supporting evidence from at least two well-designed studies. Assessments of safety require a careful analysis of the number of participants studied, their state of health and their duration of exposure to the test agent. The circumstances of studying test agents should be as close as possible to normal conditions of use.

Phase III trials in established osteoporosis

Patients participating in phase III studies of established osteoporosis will have had one or more fragility fractures beforehand. Patients entering these trials may need to be graded according to their type of fracture, their gender and other factors. Where the test agent is likely to be used by the very elderly, this group should comprise a substantial part of the trial population. In selecting patients, researchers should exclude those with nutritional disorders unless the trial agent is intended to correct these deficiencies or counteract their effects on bone.

In planning studies to show the effectiveness of an intervention, the end-points selected will depend on the findings of animal studies and the class of compound that is being tested. Ideally, the treatment would aim to reduce the risk of fracture in a patient to that of a healthy adult of the same age and sex. In practice, most study aims are less ambitious but nonetheless worthwhile. They may include preventing a worsening of fracture risk or reducing the impact of osteoporosis on enjoyment of life. In order to maximise benefits, the treatment should be applied as early as possible in the natural history of the disorder.

Increasing bone density alone is inadequate evidence of effectiveness in many situations, for example postmenopausal osteoporosis, since the architecture of bone in established osteoporosis is abnormal and an increase in bone density may not lead to a proportional increase in bone strength. Claims for a beneficial effect of a therapy are strengthened by preclinical and clinical evidence that the quality of bone formed is normal, or that fracture frequency is reduced.

Phase III bone density assessments should be made at both cortical and cancellous bone sites (see page 4) related to clinically important fracture sites for the particular population under study. Their assessment should usually be undertaken for a minimum of three years and sometimes for five to ten years. Researchers should also establish that increases in bone mass at one site are not associated with weakening of bone at another. In practice, at least two skeletal sites should be measured. The rate at which a beneficial effect wears off after therapy is stopped should also be studied.

Since the elderly population, which is most likely to experience osteoporosis, is also likely to have other illnesses and to be administered other drugs, investigators need to assess potential disease-related and drug interactions.

Where the intervention in established osteoporosis is intended to decrease falls or their severity, the standards of research should be just as rigorous as for drug trials. For example, every effort should be made to randomly allocate patients to groups receiving and not receiving the intervention and, where feasible, to restrict information about the nature of the intervention and who is receiving it, while the trial is in progress and during analysis of the results.

Phase III trials in osteoporosis

Patients accepted into phase III studies of osteoporosis should have a bone density below an appropriate cut-off value. In the case of women, this cut-off is a bone mineral measurement at the site of interest approximately 30% or more below the average for young healthy adults. For men the same absolute cut-off may be used or a bone mineral measurement giving a fracture risk greater than an acceptable norm.

Many of the points made for phase III studies of established osteoporosis apply.

In studies of postmenopausal osteoporosis where fractures are unlikely in the immediate postmenopausal period, the prevention or reduction of bone loss at specific sites are appropriate end-points for effectiveness.

Phase III trials in subjects with low bone mass (osteopenia)

Patients with osteopenia have a less severe or immediate clinical problem than those with osteoporosis or established osteoporosis. Many healthy individuals, especially in the older female population, have bone mass in this range.

Since stage of menopause has a powerful influence on the rate of change in bone mass, this should be documented. And because the osteopenic population is likely to be large, researchers should consider restricting the study to specific groups, for example individuals whose condition is related to use of cortisone-like drugs or to immobilisation. Unless the trial intervention is nutritional in type, the nutritional status of the population to be studied should be normal.

Before a trial starts, there should be substantial evidence to demonstrate the safety of the trial intervention. Studies should continue for at least three years to ensure that effectiveness and safety persist with long-term exposure. This is particularly important where interventions are targeted at relatively young populations. The size of the study should be sufficient to allow detection of small increases in clinically significant adverse events attributable to the trial intervention.

In trials of treatment for low bone mass, enhancement or preservation of bone mass is generally regarded as an acceptable end-point for effectiveness. Investigators should ensure that bone mass measurements include clinically relevant sites. In some circumstances where fracture risk is relatively high, such as in nursing home patients, fracture rates may be appropriate primary end-points. A number of secondary endpoints may be specified, including bone biopsy in some circumstances.

Phase III trials in subjects with normal bone mass

Participants in a study may be drawn from the normal population at various ages or represent a small group exposed to a particular risk for fractures.

Clinical trials in this group may be performed to assess the safety and effectiveness of new pharmaceutical agents, non-drug interventions (such as regular weight-bearing physical activity) and combinations of these.

Appropriate end-points may include bone mass measurements and sometimes change in fracture risk. Where normal subjects are studied, the future development of measurement techniques which avoid exposure to radiation is highly desirable. Studies should continue for at least three years unless the duration of risk exposure is brief, and should have enough participants to enable detection of very uncommon adverse effects of the intervention.

iv) Phase IV studies

These studies are carried out on the basis of the product characteristics for which marketing of a drug has already been approved.

They include post-marketing surveillance (for example, monitoring for adverse effects) and further assessment of how a drug is used in clinical practice and of its overall usefulness.

Phase IV studies are particularly important because agents used to prevent or treat osteoporosis are commonly prescribed for a long time and, even after treatment ends, they may be retained in bone for many additional years. Such studies are performed to further demonstrate the intervention's safety and the persistence of its beneficial effects. Approaches to re-introducing the treatment where appropriate also may be studied in phase IV. It is important to note that even serious adverse reactions may not have been detected with earlier studies if such responses occur rarely. Also, potential for adverse effects and interactions with other drugs is particularly high in elderly people who are most likely to have osteoporosis-related problems. Phase IV studies also provide an opportunity for risk-benefit studies and studies of the economic impact of targeting a drug to particular groups for whom it

seems to offer significant benefits. It is important that phase IV studies document fracture events. Phase IV studies also have the potential to detect unintended and unsuspected beneficial effects, further emphasising the value of good post-marketing research.

Most phase IV studies should include a control group. An exception sometimes arises when safety issues are being addressed and the main aim is to estimate the occurrence of adverse effects.

One type of phase IV study, known as a **cohort study**, apart from studying effectiveness (or efficacy), aims to detect non-intended adverse and beneficial effects of a therapy. This type of study compares the findings with what occurs in a non-treatment group or a group receiving another therapy by following the participants over time. The main drawback of this type of study is its non-randomised nature.

Another phase IV design, the **case-control study**, compares reports of the number of cases of a condition which may occur as an adverse event in people receiving a therapy with cases of the condition occurring in a comparable group of people not receiving it. The aim is to seek any association between the particular condition and the therapy. A major drawback of this approach is the potential for bias in selecting controls.

Another option is a **randomised controlled trial**, for example, comparing the test agent with another established treatment. If the trial is placebo-controlled, patients joining such a study should be informed that they might receive an active agent or a placebo.

Further information about osteoporosis and clinical trials

- National osteoporosis societies
- International Osteoporosis Foundation
- National Osteoporosis Foundation of the USA
- International Society for Clinical Densitometry
- International Bone and Mineral Society
- International League of Associations for Rheumatology
- American Society for Bone and Mineral Research

Further reading

Studies of drugs and other measures to prevent and treat osteoporosis: a brief guide. Also on this website.

Guidelines for preclinical evaluation and clinical trials in osteoporosis. World Health Organization, Geneva (1998).

Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Technical Report Series 843; World Health Organization, Geneva (1994).

Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. Technical Report Series 850. World Health Organization, Geneva (1995).

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