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## NEWS

# Conversations with pioneers in the bone field: Stavros C Manolagas

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When there are more things in heaven and earth than are dreamt of in the estrogen philosophy

*Editors' Note: This is the second in a new series of interviews with investigators who have made groundbreaking contributions to understanding bone health and disease. See the first interview, with T. John Martin, here* http://www.nature.com/bonekey/knowledgeenvironment/2013/130424/bonekey201373/full/bonekey201373.html.

Stavros C. Manolagas, MD, PhD, is The Thomas E. Andreoli, MD, MACP Clinical Scholar Chair in Internal Medicine at the University of Arkansas for Medical Sciences (UAMS) in Little Rock, Arkansas, USA. He is also a Distinguished Professor of Medicine; Director, Division of Endocrinology and Metabolism; Director, the UAMS/VA Center for Osteoporosis and Metabolic Bone Diseases; and Vice Chair for Research, in the Department of Internal Medicine at UAMS. He is also Chief, Endocrinology Section at Central Arkansas Veterans Healthcare System.

Professor Manolagas has spent a distinguished career studying the effects of steroid hormones on bone, making seminal contributions to understanding the cellular and molecular mechanisms by which estrogens, androgens and glucocorticoids influence the skeleton. In particular, his work has driven a paradigm shift toward thinking about osteoporosis as a disease of aging, rather than solely as a condition of estrogen deficiency. Professor Manolagas spoke recently with Neil Andrews, *BoneKEy* Features Editor, to discuss the genesis of his interest in steroid hormone action on bone, what drove his thinking away from an estrogen-centric view of osteoporosis and toward an age-related perspective on the disease, and what research questions he is pursuing now. An edited version of their conversation appears below.

*BoneKEy:* How did you become interested in studying the effects of steroid hormones on bone?

*Stavros Manolagas:* After my clinical training at the University of Athens, I was given an opportunity to do PhD work at the University of Manchester in England. My mentor then, David Anderson, had just returned from the United States, where he had learned methods for measuring multiple adrenal steroids simultaneously. David was collaborating with Robert Lindsay, who at the time was a Senior Registrar at The Royal Infirmary in Glasgow, Scotland. Robert had just reported, in studies using photon absorptiometry to measure bone density, that women who had their ovaries removed could be divided into two groups: those who lost bone quickly and those who lost bone slowly. The clinical part of my PhD thesis tested the hypothesis that adrenal steroids contributed to the variation in bone loss that follows loss of estrogens. We published that research from my PhD work in *Lancet*.[1]

For the basic research part of my thesis that would complement the clinical component, the plan was that I would study receptors for glucocorticosteroids and estrogens in bone.

David Feldman at Stanford University had just come out with evidence that glucocorticosteroid receptors were present in bone cells. I was able to detect glucocorticosteroid receptors in whole bone with different assays using rat calvaria,[2] but the estrogen receptors were quite a different story. At the time I was doing this research, Jack Martin, who was working at the University of Sheffield, had developed an osteosarcoma cell line, which he gave to me to look for estrogen receptors. To make a long story short, I managed to unbalance the ultracentrifuge required to prepare cytosol, almost destroying one of the most expensive pieces of equipment at the University of Manchester! The bottom line was that I was unable to find estrogen receptors, but having developed a method for detecting glucocorticosteroid receptors, I went on to look for 1,25 dihydroxyvitamin D receptors—the University of Manchester was the European capital of vitamin D research at the time—and found them.[3]

All of this research was the beginning of my interest in both the clinical and basic aspects of how steroid hormones work and how they affect the development of osteoporosis.

*BoneKEy:* Your early work also demonstrated that 1,25 dihydroxyvitamin D receptors were present on monocytes and T lymphocytes. How did you make that connection between those receptors and cells of the immune system?

*Stavros Manolagas:* After I finished my PhD thesis in England, I was recruited to the University of California, San Diego. Shortly after, Tatsuo Suda and his colleagues in Tokyo reported that 1,25 dihydroxyvitamin D could affect the differentiation of macrophages and monocytes. Because there were receptors in those cells for 1,25 dihydroxyvitamin D, and because we knew that there are glucocorticosteroid receptors in cells of the immune system and glucocorticosteroids have immunosuppressive properties, we went on to search for the presence of 1,25 dihydroxyvitamin D receptors in resting and activated lymphocytes. The big surprise was that the 1,25 dihydroxyvitamin D receptor was expressed upon activation of lymphocytes. That was a very remarkable observation—we wondered why an activated lymphocyte would suddenly express that receptor; in macrophages the receptor was expressed all along, independent of the state of activation. We published this work in *Science*,[4] and then several months later we discovered that 1,25 dihydroxyvitamin D had a suppressive effect on the proliferation of T cells by inhibiting interleukin-2.[5] As far as I know, that was the first real connection between the immune system and calcium-regulating hormones.

*BoneKEy:* How did your work on the estrogen receptor evolve?

*Stavros Manolagas*: The demonstration, by others, of the estrogen receptor in bone did not come until later, although we ourselves were trying all along. When I arrived at the University of California, San Diego, I spent my first year in the same office as Mark Haussler, who was the co-discoverer of the 1,25 dihydroxyvitamin D receptor. We were able to identify 1,25 dihydroxyvitamin D receptors in many other cell types, but we could not find estrogen receptors using the cell lines that were available at the time. In fact, it was very hard to demonstrate convincingly any biological effect of estrogen on bone cells *in vitro*, until many years later when we discovered that estrogens regulate bone cell apoptosis. In any case, after several years, Mark Haussler's group and Laurence Riggs's group simultaneously came out with papers in 1988 showing that there are estrogen receptors in osteoblasts, which suggested that the effects of estrogen on bone result from direct effects on bone cells.

We knew that loss of estrogen was associated with increased bone resorption, and considering our earlier work connecting calcium-regulating hormones to the immune system, we asked whether it was possible that loss of estrogens produced increased numbers of macrophages that would differentiate into osteoclasts or directly affected the differentiation of macrophages into osteoclasts. We found that following ovariectomy in mice the number of osteoclasts increased. Through a collaboration with Hal Broxmeyer, a brilliant hematologist at Indiana University School of Medicine, we were able to show that ovariectomy was causing an increase in the number of osteoclast precursors (colony-forming units for granulocytes and macrophages), which indeed resulted in increased osteoclastogenesis. We also showed that one could block the effect of estrogen loss on osteoclast progenitors using an antibody to interleukin-6.[6] We, of course, know now that receptor activator of nuclear factor kappa-B ligand is the key cytokine for osteoclast formation. Nonetheless, there is still considerable evidence that other cytokines may contribute to osteoclastogenesis.

*BoneKEy*: Your later research would identify bone cell apoptosis as a key mechanism by which estrogens, androgens and glucocorticoids have effects on bone. What led you down that path?

*Stavros Manolagas*: The impetus for the idea was Michael Parfitt's insights from enumerating osteoblasts in bone biopsy specimens from humans. He had calculated that the number of osteoblasts that are present in the basic multicellular unit on the bone surface could not be accounted for at the end of the remodeling cycle by becoming either bone lining cells or by being buried in the bone as osteocytes—two of the known fates of osteoblasts at that time. In other words, his calculations showed that most of the osteoblasts were 'missing' at the end of the remodeling cycle. At that time, Michael was a member of our research team in Little Rock, Arkansas.

We thought that maybe the osteoblasts were dying by apoptosis. We were able to test this theoretical concept, thanks to the technical capabilities we developed in our center to measure osteoblast and osteocyte apoptosis when Robert Weinstein joined our group. We found that apoptosis could account for the 'missing' osteoblasts of Michael Parfitt.[7] We went on to show potent effects of several hormones, including glucocorticoids, estrogens, androgens and parathyroid hormone on the apoptosis of osteoblasts and osteocytes, as well as effects of estrogens and glucocorticoids on osteoclast apoptosis.

Taken together with the findings that estrogens regulate osteoclastogenesis, the discoveries that steroid hormones control osteoblast and osteoclast apoptosis led us to the realization that bone-active hormones exert their effects primarily by controlling the birth and death of bone cells. Most investigators at the time had been thinking that hormones acted on differentiated cells to affect their activity or vigor. Our work revealed that bone-active hormones altered the cellular composition of bone.

*BoneKEy*: You mentioned the osteocyte, a cell type that has also been a focus of your research. Osteocytes now receive a lot of attention, but that was not always the case. Why?

*Stavros Manolagas*: Osteocytes certainly are the subject of intense study now—and deservedly so. I once mentioned, many years ago, during a lecture I gave at an ASBMR meeting,

that osteoblasts and osteoclasts are transient passersby, and someone from the audience came up to me after the lecture to ask what in the world I was talking about! But that is how the bone field was brought up: focus on osteoblasts and osteoclasts. Osteocytes, though, are the most numerous cells in bone, they are far more long-lived than osteoblasts and osteoclasts, and they are distributed throughout the skeleton, as opposed to osteoclasts and osteoblasts that occupy only a small fraction of the bone surface for only a short period of time. That realization made it obvious to us that osteocytes are indeed the permanent residents of bone, and inexorably, they must be critical factors in bone homeostasis.

The osteocyte is a truly remarkable cell buried within its own mineralized cocoon with multiple cellular processes traveling within tunnels, like buried cable, allowing it to communicate with the bone surface, the bone marrow and the circulation. But the fact that osteocytes are buried in the mineral was the reason why osteocytes were overlooked for so long—it was a big challenge to study cells buried deeply in bone. A lot of the credit for advancing the field in that direction goes to Gastone Marotti, Peter Nijweide and, more recently, Lynda Bonewald, who really pioneered osteocyte biology and convinced the rest of the field that the osteocyte is the cell that we really needed to learn more about. And we certainly do know a lot more now. Our current thinking is that osteocytes not only regulate the remodeling process during physiological bone maintenance, but later in life old or dysfunctional osteocytes are the culprits in the pathogenesis of age-related osteoporosis.

*BoneKEy*: Another area of your research focuses on the role of steroid hormones in redox homeostasis. How did you make the link between steroid hormones and that process?

*Stavros Manolagas*: For many years, I have been interested in the effects of aging *per se* on bone. I started this line of work soon after I moved to Arkansas, initially using a senescent-accelerated mouse model that had been developed by Japanese researchers to understand mechanisms. I was always intrigued by the relative contribution of sex steroid deficiency versus aging to the pathogenesis of osteoporosis. Around that time, Hui and coworkers in Indianapolis had made the striking epidemiological observation that fracture incidence increases dramatically with aging, irrespective of bone mineral density, suggesting that indeed aging *per se* was playing an important role.

With the effects of age on bone always a part of my thinking, I wondered whether osteoporosis was a disease caused by menopause in women, or whether it was a disease of old age that affected both men and women and was exaggerated by the loss of estrogens. For many years, the osteoporosis field was led by endocrinologists, including myself, who were all enamored of the idea that the estrogen deficiency at menopause caused osteoporosis and that replacing lost estrogen could prevent the disease. As we know now, estrogen deficiency contributes to osteoporosis, but it is just one of many mechanisms by which old age adversely affects bone. Our studies in mice, a model that does not experience menopause, showing that males and females have similar declines in bone mass and strength over a period of up to approximately 30 months of age, has clearly suggested that age-related mechanisms are very important.[8]

While we were conducting this work, a group in the United Kingdom led by Tim Chambers reported that ovariectomy increased oxidative stress in mice and that the effects of ovariectomy could be prevented by administering an antioxidant. From our own work, we already knew that in mice oxidative stress progressively increased with advancing age. Chambers' findings gave us a big jolt and the impetus to try to understand how estrogen affects oxidative stress, and therefore, how the menopause may contribute to bone aging. We now know that not only estrogens, but androgens, as well as glucocorticosteroids and parathyroid hormone, have potent effects on redox balance.

In fact, there is quite a bit more in the story of the effects of aging on bone, and it is not just oxidative stress that is important. I think there are other mitochondrial problems—from the latest work of Charles O'Brien in our group, autophagy seems to be a big player, as is endoplasmic reticulum stress. I strongly suspect that there will be other age-related mechanisms, which may or may not be accentuated by estrogen deficiency, that future research will show are also important.

*BoneKEy*: We have been looking back, but when you look to the present, and to the future, what research questions are you most interested in pursuing?

*Stavors Manolagas*: I am most interested in understanding why old age is responsible for so many different degenerative diseases, including osteoporosis. Are there common mechanisms of aging across the different degenerative diseases, and if there are common mechanisms, are there means by which we can affect more than one disease at the time?

*BoneKEy*: What are you working on now in that regard?

*Stavros Manolagas*: We are working with mouse models with cell-specific deletions of transcription factors, such as FoxOs, which play important roles in the defense against many different cellular stressors that accumulate with age. We are also using mouse models with cell-specific deletion of steroid hormone receptors to understand how age-related hormonal changes contribute to the aging process. Using these genetically modified mice, we are searching for new drug targets that can slow the effects of old age on bone cells—in particular, mesenchymal osteoblast progenitors and osteocytes. Some very interesting clues have already been provided from one of these models, namely a mouse with osteoclast-specific overexpression of catalase [the enzyme that converts hydrogen peroxide to water and oxygen] targeted to mitochondria. The original mouse with overexpression of mitochondria-targeted catalase in all cells was developed by Peter Rabinovitch, at the University of Washington in Seattle. His group has shown that in this mouse, several pathologies associated with old age, including insulin resistance, cardiomyopathy and some features of Alzheimer's disease, are prevented. Strikingly, we have now found that in mice with mitochondria-targeted catalase only in osteoclasts, the number of osteoclasts in bone is decreased and bone mass is increased. Even more important, these mice are protected from the loss of bone caused by loss of estrogens.

All of this work provides big new insights that there are indeed common age-related mechanisms affecting more than one system at a time. The issue is how to target those mechanisms without adversely affecting the whole organism. It is clear that systemic administration of antioxidants is not the answer in humans, and that there are likely multiple mechanisms involved in the aging process. The solution will not be simple, but the payoff of targeting age-related disease mechanisms can be huge.

*BoneKEy*: Are you optimistic that we will one day be able to intervene effectively?

*Stavors Manolagas*: Absolutely. It is so obvious that aging is a major contributor to disease, and people in many different fields, including neurodegenerative diseases, energy metabolism, muscle metabolism and bone metabolism, are simultaneously converging on compromised autophagy, stem cell exhaustion, protein misfolding, mitochondrial dysfunction, genomic instability, telomere shortening and cellular senescence as mechanisms of aging and age-associated diseases.[9] It is a very exciting area.

*BoneKEy:* Thank you so much for talking to *BoneKEy* about your research—past, present and future—in the bone field.

*Stavros Manolagas*: Thank you for the opportunity.

## Conflict of Interest

The author declares no conflict of interest.

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