VICTOR MONTORI: Good afternoon. Welcome to CCaTS Grand Rounds. I'm Victor Montori, one of the [INAUDIBLE] of CCaTS, and excited today about our presenter. It's a pleasure to introduce Dr. Joshua Farr. Dr. Farr completed his undergraduate and graduate training in physiology at University of Arizona, completing his PhD in physiology there in 2011. For the next three years, he was one of our T32 endocrine training grant postdoctoral fellows. And he did that in Dr. Khosla's lab. I asked Dr. Khosla, how did he do? And Dr. Khosla said that clearly for him, Josh is one of the best, if not the best, postdoc he has ever had a opportunity to work with. So this is high praise.

During this time, he completed several significant projects leading to five oral presentations at the annual meeting of the American Society for Bone and Mineral Research, receiving a Young Investigator Award from this organization in 2012 for his stunning work. He's offered 30 manuscripts, which are different states of publication. And in his brief career, Dr. Farr has been able to become an assistant professor of medicine and a research associate now here in the Division of endocrinology, the number one division of the chronology in the country.

Just recently received a Robert and Arlene Kogod Center on Aging, a Career Development Award; to continue his work, work that we're going to hear about today; focus on the effects of physiologic and pathologic processes in bone quality, bone strength, and fracture risk. And by applying novel methods he has helped develop, Dr. Farr hopes find the relationships between these processes and the skeleton and organ systems. It'll be important also to recognize-- that begins to connect the outcomes at the level of bone and skeletal tissue with other diseases that are affecting populations, such as type 2 diabetes. With all this, please join me in welcoming Josh as he presents changes in bone quality with growth, aging, and diabetes.

JOSHUA FARR: OK. Thank you very much for the introduction. It's an honor to speak at CCaTS Grand Rounds. I've been asked to read this disclosure. Active Life Scientific provided the microindentation instrument and probes for one of the studies I will talk about, but had no control over the outcomes or content of this presentation. So today's learning objectives can be broken down into three different sections. The first two focus on bone structure changes during two critical periods during life for skeletal health-- both growth and with aging. And the third focuses on bone material property changes in patients with type 2 diabetes.

So why are we interested in bone structure changes during growth? Well, first, the structure of the bone is the most important determinant of its strength and thus, its resistance to fracture. And as it turns out, fractures are quite common in children. In fact, they are a leading cause of hospital admission following injury in both children and adolescents. These are some classical data showing the annual incidence of all fractures in children in Malmo, Sweden. As you can see, fracture incidence increases during growth in both girls and boys. It peaks during puberty. In this particular study, fractures are more common in boys than girls. About 42% of boys and 27% percent of girls suffer at least one fracture prior to the age of 16. The distal forearm fracture is by far the most common-- the incidence of which peaks around age 11 in girls and age 14 in boys.

Now these data are consistent with data elsewhere around the world. I'm just showing a study in Canada. Again, showing this peak in the incidence of distal forearm fractures in girls around age 11 and in boys, around each 14. And if you look at this incidence of fracture risk, it's really quite high. In fact, a 14-year-old boy-- that fracture risk is not exceeded in the female lifespan until after age 53, after which estrogen efficiency results in market increase in fracture risk.

Now these data have also been reported using Olmsted County residents by Sundeep Khosla and colleagues here at Mayo. This is a high profile paper published in Jama, which actually showed over the course of three decades that there was an overall increase in distal forearm fracture incidence in boys of 32% and 56% increase over that span in girls, which suggests that these fractures are perhaps becoming more and more common.

So just to summarize thus far, approximately one in three otherwise healthy children will suffer a fracture during childhood. Distal forearm fractures are most common, and their incidence peaks during the early adolescent growth spurt. Further, there has been a dramatic increase in the incidence of distal forearm fractures in both children and adolescence. In data that I didn't show, changes in lifestyle factors, such as recreational activities or diet, only account for part of the increased risk of the incidence of distal forearm fractures. Therefore, given the large number of childhood fractures, there is an urgent need to define underlying causes.

So this prompted our group and others around the world to ask the question, what are the normal skeletal changes that occur during growth? And a number of studies have used dual energy x-ray absorptiometry or peripheral quantitative computer tomography to assess changes in bone mass, density, and structure during puberty. However, these techniques have a number of limitations when it comes to assessing bone mass and density as well as structure in pediatric subjects.

For example, DXA only provides measures of bone mass and an aerial bone mineral density, which is confounded by the thickness of the bone. So as the children grow, obviously, they get thicker bones. And therefore, aerial bone mineral density is overestimated in larger children and underestimated in smaller children. In addition, DXA does not have the ability to measure the structure of a bone-- which as I mentioned earlier, is one of the most important determinants of its strength and thus, its resistance to fracture.

So for this reason, a number of studies in kids have moved on to using standard pQCT. It has a resolution of about 400 microns, so it can measure estimates of volumetric BMD. It can also measure estimates of the structure of the bone at certain skeletal sites. However, at metaphysical sites, such as the distal forearm, with this resolution, it does not have the ability to distinguish between trabecular and cortical bone compartments, which limits our ability to understand what is happening at these sites.

We therefore move to using high resolution peripheral quantitative computed tomography, using the extreme CT scanner shown here. This is the Xtreme CT 1. It has a voxel size of about 82 microns. And CRU are you has recently received the Xtreme CT 2, which has a voxel size of 61 microns, so much better resolution. And with this resolution, it can define both trabecular and cortical bone microstructure at the distal radius and tibia. And these measures have been shown to correlate very strongly with both trabecular and cortical parameters using ex vivo micro-CT.

In addition, the technique from the images, you can construct micro-finite element models of bone strength. You can think of these as virtual simulations of crushing the bone, so it's a three dimensional stack of high resolution images. And it's like a computer modeling system that actually places force on the bone until it fails, and that is highly related to the bone strength ex vivo.

So these are just representative cross sections on the right of the most proximal and distal HRpQCT slices. This is a radiograph showing the ulna. And the radius and the region in red denotes the series of 110 high resolution CT slices extending from the most distal slice, shown here, to the most proximal slice, which is shown here. And as you can see from these cross-sections, we can obtain measures of the trabecular bone parameters, including-- that's the parameter shown in gray, sometimes referred to as the spongy bone. So for example, the bone volume fraction, the number of trabeculae, the thickness, and also their separation.

We can also measure the cortex, so that's shown in white. And this is the compact, more biomechanically relevant portion of the bone. We can measure the area, the thickness of the cortex even at these metaphyseal sites as well as the outer surface, which is denoted by this green thresholding line. It's sometimes referred to as the periosteal circumference as well as the end of cortical circumference, which separates the cortical from trabecular regions.

So the first study to use HRpQCT in children was performed here at Mayo Clinic. It was a very important study in the field. It included 140 healthy boys and girls between the ages of 6 and 21 years. And it should be noted that none of these children had a history of fractures. So the goal of this study was, really, to determine normal skeletal development throughout maturation. So the subjects were divided into five maturation groups based on their bone age using the Tanner White-House 3 method, which is still considered the gold standard for assessing maturation in kids. Group one was the prepubertal group; group two, the early; group three, the mid; group four, the late; and group five, the postpubertal.

So these are the results at the distal radius based on the HRpQCT parameters. And if you look at trabacular parameters throughout maturation, there really isn't a whole lot of, that's changing. However, in the boys, there's a significant increase in bone volume fraction during late puberty associated with an increase in trabacular number and thickness. And it can come in a decrease in the spacing of the bone, so-called better trabecular microstructures associated with lower spacing. In the girls, however, there were no significant changes throughout puberty.

What was really interesting was that when this cortical compartment was looked at-- this previously inaccessible compartment that we could only hypothesize what was changing. And what we see is that during mid puberty in both boys and girls, there is a significant deficit in cortical thickness that is mirrored by a significant deficit in the volumetric BMT of the cortex-- again, in both boys and girls.

By contrast, the outer circumference of the bone increases throughout maturation more so in boys later in puberty as compared to girls. And similarly, the endocortical circumference mirrors that increase in bone size. In terms of the strength of the bone, that also increases throughout maturation in both boys and girls mirroring the increases in bone size. These are based on the micro-finite element models.

The fall force is a calculated variable based on an individual's height and weight. And it's basically an estimate of the load which would be displaced on the forearm during a fall. That also increases throughout maturation in both sexes. The ratio of the fall force to strength, which is sometimes referred to as the factor of risk, decreases significantly throughout puberty in both boys and girls.

However, again, what's really interesting is that with this cortical thinning, there is a decrease in the load carried by cortical bone during mid puberty in girls and mid to late puberty in boys. And this is mirrored by a decrease in the ratio of the cortical trabecular bone volume fraction. And if you look at representative 3D reconstructions of the HRpQCT images, you can visualize this cortical thinning, again, in both girls and boys during mid puberty.

And if you look closely, there are pores that are beginning to appear in the cortex during this period, and that can be seen graphically here. Cortical porosity index increases during mid puberty in girls and late puberty in boys. So what's thought to be happening is that during this very rapid phase of longitudinal bone growth, the mineralization of the bone is lagging behind, which is resulting in this transient weakening of the bone, leaving it susceptible to fracture.

So just to summarize this study. This is the first study to use HRpQCT and micro-finite element analysis to describe changes in bone microarchitecture and strength in healthy children during puberty. While total bone strength increases in both sexes during puberty, the proportion of load borne by cortical bone-- in other words, the relative strength of this compartment-- and the cortical trabecular bone ratio was lowest during mid puberty during girls and mid to late puberty in boys. And this was accompanied by an increase in the porosity of cortical bone compartment. Importantly, these changes in cortical bone mirror the incidence in adolescence distal forearm fractures from similar populations in Rochester and elsewhere around the world that I showed you earlier. So the obvious question then becomes, do children with distal forearm fractures have even more severe skeletal deficits as compared to their non-fracture control peers?

So this study was also performed at Mayo Clinic. It included 223 healthy boys and girls between the ages of 8 and 15 years, 115 of which had sustained a recent distal forearm fracture along with 108 non-fracture controls. Exclusion criteria included diseases or drugs which could affect bone metabolism. And we also excluded cases with the distal forearm due to severe trauma because we hypothesized that the severe trauma would cause the fracture regardless of the underlying strength of the bone. And we specifically measured bone parameters in the non-fractured radius and excluded those with bilateral distal forearm fractures. Because again, casting or immobilization can cause reductions in bone strength.

So we use Landin's modified criteria to assess trauma levels, again, excluding the severe trauma fractures and then stratifying the remaining cases based on mild versus moderate trauma. These are the results. The non-facture controls are shown in orange, the mild trauma distal forearm fracture cases are shown in yellow, and the moderate trauma distal forearm fracture cases are shown in green. As you can see, both boys and girls had significant deficits in cortical area and cortical thickness as compared to sex-matched, non-facture controls, whereas the moderate trauma fracture cases showed no differences in cortical bone parameters as compared to their non-fracture peers.

Consistent with those decreases in cortical bone deficits, there was also a significant decrease in the failure load or bone strength in the mild trauma distal forearm fractures as compared to non-fracture controls in both boys and girls, whereas no differences were observed between the moderate trauma distal forearm fractures for both boys and girls. By contrast, the load to strength ratio was significantly higher in the mild trauma forearm fracture cases-- higher levels representing worse values. And interestingly, the boys with moderate trauma distal forearm fractures actually had lower, or better, factors of risk. There were also significant deficits in trabecular bone parameters in the boys with mild trauma distal forearm fractures. They had significantly lower bone volume fraction, trabecular number, and significantly higher trabecular separation, whereas the girls had significant trabecular thinning.

So just to conclude this study, children and adolescence with the distal form fracture due to mild, but not moderate, trauma have significant cortical thinning, deficits in trabecular microstructure, and suboptimal biomechanical strength as compared to non-fracture controls. Therefore, we infer from these findings that distal forearm fractures during growth have two distinct etiologies-- those due to underlying skeletal deficits leading to fractures with mild trauma versus those due to more significant trauma in the setting of normal bone strength.

So if you think about it, these the subjects who are suffering fractures due to moderate trauma, that's just based on the fact that there is this normal cortical thinning that's occurring during mid to late puberty. And that's just a result of that normal trauma, and they don't necessarily have poor bone strength. However, there's a subset of patients who suffer mild trauma fractures, and these are the ones that we may perhaps need to closely monitor for skeletal deficits later in life as they tend to have skeletal deficits during growth.

So that was the next question. Do the deficits persist later in life? And this study, again, was performed at Mayo. We recruited 75 women and 75 men between the ages of 20 and 40 years. These were all Olmsted County residents who had suffered a childhood distal forearm fracture and remained in the community, and we followed up with them. We also recruited 150 agents sex-matched, non-fracture controls also from on Olmsted County with no fracture history. We used the identical protocols used for the kids' study.

And what is really quite remarkable is that the results virtually mirrored those that we saw in the kids. So both the men and women who had a childhood distal form fracture, still in adulthood, had deficits in bone strength whereas the moderate trauma fracture cases showed no differences, suggesting that these minor trauma cases, although they had a bone fracture, they developed normal bone strength into adulthood.

So that led us to the next question. Does a childhood fracture predict an increased risk of fracture later in life? And this work was done by Shreyasee Amin and Joel Melton here at Mayo using the Rochester Epidemiology Project. This is a difficult question to answer because it's very difficult from records to decipher between mild and moderate trauma. And I'll come back to that. But basically they identified a population-based cohort of over 1,700 children from Olmsted County who had suffered a distal forearm fracture between 1935 and 1992.

In these patients, they identified incident fractures occurring after age 50 using the REP. These were observed nonpathological fractures resulting from no more than moderate trauma. So they are considered fragility fractures. Severe trauma fractures were excluded. But, again, because of the relative lack of details from clinical records it just simply was not possible to decipher between the mild and moderate trauma. And these fracture rates were compared with the expected numbers estimated from fracture incidence rates based on age, sex, and calendar year from Omsted county.

So these are the results. As you can see quite clearly, older men who had a childhood distal forearm fractured had a significantly higher risk for any moderate trauma fracture later in life, any fracture at a osteoporotic site, and any nonosteoporotic fracture. By contrast, the women who had a childhood distal forearm fracture had no elevated risk whatsoever for any of these fracture types. So what it suggests is that a childhood distal forearm fracture in boys does predict an increased fracture risk later in life in men.

However, there was a lack of an effect in women. And it's possible that any lack of tracking from childhood could have simply been overwhelmed by the menopause, which is a very significant insult on the skeleton. And therefore, it may have masked any tracking that could have occurred from childhood. Now it's also possible that the effect is weaker or that we may have missed it due to the lack of distinction between mild versus moderate trauma. So I think more work is needed here, but it certainly suggests that these traits could be tracking into later in life. So that concludes bone structure changes during growth.

Move onto bone structure changes with aging. So there's a lot of data out there for many years that the bone quality deteriorates independent of aging. So previous studies have shown using DXA that the chronological age itself of an individual is a major predictor for fragility fracture regardless or independent of an individual's areal BMD. So for example, if you take a 7-year-old woman and a 30-year-old woman who have the exact same areal BMD, the 7-year-old woman has a greater than tenfold increased risk for fertility fracture as compared to the 30-year-old. And that can be seen here in this classical study published in JCI. For a given radius areal bone marrow density, fracture risk increases dramatically with age, even though areal BMD by DXA is the same.

So has led to the hypothesis that age-related changes in bone quality that are not being captured by DXA are contributing to this increase fracture risk. And some of hypothesized trabecular thinning or loss of connectivity. Others cortical thinning or increased cortical porosity like we saw in kids, or altered bone material properties, which all come back to later. So because we had access to the HRpQCT and a large cohort that Larry Riggs characterized of Rochester, Minnesota residents-- 44 women who are younger-- mean age, 41 years-- were matched with 44 women who are older-- mean age, 63 years-- based on their ultradistal radius areal BMD score. Similarly, 57 men who are younger were matched with 57 older men. All of the measurements for DXA were performed using a Lunar Prodigy scanner. And we use NHRP QCD to test this question as to whether there is an aerial BMD independent effect.

So just to orient you, the young subjects are shown in orange. The older subjects are shown in green. The women are shown on the left panel, the men on the right. And given the fact that we matched subjects, older and younger, for aerial BMD, there were no differences whatsoever between the women and men. And interestingly, none of the trabecular parameters were significantly different with aging, suggesting that the trabecular parameters don't explain this areal B independent effect.

Now when we looked at cortical volumetric BMD, older women had lower cortical volumetric BMD as compared to younger women, although there was a modest decrease. No effect was seen in men. Neither was there effect in cortical thickness in either women or men, nor in the outer circumference or in cortical circumference of the bone. The one parameter that came out as a very important distinguisher between the older and younger subjects was cortical porosity. Here you see very high levels of cortical porosity in the older as compared to younger women, and older as compared to younger men. And other measures of porosity, including the poor volume, the diameter, the heterogeneity of the pores, were also elevated with aging.

So this can be seen using representative cross-sectional slices of HRpQCT scans. This is a 33-year-old female which is matched by areal BMT with a 77-year-old female. And you can see in the cortex in the 77-year-old that there are these pores that are starting to appear that are perhaps significantly contributing to fracture risk. Again, this is a 36-year-old male which is matched with a 79-year-old male. They have the exact same areal BMD and very similar trabecular bone parameters. However, again, these pores are appearing in the cortex that we hypothesize are contributing to fracture risk.

Now since the publication of that paper, Rogers Zebaze and colleagues at the University of Melbourne have developed a novel approach to assessing cortical porosity. And in collaboration with that group, cortical porosity and areal BMT were measured in 68 postmenopausal women with distal forearm fracture and 70 age-matched community controls from Olmsted County residents. Interestingly, in women who had osteoporosis-- so this is an altered distal radius areal BMD t-score as defined by less than minus 2.5, WHO criteria-- adding a measure of the porosity to the areal BMD actually did not identify more women with fractures than just having aerial BMT alone. So what it suggests is that when areal BMD is reduced into this range, porosity isn't adding anything.

However, in the patients with osteopenia, areal BMD alone-- this is areal BMD t-score between minus 1 and minus 2.5-- areal BMD alone did very poorly at predicting fracture risk. However, there is a subset of patients with osteopenia who also have high cortical porosity. So when you add porosity in with areal BMD, there's an independent effect that significantly increases fracture risks. The odds ratio of for four.

So what this suggests is that in the patients with osteopenia, there are these pores, again, that are appearing in the outer compact up here in cortex, and the inner and transitional zone shown in red, which are significantly contributing to fracture risk, yet not being picked up by DXA. And in the Keeps trial, this is a four year study of recently postmenopausal women. We showed that a low dose estrogen treatment actually significantly attenuated an increase in cortical porosity. So in the placebo group, over four years, there was a 30% increase in porosity that was significantly attenuated with low dose estrogen therapy.

So just to summarize, aging is associated with increased cortical porosity, which is not captured by DXA. If areal BMD DXA is reduced into the osteoporotic range however, adding a measure of porosity does not improve fracture prediction. However, in the much larger group of patients with osteopenia, which also is the group which suffers about threefold more fractures than those with osteoporosis. This group remains the most ambiguous when it comes to treatment decisions. Areal BMD alone is a poor predictor of fracture risk. However, including cortical porosity may significantly improve fractures prediction. So we're in the process of applying for funding to look at these analyzes in larger cohorts of both women and men.

So finally, I'll end up today talking about bone material property changes in patients with diabetes. So these are data from the Women's Health Initiative, a very large study of postmenopausal women. And from this study, you can see quite clearly that fracture risk is significantly higher at skeletal sites, such as the hip, proximal humerus, foot, ankle, and spine. And these data are consistent with data from Joe Melton and colleagues here at Mayo, again, showing that fracture risk is higher in patients with type 2 diabetes. Now this presents a little bit of a paradox because clinically, these patients present with higher areal BMD measures at least before adjusting for BMI.

And Schwartz and colleagues used a very large cohort of patients around the US at different centers and actually showed that using the FRAX algorithm, the 10 year hip fracture risk for women with diabetes and men with diabetes was significantly underestimated for a given femoral BMD T-score. So here, if we just look at a particular BMD T-score, I've chosen minus 2.54. For a given areal BMD, fracture risk, according to FRAX, is significantly underestimated in patients with type 2 diabetes as compared to non-diabetic subjects, suggesting that other factors are responsible for this increased fracture risk.

So we hypothesize that type 2 diabetes results in altered or compromised bone quality, in other words, altered bone material properties or altered bone microarchitecture, rather than reduced bone marrow density. So the study aim was to determine whether bone material strength in index of bone material properties, bone imaging parameters derived from DXA or HRpQCT, and/or bone turnover are altered in patients with type 2 diabetes as compared to age-matched, non-diabetic controls.

We also examined the association of this index of bone material properties with the duration of type 2 diabetes and circulating levels of glycated hemoglobin. The study subjects included 60 normal postmenopausal women, 30 patients diagnosed with type 2 diabetes for greater than 10 years, and 30 age-matched, non-diabetic controls. All of the women were postmenopausal. We excluded subjects if they had low stores of vitamin D. They were all vitamin D sufficient. And we also excluded subjects with any fractures within the previous six months, as fracture has been shown to elevate bone turnover within this time period. So we also excluded any subjects with any conditions that could alter skeletal structure function as well as those with a history of traumatic or pathological fractures, or those on any medications that could affect bone metabolism.

So historically, it has not been possible to assess bone material properties in humans in vivo because of the relative invasive nature of the measures required to assess these parameters. However, there have been some recent technological advances which allow for a very minimally invasive assessment of bone material properties using the OsteoProbe shown here, which is a handheld microindentation instrument which is designed for in vivo measures of the bone material strength index.

Now the measurements are performed at the non-dominant anterior tibia. The site is injected with lidocaine to numb the region, and that basically is the worst part of this procedure. There a little bit of a bite while the lidocaine is administered. However, during the procedure when you insert the disposable probe into the skin and through the soft tissue lying on the periosteum, the subject basically doesn't feel anything whatsoever.

So the measurement is actuated by slowly depressing the outer housing unit of the device, which initiates an internal impact within the spring, which causes the probe to drive into the bone. And this is a single impact. It's not a repetitive loading. It's just a single impact. We typically perform between 5 and 10 measurements. The indentations are very small. They average on less than 200 microns, and this technology has been well validated in both previous prototypes in humans and in animal studies. And it's been shown to be a very robust measure of material properties.

So our study was the first to assess bone material properties in patients with type 2 diabetes. The average duration of diabetes was 17 years. Given age-matching there were no differences whatsoever in age, no differences in height. However, the patients with type 2 diabetes were significantly heavier and had higher BMI as compared to the non-diabetic controls. In terms of the serum biochemistry, as expected, the hemoglobin A1c levels at screen visit were significantly higher in the patients with type 2 diabetes as compared to the non-diabetic controls.

We also assessed an index of chronic glycemic control. From clinical records, we measured the 10 year average hemoglobin A1c level. And I'll come back to that. We're also interested in bone turnover, so we measured a measure of bone formation, P1NP, and also a measure of bone resorption, CTX, which were both significantly lower in patients with type 2 diabetes as compared to non-diabetic controls. By contrast, there were no differences in vitamin D levels.

So this was the primary outcome of the study. The bone material strength index, which was about 12% lower in patients with type 2 diabetes as compared to non-diabetic controls in unadjusted analyzes, and 11% lower in patients with type 2 diabetes as compared to non-diabetic subjects after adjusting for BMI. And these differences were highly significant. They remain significant following adjustments for hypertension and other diabetic complications. Now when we looked at the areal BMD measurements by DXA, in unadjusted analyzes not shown here, the patients with type 2 diabetes had significantly higher regional areal BMT. However, when you adjust for BMI, those differences go away.

In terms of the HRpQCT parameters, there were no differences in any of the parameters between the patients with type 2 diabetes and non-diabetic controls. However, consistent with a small study by a group at the University of San Francisco, they showed that higher cortical porosity was observed in patients with type 2 diabetes. And we at least saw a trend in that direction. However, we may have been underpowered to detect that difference.

So finally, we looked at an index-- we looked at correlations between the index of bone material properties and things like duration of diabetes and chronic glycemic control. While the relation between BMSI and the duration of type 2 diabetes was not significant, interestingly, the 10 year average glycated hemoglobin-- there was a very strong negative correlation between BMSI and the ten year A1c, suggesting that subjects with worse glycemic control also had worse bone material properties.

So just to conclude this study, these findings represent the first demonstration using a direct in vivo measure of bone material strength, of compromised bone material properties in patients with type 2 diabetes. These results confirm previous studies demonstrating that patients with type 2 diabetes have low bone turnover. And they also highlight the potential detrimental effects of prolonged hyperglycemia on bone quality. So thus, we infer from these findings that the skeleton needs to be recognized as another important target tissue subject to diabetic complications.

So our working model for the pathogenesis of skeletal fragility in patients with type 2 diabetes is that with diabetes, poor glucose control leads to an elevation of advanced glycation end products. And I didn't have a chance to talk about these, but these are intermediate protein products that have been shown to be elevated in diabetes that have negative effects on osteoblasts in vitro. And so, really, what's hypothesized is that this defect in bone formation results in low bone turnover due to coupling between bone formation and bone resorption.

So that, overall, low status of bone turnover increases the lifespan of type 1 collagen in bone. And normally, we want our college to be remodeled so that the micro damage can be repaired. And in this case, it's not, where as it particularly the leaves that type 1 collagen that's not being remodeled to increase damage from advanced glycation end producted. So this creates a positive feedback loop, and essentially a vicious cycle that we hypothesize results in reduced bone material strength and increased fracture risk in these patients.

So with that, that concludes today's learning objectives. I'd like to think my mentor, Sundeep Khosla, in particular, who is the PI and the real driving force for many of these studies. I also like to think my male collaborator, Shreyasee Amin, Joe Melton, Matthew Drake, Salman Kirmani; our external collaborators at the University of Melbourne as well as our study coordinators, Louise McCready and Amanda Tweed, with help with data collection; and subject recruitment; our statisticians, Beth Atkinson, Sara Achenbach, and Terry Themeau; and Active Life Scientific. I'd also like to thank research support from the National Institute on Aging, NIAMS, and the Robert & Arleen Kogod on and Aging as well as Mayo CCaTS. Thank you.