Measurement of Bone Quality in Growing Male Rats Using Dual Energy X-ray Absorptiometry and Bone Ash Content

A thesis presented in Partial fulfilment of the requirements for the degree of Master of Applied Science in Animal Science at Massey University, Palmerston North, New Zealand

Katherine Elizabeth Brown
2001

Abstract

Growing male rats have been considered and used as a model for bone growth and prevention of osteoporosis because of their high bone turnover and demand for calcium. Dual Energy X-ray Absorptiometry (DEXA) is a useful tool for identifying minimal changes in bone mineral density and has recently been adapted for use in small animal models. The objective of this trial was to identify the changes in Bone Mineral Density (BMD) in relation to age and to identify how BMD varies from site to site.

Sixty male Sprague-Dawley rats were split into six groups to allow measurements at one, two, three, four, five and six months of age (n=10 per group). At each time point a group of rats was scanned using a QDR4000 DEXA machine from Hologic. Duplicate BMD measurements were obtained for the whole body, spine and both femurs *in vivo*. The rats were then euthanased and the spine and both femurs were excised for *ex vivo* DEXA scanning and ashed calcium analysis.

BMD increased almost linearly to four months and then formed a plateau. This indicates that from weaning to four months is an especially sensitive time for manipulating bone growth in male rats. There was a significant difference in BMD between groups ($P<0.001$), which is to be expected in growing rats. There was also a significant difference in BMD within groups ($p<0.001$), believed to be due to variation at two and five months of age. There was a very strong positive correlation between weight and BMD and age and BMD at all sites, indicating that BMD is a strongly related to both weight and age. All sites were strongly correlated to each other and to the ashed calcium values. The excised femur had a lower BMD value than the *in vivo* femur, although the two values were strongly correlated. This is believed to be due to differences in positioning and indicates that the two methods cannot be used interchangeably.

These results indicate that bone mineral density is the gold standard for following changes in bone growth over time in the growing rat. Alternatively, ashed bone calcium content can be used, but only as a once off endpoint.

Acknowledgements

I would like to express my sincere thanks to my supervisors, Dr Marlena Kruger and Dr Linda Schollum, for their encouragement, patience and commitment throughout this study. I would also like to thank Dr Barbara Kuhn Sherlock for her invaluable knowledge and assistance with many aspects of this study.

Thanks are also due to Miss Nicky Frearson for all her good advice and assistance, to Mr Philip Moore and Miss Dianna Crosbie Caird for their excellent animal handling skills, to Miss Gabrielle Collett, Miss Lee Layton and Ms Chungli Feng for their assistance in sample preparation and to Mrs Margaret Brown, Mrs Judith Stanton and Mrs Debbie Chesterfield for their help with daily animal care. Thank-you to Mr Colin Hughes and the analytical Chemistry team at DRI and to Dr Kathy Parton for her veterinary knowledge of anaesthetics and recovery.

I would like to give a big thanks to all the people of the Milk and Health Research Centre in the Institute of Food Nutrition and Human Health at Massey University. I gratefully acknowledge the financial support of the Milk and Health Research Centre and the New Zealand Dairy Board.

Finally I would like to thank my parents Chris and Graeme for their unending support, love and understanding.

Table of contents

List of Tables

List of Figures

List of Abbreviations

ACP = Acepromazine

Ashed Ca = Ashed calcium content

BMC = Bone Mineral Content

BMD = Bone Mineral Density

DEXA = Dual energy x-ray absorptiometry

LF = Left Femur BMD

Lfex = Ex vivo Left Femur BMD

Mth = Age in months

PBM = Peak Bone Mass **RF =** Right Femur BMD

RFex = Ex vivo Right Femur BMD

Spine = Spine BMD

Spex = Ex vivo spine BMD

Wt = Rat weight (g)

General Introduction

Osteoporosis

Osteoporosis is a wide spread disease in postmenopausal women and the elderly. It is estimated that in the U.S.A alone 15-20 million women over the age of 45 have osteoporosis (Baran *et al*, 1989; Petley et al, 1996). Osteoporosis describes a condition of low bone mass that results from excessive loss of bone after maturation or from inadequate development of the skeleton during maturation. Osteoporosis and its consequences have become one of the highest costs to our society (Aufdemorte *et al*, 1993).

With new advances in science and medicine, the average life expectancy has increased. Diseases that were previously fatal are now treated effectively by a range of drugs and practices. This has led to an increase in the number of elderly people in the population, resulting in an increase in the number of cases of osteoporosis. Loss of bone mass is an almost universal occurrence in the elderly and leads to an increased risk of fracture (Baran *et al*, 1989).

The World Health Organisation (WHO) definition of osteoporosis is "A systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (Kanis, 1994; Petley *et al*, 1996)). There is a rapid loss of trabeculae in spongy bone and a slower loss in cortical bone. This gives the bone a porous look. Although bone mass is decreased, mineralisation of the remaining bone is normal.

Bone is a living tissue that is constantly being remodelled. This happens in two phases. The first is bone resorption. Once activated, osteoclast precursor cells clump together and become an active multi-celled unit, which chews through bone. Tunnels are formed in cortical bone and lacunae are formed in trabecular bone. This process takes about one to three weeks. The osteoclasts then disappear and are replaced by osteoblasts. The osteoblast's role is to repair the tunnels and lacunae by filling them in with new bone. This process takes several months (Kanis, 1994). In healthy young adults the net balance is zero.

There are two types of osteoporosis, senile and postmenopausal. In senile osteoporosis, it appears that the cavities that are formed in bone are only being partially filled. This indicates a decrease in bone formation. It is likely that senile osteoporosis is a natural part of the aging process. Senile osteoporosis could act through an increase in bone resorption, a decrease in bone formation or a combination of both factors. Women are affected at a ratio of 2:1 compared to men. There are two main reasons for this. Firstly women are smaller in stature than men, so they have less bone mass to start with (refer to Figure 1). Secondly there is a definite hormonal link. The sex hormones have a protective role against the catabolic action of Parathyroid Hormone (PTH). Androgens are responsible for skeletal integrity in males (Rosen *et al*, 1995). Testosterone has a higher anabolic effect on bone than oestrogen although the levels of both decline with age. The circulating levels of estrogen decrease dramatically in women after menopause.

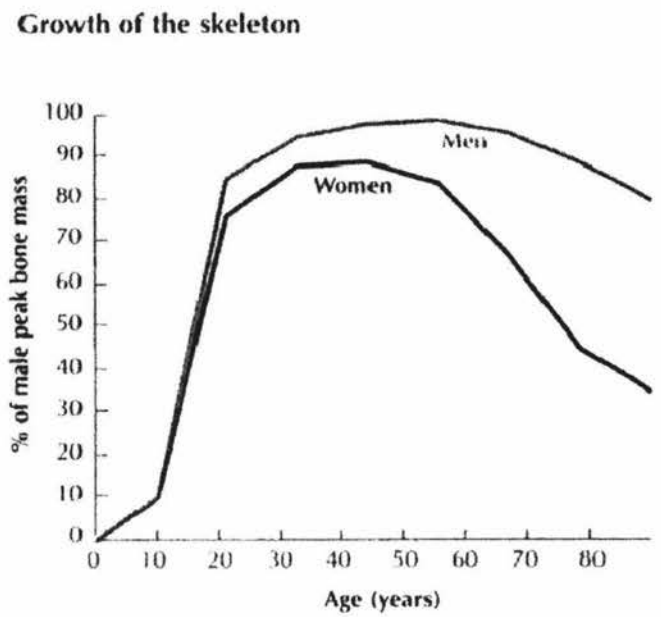

Figure 1 Growth of the skeleton

In postmenopausal osteoporosis, an increase in bone turnover occurs due to a lack of oestrogen (Ke *et al*, 1995). The circulating levels of oestrogen decrease after menopause. Oestrogen has an inhibitory effect on PTH, which is known to stimulate osteoclasts (Bagi, 1997). In postmenopausal osteoporosis the number of osteoclasts present increases. This results in very large cavities forming in the bone. Unfortunately there is no subsequent increase in the activity of the osteoblasts. This leads to a thinning of the bone's structure, especially in trabecular bone (Kanis, 1994).

Secondary osteoporosis may develop as a result of other factors (Nordin, 1984). Some genetic disorders such as Pagets disease and hypogonadism can lead to osteoporosis. Alcoholism and several drug therapies, such as the use of glucocorticosteroids, also lead to osteoporosis and increase the risk of fracture (Loré, 1989).

While treatment can increase bone mineral density (BMD), the damage to the architectural structure of the bone may never mend completely. The weakening in the bone's structure means that fractures may still occur despite positive responses in BMD.

Osteoporosis is a serious medical condition. While brittle bones in themselves do not cause a problem, there is a subsequent increase in fracture risk. Low bone density increases the risk of a debilitating fracture. A loss of height is the most recognisable sign of osteoporosis. In normal cortical bone, 95% of the area is taken up by bone material; in osteoporosis as little as 30% of the area may consist of bone (Sissons, 1962). This gives the bone a porous appearance and can lead to weakening and collapse of the bone structure. The lumbar spine is usually the first area to be affected, as it has a small area in comparison to its weight bearing capacity. This often results in a collapse of the vertebrae and causes a reduction in the patient's height.

The hip is probably the most serious site of fracture and is associated with a higher degree of morbidity and mortality (Sissons, 1962). It is usually the result of a fall and thin women with little padding around their hips are particularly susceptible. It is associated with a high cost to our society, as patients are in need of immediate hospitalisation. There is also a loss of mobility and increased pain, which can lead to a loss of independence. Patients may need to go into a nursing home or be cared for by family members.

The recommended course of action is a hip replacement. The patient is mobilised almost immediately. This stops muscle atrophy, which is associated with a decrease in blood supply to the bone. This course of action is usually highly successful (Kanis, 1994). If left in traction the fracture can heal of it's own accord but long-term

immobilisation is linked with a greater loss of bone mass, an undesirable side effect in osteoporosis. Hip fracture is unlikely to be a main cause of death. The higher degree of mortality is probably associated with patients who are too unhealthy to undergo hip replacement surgery or who acquire secondary complications unrelated to the actual fracture (Sissons, 1962).

The risk of osteoporosis can be significantly reduced by:

1. Maximising the development of high peak bone mass (PBM, see chapter 1.1.1).
2. Maintaining bone mass after PBM is achieved
3. Reducing bone loss as much as possible.