

Hormones and bones

Dr Rachel Davey discusses her research into metabolic bone diseases which has a focus on the physiological role of hormones to increase new bone growth



Interview contributors: Drs Rachel Davey and Helen MacLean

How would you summarise the overall goal of the project?

RD: The goal of our project is to gain a better understanding of how the male sex hormones, androgens, act to control both the formation of new bone and the breakdown of old bone. We aim to understand how androgens act in two ways: firstly, how they control the bone forming cells (osteoblasts) at different stages of their development to increase bone formation during growth and maintain bone in adulthood, and secondly, how androgens control the bone resorbing cells (osteoclasts), to inhibit bone resorption.

Why aren't androgens more commonly used to increase muscle mass and strength?

HM: Despite the fact that androgens can increase muscle mass and muscle strength in both men and women, they are not widely used as a therapy because of their negative side effects. In women, androgens have unacceptable masculinising effects. In men, depending on the dosage, androgens may potentially increase the risk of stroke, prostate cancer and cardiovascular disease.

For these reasons, higher than normal doses of testosterone which may be required

to build muscle mass are not routinely administered. Research is focussed on developing selective drugs that will have the anabolic effects of androgens in muscle and bone without the negative actions in other tissues.

What makes your research unique and how has this been beneficial?

RD: We are one of the few groups internationally that are using tissue-specific androgen receptor knockout models in order to provide insights into the precise mechanism by which androgens exert their effects on bone. As such, this has provided us with the opportunity to establish collaborations with leaders in the field of bone research both nationally and internationally, thereby raising the profile of the medical research being conducted in Australia in this field.

What advice would you give to a person who is looking to prevent osteoporosis?

RD: As calcium is not made by the body, when calcium levels are low, the body breaks down bone to meet the demand for additional calcium. It is therefore essential that levels of calcium are maintained by eating a diet containing calcium-rich foods and/or taking calcium supplements.

In addition, vitamin D, a key hormone in the absorption of calcium from the gut, is also required. Sun exposure of 10-15 minutes duration, two to three times a week is sufficient to provide adequate vitamin D levels. The benefits of weight-bearing exercise on bone health are well documented; not smoking and limiting alcohol consumption also promote healthy bones.

Could you expand on your work on the hormone calcitonin and how it relates to bone health?

RD: We are currently studying the physiological role of the calcium regulating hormone calcitonin. Calcitonin, acting via its receptor (CTR), is well known to potentially inhibit osteoclasts and is used to treat disorders



FIGURE 1. 3DmicroCT images showing that removal of the androgen receptor (right panel) results in smaller and thinner bones of decreased density.

characterised by increased bone resorption. However, individuals with calcitonin deficiency do not exhibit any bone abnormalities leading many to question whether calcitonin has a physiological role to play in regulating bone and calcium homeostasis.

Our unique studies examining the actions of calcitonin in bone *in vivo*, using global- and osteoclast-specific CTR knockout mice, demonstrate a surprising role for the CTR in inhibiting bone formation. We also provided the first *in vivo* evidence that a major physiological role of the CTR is to protect against high serum calcium levels. We showed that the CTR exerts this protective effect predominantly via its actions on osteoclasts to inhibit bone resorption. Our current research aims to further understand these functions of the CTR. The elucidation of a new pathway regulating bone formation is of major significance and may lead to a new class of anabolic treatments for osteoporosis, acting to increase bone formation rather than inhibiting bone breakdown.

Could you outline some of your greatest achievements to date so far over the course of your research?

RD: Establishing my own research group and successfully obtaining grant funding in order to study the hormonal control of bone cell metabolism is one of my main achievements. The recognition of my research by my peers through being the first Australian to be awarded the Early Career Excellence in Teaching Award by the American Society of Bone and Mineral Research in 2008 is also a major achievement for me.



The anabolic actions of androgens

The effect of declining oestrogen levels on women's bones is well known, but a project at the **University of Melbourne** is investigating the lesser-known role of male hormones in the formation and breakdown of bone

ONE IN TWO women and one in three men over the age of 60 will suffer a fracture as a result of weak bones. In an increasingly ageing society, it is essential to address this major public health concern. The role of sex steroids in the development of osteoporosis has been extensively studied in women, mainly as a result of the well-documented bone loss associated with menopause due to oestrogen deficiency. Despite the incidence of fracture in men also being high, the role of male sex hormones, androgens, in the development of osteoporosis in men has not received the same attention.

This disparity is likely due to the fact that the decline in testosterone levels in men does not occur as rapidly as the decline in oestrogen levels in women after menopause. However, the role of androgens in the development of osteoporosis in men has been the subject of increased investigation following the finding that men have a higher rate of mortality than women following a hip fracture.

AMAZING ANDROGENS

Androgens are essential for skeletal growth and bone accrual during puberty, and for post-puberty bone maintenance in males, determining both the size and strength of adult bone. These hormones directly stimulate the outward growth of bone by a process known as periosteal apposition. Because of this process, the actions of androgens play a significant role in determining peak bone mass, or maximum bone density, which is achieved by the time a male reaches his early twenties. This in turn plays a critical part in determining bone strength in old age: everyone experiences age-related bone loss, but a higher peak bone mass is associated with a decreased risk of developing osteoporosis later in life.

The mechanisms by which androgens exert these effects on bone are not well understood.

The molecular building – or anabolic – actions of androgens, together with their potential but less well defined action to halt breakdown of bone – known as anti-catabolic action – make androgens a key candidate for understanding the emergence of bone fragility associated with ageing.

BONE FORMING CELL CYCLE

Dr Rachel Davey, Senior Research Fellow in the Department of Medicine (Austin Health) at the University of Melbourne, is leading a research project which is investigating how androgens control the breakdown and formation of bone, using a unique combination of bone physiology and genetically modified mouse models.

The formation of bones is controlled by cells known as osteoblasts, which have three distinct stages of their cell cycle. The proliferation stage occurs when the cells divide and multiply. Next, the matrix synthesis stage occurs when collagen and other structural proteins are synthesised, providing a scaffold to give the bone support. Finally, in the mineralisation stage, this scaffold is strengthened by adding calcium and phosphate to form mineralised bone matrix. Davey's team is testing the idea that androgens have different effects on osteoblasts at different stages of their cycle.

ARKOS

The researchers have carried out experiments with genetically modified mice that have had the androgen receptor (AR) removed from all tissues including osteoclasts and osteoblasts at every stage of their cell cycle. This is known as global Androgen Receptor Knockouts (ARKOs). It has shown that ARKO male mice have smaller bones than normal male mice due to loss of both the outer compact, or cortical, bone and inner sponge-like trabecular bone. Loss of bone was due to an increase in both

the breakdown and formation of bone, referred to as bone turnover, with the rate of breakdown exceeding the rate of formation. These results demonstrate that these actions of androgens on bone are mediated directly via the AR.

The molecular building actions of androgens make them a key candidate for understanding the emergence of bone fragility associated with ageing

The team moved on to study in more detail the effect of androgens at the different stages of the osteoblast development cycle. They generated two different mice models in which the AR was deleted in osteoblasts at the matrix synthesis and mineralisation stages of their development: osteoblast-specific ARKOs. Comparing the phenotypes of these different mice models has enabled them to determine the effects of androgens at these two stages. "We showed that androgens act on osteoblasts at both the matrix synthesis and mineralisation stages of their development to maintain the inner trabecular bone by regulating the breakdown of bone," Davey explains. "Androgens also act via the AR at the mineralisation stage to regulate the matrix mineralisation process." In contrast to their findings in the global ARKOs, the bone size of these osteoblast-specific ARKOs was not affected, suggesting that androgens act on osteoblasts at different stages of their development to exert different effects.



More recently, the research group has extended these studies to identify genes that are different in the mice lacking the AR specifically in the bone forming cells at the mineralisation stage of their cycle compared to normal mice. They have demonstrated differences in genes involved in the growth of the bone forming cells, bone breakdown, bone and skeletal growth and carbohydrate metabolism.

OSTEOBLASTS AND OSTEOCLASTS

The next stage of the team's research will determine the relative contribution of androgens acting via the AR to increase bone size and to maintain bone volume in the proliferation stage of the osteoblast cycle, as compared to the mineralising stage. To investigate this, they are putting the AR back into the osteoblasts of ARKO mice (which have no AR in any tissue) at the proliferation and mineralisation stages of their cycle, in order to compare and contrast the effect this has on bone.

They will also examine the effect of androgens on cells involved in bone breakdown, known as osteoclasts. Whilst research so far has shown that androgens indirectly inhibit osteoclastic bone breakdown through the AR in osteoblasts, their actions directly on osteoclasts are less well defined. Davey and her colleagues have demonstrated that osteoclasts express the AR, however, findings from cell culture studies are conflicting with reports of no effect or decreased osteoclast activity and bone breakdown following androgen treatment. They propose that

androgens act directly via the AR on osteoclasts to inhibit bone breakdown. To test this, they are generating genetically modified mice in which the AR is removed only in osteoclasts and the effect of this on bone will be examined.

IMPROVING THERAPIES

The knowledge gained from these investigations has potential for the development of improved therapies for the treatment of metabolic bone diseases, such as osteoporosis. "Understanding the precise mechanisms by which androgens exert their effects on bone may provide new avenues for therapies which target the specific actions of androgens via the AR, within specific bone cell types that lead to increased bone size and volume, thereby improving bone health," Davey elucidates. "This is particularly important given that the current forms of androgen therapy may have undesirable side effects in ageing men, whilst they cannot be used in women." It is also hoped that these results may assist in increasing public awareness of male osteoporosis and the importance of undertaking research in this area, and will encourage men to be more aware of their bone health.



DAVEY AND MEMBERS OF HER RESEARCH GROUP

INTELLIGENCE

THE MECHANISMS OF THE ANABOLIC ACTIONS OF ANDROGENS IN BONE

OBJECTIVES

The overall goal of the project is to have a better understanding of how the male sex hormones, androgens, act to control both the formation of new bone and the breakdown of old bone.

KEY COLLABORATORS

Professor Jeffrey Zajac, MBBS, PhD and **Dr Helen MacLean, PhD**, University of Melbourne

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FUNDING

National Health and Medical Research Council of Australia

Austin Health Medical Research Foundation
University of Melbourne

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DR RACHEL DAVEY completed her PhD in Physiology at The University of Adelaide in 1997, studying the effects of oestrogens and androgens on bone cell metabolism. She is currently Head of the Bone Biology lab in the Department of Medicine, Austin Health at the University of Melbourne. The focus of Dr Davey's research includes identifying the physiological role of calcitonin and the mechanisms by which androgens exert their actions on bone.



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