Salvatore Minisola: Good afternoon, my name is Salvatore Minisola. It is my pleasure to co-chair this terrific session with Dolores Shoback. Before starting the session I have two important announcements to make. The first one concerns the use of audio and videotaping, which is I’m sure strictly prohibited.

The second announcement regards the fact that all attendees will receive an e-mail immediately following the meeting inviting them to complete an online meeting evaluation. We encourage you to take a few minutes to share your feedback. The first speaker is Juliet Compston.

I think that she is very known to all of us and she is a Professor of Bone Medicine at the University of Cambridge School of Medicine in UK. So I invite her to deliver a speech that concerns Oversuppression with Bisphosphonates: Is It Real?

Juliet E. Compston: Thank you very much and good afternoon ladies and gentleman. For those of you who want to leave early, I will tell you now I don’t know the answer to the question posed by the title of my talk. But what I would like to do in the next 25 minutes or so is spend some time discussing what we actually mean by bone turnover, how we can measure it and what the different approaches to measurement actually mean? I will then look at the...what evidence there is that there are harmful effects of long-term suppression of bone turnover focusing predominantly on human studies and finally look at some of the potential mechanisms for these effects, should they exist. So, first of all, what is bone turnover? Well, bone turnover describes the amount of bone, which is moved and replaced within a given volume of bone tissue in a given time.
And as such, it is a composite of the remodeling rate, that's a number of remodeling units on the bone surface at any time point and the remodeling balance, that is the amount of bone resorbed and formed within individual bone remodeling units. Now, bone turnover can be assessed by histomorphometric examination of bone biopsies using double tetracycline labeling, and the indices most commonly used are the bone formation rate and the activation frequency. The bone formation rate is a true measure of bone turnover. It encapsulates both the number of remodeling units and the focal balance within each of those units.

It is, however, based on the critical assumptions that resorption is always followed by bone formation and that bone remodeling is in a steady state. Activation frequency is conceptually different. It's defined as the probability that a new remodeling unit will be initiated at any point on the bone surface and hence it represents remodeling rate, but not bone turnover. Now activation frequency can be derived by calculation from the bone formation rate and the wall width, which is the amount of bone formed within individual remodeling units. And this mathematical equation assumes a functional dependence of remodeling rate on focal balance, which in fact is not seen in the adult human skeleton and I don't have time to go into this in more detail at the moment, but I shall be presenting it in more detail at the ISBM meeting, which follows this ASBMR meeting.

But the bottom line is that when you use activation frequencies as calculated from bone formation rate and wall width, you may overestimate or underestimate remodeling rate when changes in remodeling rate and focal balance differ in terms either of their direction or magnitude.

And a prime example of that is during ageing when remodeling rate increases, but wall width decreases. The other approach to assessing bone turnover is of course using biochemical markers and the main ones that we used are showed on this slide. Main formation markers are bone-specific alkaline phosphatase, osteocalcin, and procollagen type-1 N-propetide and a bone resorption, the collagen type 1 telopeptide, CTx and NTx, deoxypyridinoline, and tartrate-resistant acid phosphatase type 5b. Now these biochemical markers reflect whole body bone turnover, both cortical and cancellous bone and they mainly reflect the remodeling rate.

Under most circumstances, they are not able to detect changes in focal remodeling balance although they may do so in certain situations where there are large changes in focal balance, for example, during growth and also in response to anabolic agents.

In contrast histomorphometric assessment of bone turnover is obviously limited to the site of biopsy, which for all practical purposes is the iliac crest. And generally measurements are made only in cancellous bone, although, of course, they can also be made in cortical bone.
But a big advantage of histomorphometry is that the remodeling rates and the focal balance can be separately assessed and so their relative contributions to bone loss or bone gain can be determined. So if we compare these two approaches, biochemical markers and histomorphometry, first of all in terms of the skeletal site, biochemical markers look at whole body bone turnover, remember 80% skeleton is cortical bone, whereas histomorphometry is usually confined to the iliac crest. Both approaches are associated with really quite a substantial variance.

This is probably more marked in the case of histomorphometry and its worth pointing out that even in a normal bone biopsy from a healthy person, it may be necessary to look through many sections before seeing any double tetracycline labeling. Biochemical markers, we know are affected by the presence of a fracture, so they are elevated when fractures occurred and this probably is not the case for histomorphometry. And finally, biochemical markers cannot usually distinguish between changes in remodeling rate and focal balance whereas histomorphometry can.

So although biochemical markers and histomorphometry have undoubtedly been very valuable in assessing bone turnover, they clearly have some limitations and these are emphasized by the data shown on this slide, which shows the variations in bone turnover at different skeletal sites in a woman with osteoporosis.

This was a woman with postmenopausal osteoporosis, who underwent a bone biopsy and died suddenly 11 days later, I believe for reasons unrelated to the bone biopsy, and as you can see, this was 1987. So the investigators were able to remove bits of bone from all over her skeleton and assess bone formation rate.

What you can see is that there is a substantial variation in bone formation rate, both within and also between these different skeletal sites. Now some of this may, of course, be attributable to measurement variance, which is unlikely to be the whole answer.

And this really emphasizes the need we have for methods, which can assess regional bone turnover at the sites of clinical significance, particularly of course, the spine and the proximal femur. And in this respect, the method of fluoride PET scanning, which is being developed, at Guy’s Hospital by Mr. Fogleman (ph) and his colleagues holds great promise and it appears to reflect both osteoblast number and also osteoblast activity. Now given the differences between biochemical and histomorphometric methods for measuring bone turnover, we shouldn’t be surprised that they generate different figures when we look, for example, at the degree of suppression of bone turnover. And this slide shows data from one study in which biochemical markers and bone histomorphometry was performed at fairly similar time points in women treated either with alendronate or conjugated estrogens or a combination of the two.
And here, you can see that the bone turnover was assessed histomorphometrically using the mineralizing surface and biochemically using bone-specific alkaline phosphatase and urinary NTx. And if we just look in the combination group, you can see that histomorphometry indicates almost total suppression of bone turnover, but when we look at the biochemical markers these indicate something in the region of 60 to 70% bone turnover, and this is a pattern that in general is reflected in other studies, namely that histomorphometry tends to indicate greater degrees of suppression of bone turnover than do biochemical markers.

Now the implication in the term oversuppression of bone turnover is that it's harmful and of course in clinical terms, this means that it increases bone fragility and hence increases fracture risk. Most phase III studies with the bisphosphonates have basically been designed to last for three years and so robust fracture data for longer time periods are not available. But this slide shows, for risedronate the date of a vertebral fracture is in two two-year extension studies. Here, you have the main phase III study showing significant reduction in vertebral fractures.

In years four and five, the randomization continued between the risedronate featured in placebo group, and then in years six to seven all the women received risedronate 5 mg daily. And you can see that there is quite a significant attrition rate of women in the study over the seven years and also of course there is no placebo group in the years six to seven. I think all one can say from these data is that they certainly do not suggest that there is any increase in fracture rate given all the limitations of this study and that the anti-fracture efficacy appears to be maintained for at least up to five years.

Now similar limitations apply also to the long-term data for alendronate, because in this 10-year study, there was again significant attrition rate of the women in the study from 994 at year one to 247 at year eight. There is no true placebo in years 6 to 10. There were some changes in the study designed during the 10 years and some significant imbalances in baseline characteristics in the women, studied during years 6 to 10. What this slide shows are data for morphometric fractures and for non-vertebral fractures, again you have years one to three showing significant reduction in vertebral fracture and a reduction in non-vertebral fracture, and then in years 6 to 10 we have the group who discontinued alendronate after five years, those who continued to take alendronate in a dose of 5 mg and 10 mg.

If we look at the morphometric fractures, it looks as if there is an increase possibly in those women taking 5 mg daily. But in fact these women had a much higher prevalence of baseline vertebral fracture than the other group, so I think it’s very difficult to interpret these data.
And if we look at non-vertebral fractures, again there doesn’t seem to be any increase in fracture risk in those who continued on alendronate, but these data are far from robust. Now a lot of interest is being generated by the case reports described by Odvina and her colleagues and reported in the JCEM last year.

These were nine patients, one man and eight women, who had received alendronate mostly for over six years, three of them were also receiving HRT and two were also receiving prednisolone. And these patients presented with unusual fractures, spontaneous fractures, some at the femoral shaft, for example, many of which showed reduced or failure to heal. And they carried out bone biopsies in all these patients and found that in all of the bone biopsies, there was complete absence of double tetracycline labeling and very few bone cells were seen on the bone surfaces.

Now these data obviously fall far short of proving a causal association between long-term alendronate therapy and the fractures that these patients presented with, but there are some interesting aspects to these cases, which I will examine on the next two slides.

So firstly, if we look at the biochemical markers of bone turnover in these patients, you can see here, we have got bone specific alk phos, osteocalcin, and urinary NTx and the normal range is shown in these shaded areas. You can see that for alkaline phosphatase, all of the patients had normal levels or levels were elevated in some cases.

For osteocalcin, many levels were normal, some were slightly reduced, and these two last patients were the ones on steroids, so that would be expected. And urinary NTx levels were normal in all patients. So again, you see this discrepancy, but rather more marked in this case between biochemical assessment and histomorphometric assessment of bone turnover. In the seven patients in whom bone density was measured, only two had osteoporosis as defined by femoral neck T-score of -2.5 or below. And you can see that these patients, this one, this one and this one, they have levels between...with T-scores between -1.5 and 0, which we would essentially consider as pretty normal. And the final interesting fact about these patients is that six of them had never sustained a fracture previously. So these aren’t typical patients with very severe osteoporosis and all these observations raise a possibility that there were additional factors perhaps related to various aspects of bone quality, which were contributing to bone fragility in their case. And I think measurements of bone quality in these biopsies would be of great interest. So I think just to summarize so far, there really is no compelling evidence to suggest that long-term suppression of bone turnover with bisphosphonates is associated with increased bone fragility and an increase in fracture risk.
There may be hints here or there, but nothing compelling. But nevertheless it’s interesting to look at some of the potential mechanisms by which adverse effects on bone strength might result from suppression of bone turnover and one of these is an increase in the degree of mineralization and perhaps also it’s homogeneity, an increase in bone age and of course also changes in the composition of bone matrix and mineral about which we know very little.

Another theoretical possibility is that there is increased production and reduced repair of microdamage and then thirdly, there may be reduced replacement of osteocytes. So the first question is how much do bisphosphonates suppress bone turnover and as I have already told you, it depends how you measure it, but if we look at histomorphometric measurements, then we have data here for alendronate, zoledronate, risedronate, etidronate, ibandronate. Now these are not head-to-head studies. And the other important factor noticed is that these represent the doses in which the bisphosphonates are used clinically.

So the differences you see on this slide may not be due to differences between bisphosphonates per say, but simply reflect the doses that you use clinically. But for what it’s worth, it appears that alendronate and zoledronate suppress bone turnover rather more than these other bisphosphonates.

Histomorphometric data in women receiving bisphosphonates have mainly been collected during the first three years of treatment and there really are very few long-term data, which of course is what we want, because safety data should reflect the normal duration of treatment in osteoporosis, which is often 10 years or so.

Bob Recker, two years ago, presented at the ASBMR, some data in women who had received alendronate for 10 years. These were women from the flex trial and after six years, they were randomized to placebo or alendronate 5 mg daily. And all the biopsies in the women who received alendronate for 10 years showed double tetracycline labeling and the mineralizing surfaces, which is a measure of remodeling rate, were only fairly modestly reduced by about 50%. So these data, at least at first sight, appear quite reassuring, but we should remember that the dose here of alendronate is 5 mg not 10 and there are data from other sources, which indicate a dose response in terms of suppression of bone turnover. And of course, there may also be questions about persistence with therapy in women given alendronate for 10 years. Now in line with the suppression of bone turnover, as we would expect, there is an increase in the degree of mineralization and at least initially in the homogeneity of mineralization of bone in women treated with bisphosphonates. And this slide shows data for alendronate, risedronate, conventional and high-dose HRT here just for comparison.
Again, these are not head-to-head studies. The methodology used was not always the same, but they show fairly modest degrees of increase in mineralization, usually in the first three years of treatment. Now the effects of this magnitude of increase in mineralization on bone strength are unknown,

but it’s really very unlikely that they would have adverse effects on bone strength. And there might even be positive effects with an increase in bone strength, as has been suggested by others. What we really need are these sorts of data for people who have been receiving bisphosphonates for 10 years or longer.

In terms of microdamage accumulation, the effects of bisphosphonate therapy in women receiving treatment for osteoporosis have not yet been established. But there have of course been studies in dogs and this slide shows data from one of those studies, here the beagles were treated with very high
doses of risedronate or alendronate, probably six to eight times what we would use in postmenopausal women and bone turnover was decreased by 90 to 95%, so almost completely suppressed. And they showed that there was significant microdamage accumulation.

But in fact, when they looked at the ultimate load, which is a measure of bone strength, they found that it was significantly increased not decreased, as you might expect, in the dogs treated with bisphosphonates, although they did actually show that there was a small reduction in toughness.

So they may be conflicting changes in different aspects of bone strength. So what these data indicate is we know the bisphosphonates suppress bone turnover, we know that they increase the degree of mineralization and at least initially increase the homogeneity of mineralization.

We don’t know what happens in terms of microdamage accumulation nor do we really know much about what happens to the composition of matrix and mineral with long-term treatment with bisphosphonates. And finally, the term oversuppression of bone turnover is usually applied in the context of

bisphosphonate therapy, but of course, it may in theory apply to any potent antiresorptive agent. And in this respect, I think the effects of RANK Ligand in addition with denosumab will be of great interest because as you can see here, there is a very potent suppression of bone turnover with biochemical marker suppressed down to about 80%.

So in summary, bone biomarkers and bone histomorphometry provide different information about bone turnover and moreover, bone turnover varies throughout the skeleton and as yet we do not have methods for assessing the suppression of bone turnover by bisphosphonates in clinically relevant sites such as the spine and the proximal femur.
Suppression of bone turnover is associated with increased mineralization, possibly with microdamage accumulation, but the effects of these on bone strength is not well understood nor do we know what happens to the composition of bone matrix and mineral with the long-term treatment.

So the adverse effects of oversuppression of bone turnover on fracture risks so far have not been demonstrated, but they remain a potential concern. What we need ideally a robust long-term fracture data, but I think it’s extremely unlikely that we will ever have those and as a second best, we need to understand better the effects of suppression of bone turnover on bone quality at different skeletal sites, particularly in the spine and the femoral neck. Thank you for your attention.

Salvatore Minisola: Thank you Dr. Compston for your lecture. It is now open for discussion. Microphone number 2.

Male Speaker 1: Juliet, I was a bit concerned regarding the conclusion that you have drawn because you are talking about the consequences of oversuppression.

The real issue is whether during bisphosphonate treatment you do get oversuppression. What you badly need, that’s my opinion, solid and robust data on the degree of suppression in comparison between patients treated with bisphosphonates, very high doses of bisphosphonates and premenopausal women, normal women.

Juliet E. Compston: Well, I showed some data, which indicate that the suppression of bone turnover with some bisphosphonates is certainly greater than...they suppress bone turnover at levels that would be below what you would see in normal premenopausal women.

I think and I agree with what you say, but the problem is which site do you look at and which method of assessing markers do you use, because if you use the biochemical markers and say we suppress them into the premenopausal range that doesn’t necessarily tell you that within the spine, for example, or proximal femur, you are getting that level of suppression of bone turnover that you feel is optimal.

Male Speaker 1: Okay, then you agree that you need this sort of information? Juliet E. Compston: Sorry?

Male Speaker 1: And then you agree that we need that sort of information before drawing the conclusion that with bisphosphonates you had oversuppression of bone turnover.

Juliet E. Compston: I agree and that’s why I didn’t draw any conclusions.

Salvatore Minisola: Microphone number 2.

Male Speaker 2: That was a lovely talk. Isn’t it part of the problem that we don’t really
understand why bone remodel? So, for example, men remodel more slowly than women, blacks remodel more slowly than whites.

00:24:58
If we suppress somebody’s remodeling who is at the 95th percentile now to the 50th, will that cause increased fragility compared to someone suppressing someone at the 50th percentile down to the 5th percentile? So what is the driving force for the variability in bone remodeling?

00:25:17
Is it something about damage? What is the definition of damage? Is it apoptotic death or microdamage? Do you want to comment on where we should be going with these sorts of questions?

00:25:30
Juliet E. Compston: Well, there are big questions Ego. I think one thing which is very interesting is whether people...if you give a bisphosphonate to people who have low bone turnover to start with, is it going to be more damaging potentially in terms of increasing bone fragility than somebody who has very high bone turnover.

00:25:47
I mean intuitively, you would think that there would be, but I think until we have better methods of looking at bone turnover at different skeletal sites, it’s going to be a difficult question to answer.

00:25:58
Salvatore Minisola: Microphone 1.
Male Speaker 3: The accumulation of microdamage may be quite significant although I agree that osteonecrosis of the jaw is associated with local factors as well, there does seem to be some evidence suggesting that the frequency is associated with the potency of the bisphosphonates.

00:26:18
With the monthly intravenous zoledronic acid, it is about 10% in 4 years. With intravenous monthly pamidronate, it is about 4%, whereas with the oral alendronate and risedronate, there are only sporadic cases.

00:26:32
Juliet E. Compston: Yeah, I meant to say, but I didn't that I wasn't going to talk about ONJ simply because there is a talk devoted to that topic later this afternoon. But what you say is perfectly true. Of course, there are other mechanisms by which bisphosphonates may contribute to ONJ.

00:26:46
Depletion of gamma, delta T-cells is one, inhibition of neo and diogenesis is another. So it’s quite conceivable that more potent bisphosphonates, potent in whatever action, might predispose people more to the development of ONJ and I agree that oversuppression of bone turnover may be one factor, but it’s probably one of many.

00:27:11
Male Speaker 3: Thank you.
Salvatore Minisola: Number 4.
Raul: Juliet, Raul from Detroit, thanks for the review. I was wondering on your thoughts. If the...most of the benefit in the FIT trial was accrued from the 5 mg dosage and since other bisphosphonates, agreeing they are not head-to-head
comparison, seem to have similar benefit, do we really need to suppress more than 50%, do you have any thoughts?

Juliet E. Compston: That’s a very good question and the answer is probably no, we don’t need to, and of course, zoledronate 5 mg, as you say, appears to have very similar anti-fracture efficacy as far as we can tell, and does appear also to suppress bone turnover less than 10 mg daily. So, I think one could have made a strong rationale of the time for using 5 mg daily for treatment, but that didn’t happen.

Salvatore Minisola: Thanks again, Dr. Compston for your contribution. Now let’s move to the next speaker.

Dolores Shoback: We are going to move on to our second presentation for this afternoon, which is being given by Dr. Andrew Stewart, Chief of Department of Endocrinology at the University of Pittsburgh.

Andrew F. Stewart: Thank you Dolores, and thank you all for coming and joining us in what is obviously a meeting full of lots of competing and very interesting and important thoughts. So my mission is to tell you today about what the connection is between PTHrP, which is where I am starting, and hypercalcemia, malignancy and lactation.

And I hope to tell you that I think and I would love to get your feedback that actually there is a very natural connection and that they basically represent different degrees of the same phenomenon. I want to acknowledge at the outset, conversations and thinking about this with Chris Kovacs and John Wysolmerski and I am going to show you some of their work as we go along. So without further ado, here are my disclosures. All the work I am going to show you, by the way, is funded by NIH. None of it is Pharma. So, first of all, cancer or hypercalcemia, so this story begins in the 1920’s and suffice it to say that it’s common, it’s serious, and it’s grave. In this study by Stuart Ralston in 1990, he showed that no matter how patients were treated from the time that they were diagnosed as having cancer or hypercalcemia to the time that 50% of them died, that time was only 30 days and I think nobody has done a study like this since, but I don’t think much has changed in the intervening 15 or 16 years. So it’s a serious disorder that comes in several different flavors, the most common of which I am going to talk about today, that’s humoral hypercalcemia malignancy or HHM.
From the 1940’s to 1970’s, most people thought this was due to secretion of parathyroid hormone and it was called ectopic hyperparathyroidism or pseudo-hyperparathyroidism, now most people refer to this as HHM. The tumors that cause it are listed here or at least the most common ones and particularly, squamous and renal carcinomas are common. These patients are characterized by having little in the way of skeletal metastases shown here and finally in unselected prospective series, it’s by far the most common cause and that’s worth a cause of hypercalcemia in patients with cancer. That’s worth mentioning because many people still think that this is the most common kind and I don’t have time to talk about all of them but I have just to bring that to your attention now. My interest in this started in the late 1970’s when HHM was ectopic hyperparathyroidism, and in a study that I did, but I want to acknowledge Art Broadus, who was my lifelong mentor in this.

We looked at nephrogenous cyclic AMP and the tubular maximum for phosphorus in patients with HHM and we found that patients with HHM had increases in nephrogenous cyclic AMP excretion and they were also phosphaturic. They had reductions in their TmP for GFR.

And in those ways they resembled the patients with primary hyperparathyroidism shown here and here. So whatever caused HHM was something that activated phosphate excretion and/or inhibited phosphate reabsorption and stimulated cyclic AMP generation in the proximal tubule.

When we looked at other renal functions, we found that the two syndromes were in fact quite different. In the distal tubule, renal calcium handling seemed to be quite different in patients with HHM as compared to those who are hyperpara, and also in the proximal tubule, where 125 vitamin D is made,

125 levels were undetectable or low in patients with HHM and yet elevated in patients with hyperpara. And I am going come back to that. When reliable immunoassays for PTH came along in the 1980s...so this is a two-site immunoassay from Sam Nussbaum and Gino Segre just making the point that PTH immunoreactivity is absent in the plasma of patients with HHM and that’s of course very different from the situation in patients with primary hyperparathyroidism. If you look at bone biopsies that we did in the 1970’s in patients with hyperparathyroidism, you can see that there is an increase in osteoblastic bone formation shown here and a coupled increase in osteoclastic bone resorption shown, for example, here. So hyperpara is characterized by increases in bone formation and also by increases in bone resorption. When we did biopsies on patients on HHM, the histology was really quite different.
So first notice that there is no tumor in the bone marrow here, so this is not due to a metastatic bone disease and metastatic tumor in marrow. Second, there is a lot of osteoclastic activity, so here is an osteoclast, here is one, here is one, here is one up here, here is one, here is one, here is one, they are all over the place.

So there is more osteoclastic activity in HHM than there is in hyperpara and yet, when you look for evidence of bone formation it’s not there. So there are no osteoblasts lining the bone surface in these biopsies and there are no osteoid seams. So you can get quantitative about that and this is work that was done in collaboration with Roland Baron and Art Broadus at Yale. If you look at patients who got primary hyperparathyroidism, secondary hyperparathyroidism, or Paget’s disease, there is a nice correlation between indices of bone formation and indices of bone resorption.

But when you look at patients with HHM despite having in some cases really tremendous bone resorption, there is no increase in bone formation. So in HHM, osteoblastic and osteoclastic activities are uncoupled and that’s very different from hyperpara.

So just to summarize what I have shown you in the last several slides, as I realize I am going quickly because I think a lot of this is old and I want to show you some newer stuff. These slides show that there are similarities between patients with primary hyperparathyroidism and there are differences.

The similarities are that both groups are hypercalcemic, both groups have increased osteoclastic bone resorption, both groups display phosphaturia, both groups display increases in nephrogenous cyclic AMP excretion, and both syndromes are due to circulating humors.

In the case of hyperpara, the humor is PTH and in the case of HHM, in the 1970’s, the humor was unknown and I will come back to that in a second. So there are similarities, but there are also differences and you can see, as I summarized that 125 vitamin D levels were increased in hyperpara, but reduced in HHM.

Osteoblastic activity is increased in hyperpara, but reduced in HHM, and finally renal calcium absorption is increased in hyperpara, and the data that I showed you a moment ago suggests that it is decreased in patients with HHM. So this set a sort of physiological stage that looked like this.

So this is what HHM looked like in 1980. Somebody would have a squamous carcinoma for example or a renal carcinoma and it would be making this HHM factor that would selectively stimulate osteoclastic bone resorption and stimulate it robustly. It would not lead to activation of formation.

If anything, bone formation is suppressed. So you would have a huge unidirectional flux of calcium from the skeleton in the extra cellular fluid. That would exceed the ability of the
kidney to clear calcium and that would result in severe hypercalcemia in this extracellular fluid black box.

That would lead to suppression of PTH, suppression of 125 vitamin D, and inability to absorb calcium from the GI tract. So this slide summarizes what we were thinking in 1980 and the big question in that era was what is the HHM factor. Well, a lot of us spent a lot of time trying to figure out what it was, where we purified and sequenced it and cloned it and so did Jack Martin and his group in Australia and so did Buck Strewler and Bob Nissenson in San Francisco, and Mike Rosenblatt in Boston at the time. So I don’t have time to spend a lot of time on this but I suspect this audience knows a lot.

I just want to draw your attention to the fact that both PTH and PTHrP are homologous in their amino...terminal 14 amino acids and then the sequences diverge completely. It turns out that PTHrP is a prohormone. It undergoes, like most neuroendocrine peptides, extensive post-translational processing.

And this slide summarizes or shows you the initial translation product of PTHrP and then the family of peptides that are generated by intercellular post-translational processing of PTHrP. So in other words, these are the secretory forms of PTHrP, it’s not a protein, it’s a family.

The one that I am going to focus on in the rest in the talk is this one, which is PTHrP (1-36), which is generated normally by a cleavage here at this arginine and by this lysine-arginine cluster and importantly, it contains the PTH-like region that’s homologous to the PTH sequence.

So one thing we wanted to do is to develop an immunoassay for PTHrP and together with Bill Burtis, we developed a two-site IRMA and you can see that PTHrP levels are elevated in patients with HhM. You can also see that if you look at patients with hyperpara shown here, PTHrP levels are not elevated.

If you look at people with myeloma or other kinds of cancer where bone metastases are the cause or prior bone marrow involvement is the cause of the hypercalcemia, PTHrP is not elevated and it’s not elevated in renal failure. So in terms of fulfilling Koch’s postulates, PTHrP is uniquely elevated in patients who have by other criteria the HHM syndrome. I am going to come back to this group, but just for the moment, let me just show you that the black dots here are human milk, the open circles are bovine milk from the local supermarket. Buck Strewler had shown very similar results around the same time, but the point is that the levels of the PTHrP in milk are around 30,000 picomolar, that’s about a 1000-fold higher than the levels in the plasma of patients dying of cancer hypercalcemia and that’s about 10,000 times higher than the levels in you and me.
So there is a milk story here and I’m going to come back to that. Okay, so now then, in terms of pathogenesis the syndrome becomes easy to understand. You have got a peptide that binds to the PTH receptor just like PTH and signals through the same kinds of signaling pathways that PTH does in bone and kidney.

I won’t show you the data, but lots of labs including ours have shown that that’s true, PTH and PTHrP bind with equivalent affinity to the receptor and they activate the same downstream signaling pathways in bone and kidney. In terms of fulfilling Koch’s postulates, it was necessary to infuse PTHrP in the animals, in this case rats, to show that you could cause hypercalcemia and the rest of the syndrome and that clearly happens if you infuse PTHrP into the rats, the serum calcium goes up to around 19 or 20 and when you discontinue the infusion, the serum calcium returns to normal.

So all of the data that I have shown you up till now are from the 1980s and 90s, so what I want to do now is show you the kinds of things we are doing now. So one question is, can you reproduce the HHM syndrome in humans by infusing PTHrP? In a study that we did years ago...so this is 1996, 10 years ago, we showed that if you infuse PTHrP for six hours into healthy volunteers, you generated large increases in nephrogenous cyclic AMP. That was important because it showed that you could reproduce, what was at the time, the main hallmark of the syndrome, nephrogenous cyclic AMP excretion.

It showed that in vivo PTHrP could in fact activate the renal PTH receptor. Placebo infusion or vehicle infusion did nothing. More recently, together with Mara Horwitz at the University of Pittsburgh, we have been doing head-to-head comparison studies, comparing PTHrP to PTH (1-34), and we have been doing these studies over 48 hours.

Most studies looking at parathyroid hormones had been for six hours, eight hours, four hours, there are some that are 24 hours, but even for PTH, no one has really looked out for the second day of continuous IV infusions. So in these slides and all the next several slides, PTHrP is shown in red and these are different doses.

PTHrP is shown in black and these are different doses. I won’t dwell on it, but obviously, both peptides cause hypercalcemia when you infuse them into normal human volunteers. When you make normal human volunteers hypercalcemic, they suppress endogenous PTH (1-84) secretion and that’s exactly what’s shown here, no surprises.

Remember, that PTH, the hyperpara and HHM are associated with phosphaturia or reductions in the tubular maximum of a phosphorus and what this slide shows is that is true if you infuse these peptides as well. This little blip here is because after they have been fasting for 24 hours, we allow them eat, but
for...the take home message is that both peptides induce phosphaturia. This is a proximal tubular effect and it’s comparable for both peptides. This is a complicated slide and I am not going to go through all of it, but I just want to use it to give you the message that it shows very clearly two things.

One is that parathyroid hormone is anticalciuric in humans and, believe it or not, that’s not something that has been shown very convincingly prior to this. It of course has been in laboratory animals. But importantly for the current discussion, it shows that PTHrP is also a potent anticalciuric in humans and it’s equivalent in potency, in anticalciuric potency to PTHrP. Now, Stuart Ralston, Rene Rizzoli, Philippe Bonjour have been saying for years that this was true, but it needed to be done and it turns out they are exactly right. For the pathophysiology of HHM, it indicates that it’s not purely a bone resorption osteoclast disease.

That’s of course very important, but also PTHrP limits the ability of the kidney to excrete calcium and so it also contributes to the hypercalcemia in patients with HHM. So I have shown you that you can reproduce the biochemical features of the syndrome, increases in nephrogenous cyclic AMP, reductions in the renal phosphorus threshold by infusing PTHrP. I have shown you that in fact these original observations are likely incorrect. PTHrP seems to be just as anticalciuric as PTH and this is probably an artifact to trying to assess GFR in cachectic end-stage dehydrated patients with cancer, in whom it’s difficult to calculate GFR, which you need for the fractional calcium excretion measurements. So I want to turn now to the 125 Vitamin D measurements, which are very different between the two syndromes. So when we infused PTH and PTHrP into normal volunteers, here’s what’s happened.

The red lines are PTH and the different numbers here represent increasing doses in groups of...I have forgotten exactly, but I want to say, seven patients. So, if you infuse 8 picomoles/kg/hour PTH, 125 goes up this much. If you do 12 picomoles/kg/hour it goes up this much.

And if you do 16, it goes up this much. So there is a dose-related increase in 125 levels as you escalate the dose of parathyroid hormone. What happens with PTHrP? Well, it turns out that PTHrP is a poor agonist of 125 vitamin D. So here is 8 picomoles/kg/hour.

It’s less, it has really very little effect and is clearly less effective than the comparable dose of PTH. 12 picomoles/kg/hour really does nothing and that’s much less than the comparable dose for PTH and even if you give whopping doses of PTHrP, you still don’t achieve the degree of 125 activation that you see with lower doses of PTH.

So in human beings, PTHrP is a poor agonist of 125 production. So this suggests that this paradigm that we have also sort of been using for years is probably incorrect. It is true that
they bind in the receptor comparably, it is true that in rodent systems they are very similar, but honestly no one has really looked carefully at human renal tubular cells with human PTH receptors in them to look at signaling, to look at 125 activation and that’s something we are doing right now. Okay, so what about the bone turnover side of this thing? So we infused PTH and PTHrP and we also looked at bone resorption, and what we found is exactly what we expected. We found that both PTH and PTHrP stimulate bone resorption as assessed by CTx. We also looked at the serum NTx and the results are comparable. So both peptides, as expected, stimulate bone resorption.

Now we expected, since hyperpara is associated with increased bone formation, that when we infused PTH we would see increases in bone formation markers. What we saw in fact is actually the opposite. So then, the red lines infusing PTH for 48 hours leads to actually a suppression of bone formation and we have just been tutored on the quantitative aspects of that, so this is about a 50% reduction in bone formation as assessed by P1 and P levels. So that was counterintuitive, we didn’t expect that. We also found that PTHrP did exactly the same thing, both in quantitative and qualitative terms.

So we thought, well, this is surprising but probably we just didn’t go long enough and if we infuse these things for a couple more days then we would get an increase in bone formation. So we extended these studies and this time we used 96 hours of infusions or a four-day infusion instead of a two-day infusion at the lowest dose of PTHrP 8 picomoles/kg/hour. What I love about this slide is it absolutely perfectly reproduces the pathophysiology of HHM. So if you infuse PTHrP, you get an increase in serum calcium up into the high 10s. Serum CTx, a marker of bone resorption increases just as it does in HHM, and again in this cohort, a different cohort, P1 and P fell for the first 24 hours. We had guessed that it would turn around and start going up, but it didn’t, it actually went down further over the second 48 hours. So it turns out that PTHrP and PTH actually suppress bone formation, at least markers of bone formation, when you infuse them. So we thought, this is really surprising, I can’t believe that we have found this and no one else have ever seen this, and it turns out they have seen it and it’s actually been seen a lot. So, here is a study by Felicia Cosman and May Parisien in Haverstraw, New York at Columbia and they showed that if you infuse PTH for 24 hours into black or white women, that it leads to suppression of a formation marker, P1CP. They have done previous studies in premenopausal and postmenopausal women looking at bone specific alkaline phosphatase and found exactly the same thing.
So in the hands of the Felicia Cosman Group, PTH infusion, at least over 24 hours, suppresses bone formation. This is a study from Ben Leder and Joel Finkelstein at Massachusetts General and this study is in men and these were men who were either androgen replete or androgen deficient.

And they infused PTH and used a different formation marker to follow them. And what they found is that continuous infusion of PTH suppressed bone formation in both kinds of men whether the androgen was there or not. So this notion that infusion of PTH continuously or infusion of PTHrP continuously leads to suppression of formation markers is not new and in fact it's pretty old, some of the data go back... this is a study by Dave Goldsmith and Larry Flair (ph) going back to 1992. So what about in animals? Well, this is a study that's used all the time to explain the anabolic... the paradoxical anabolic effect of PTH when you give it as a single daily injection but there is another part of it. This is a study by Russ Turner and Harald Dobnig and they did continuous PTH infusions for six days in rats and they found that it suppressed histologic evidence of bone formation and stimulated resorption.

So in fact, it's exactly what you see in HHM. When they looked at the histology, they found that a single subq dose stimulated osteoblastic activity, a one-hour infusion stimulated osteoblastic activity, a two-hour infusion stimulated osteoblastic activity and osteoids beginning to appear here. But if you went for six hours or gave continuous infusions there weren't any osteoblasts, there was just this proliferation of fibroblastic cells surrounding trabecula. So their conclusion was the same that actually continuous exposure to PTH suppresses osteoblastic bone formation.

And what about in vivo, can this stuff happen in vivo? Again, lots of studies showing this, and I am just going to show you one that I think is very convincing. So this is the study from David Rowe at University of Connecticut. He has made transgenic mice that over-express... that expressed green fluorescent protein under the control of the collagen 2.3 promoter and the point for the clinicians here is that this is a marker of mature osteoblast. So if you take calvarial cells from these mice, put them in culture, and just allow them to differentiate, they turn green, that means they are turning on the COL1 promoter.

It means they are turning into osteoblasts and they mineralize. The red here is mineralization. So, if you don’t have PTH in the cultures, these things go through mineralization sequence normally and they mineralize or the maturation sequence or differentiation sequence.
On the other hand, if you have PTH in the cultures continuously for 21 days, nothing happens. They won't differentiate, they won't turn into osteoblasts, and they won't mineralize. So here now is longer-term data in an in vitro system showing exactly the same kind of thing.

If you look in the literature there are lots of other examples. So they interpreted these data, the David Rowe Group, to mean or to indicate that continuous exposure to PTH interrupts the final differentiation step in the osteoblast differentiation pathway.

So you start out with stromal cells or lining cells or stem cells in bone marrow, a little bit of PTH and other things makes them differentiate into pre-osteoblasts, which look a lot like osteoblasts under the microscope. But continuous exposure to PTH prevents that next step from occurring, so they are arrested.

They can't turn into osteoblasts and so you get fibroblasts in marrow but you don't get osteoblasts, and you will get osteoblast markers. Okay, so now that you have seen all that, one of the questions we have been asking for years is how come bone formation is suppressed in patients with HHM, and I think it seems pretty obvious.

If you use continuous PTHrP infusion or if you have a squamous carcinoma that's continuously secreting PTHrP in a patient with HHM, it's going to suppress osteoblastic activity. It's going to explain the histology that I have shown you. So the question that is so why would this be, why did we evolve a system to do this?

Why did God or evolution...in Western Pennsylvania, where I am from, of course it's God, but maybe evolution is involved. That's a joke; you are supposed to see if you are paying attention. So I think the answer to this is that it's all about lactation.

And these studies that I am showing you here, if you are interested in this I urge you to read by Josh Van Houten and John Wysolmerski, they make a really lovely case in mouse models that either express or don't express PTHrP that either do or don't have estrogen that this is exactly what's going on.

So normal skeletal calcium losses in you and I are in the range of 10 to 30 mg a day. During lactation, if you have a single baby, you put about 300 or 400 mg a day of calcium into milk. If you have twins, you put about a 1000 mg a day into milk, and it all comes out of the skeleton.

This results in striking skeletal BMD losses. In humans, 10% of skeletal mass is lost during six months of lactation. In rodents, they lose 30% of their bone density or bone mass during lactation. In both cases, they are rapidly reversible; in humans when they stop nursing, they regain their baseline bone mass within a period of six months or so and in rodents, they get back to their original bone mass within about three weeks. Lactation is also associated with estrogen deficiency, and so that's part of the story here and again, I don't have time to go into all the details, but clearly
the degree of bone loss in lactation is much greater than the degree of bone loss with estrogen deficiency with ovariectomy, with menopause, etc. And so there is a very compelling story that I urge you to read if you are interested in this, where PTHrP drives bone resorption and it’s worse, it’s more accentuated in the setting of estrogen deficiency that occurs in lactation. So what I am telling you that in physiologic terms is here is what goes on in normal physiology, I won’t review that because I think you all know it. In lactation what happens? The mammary glands starts making PTHrP and it puts it into the circulation, it also puts it into milk but it puts into the circulation and circulating levels of PTHrP are in the 2 to 3 picomolar level during lactation. That induces selective osteoclastic bone resorption and you want that; if you had stimulated comparable degrees of bone formation then no calcium would come out of the skeleton, no net calcium would come out. But if you just had unilateral bone resorption or unidirectional then you get nice net skeletal bone delivery in the extracellular fluid. If that’s all you did, this calcium would then just get dumped in the urine but now we know that PTHrP is a potent anticalciuric and it blocks renal calcium losses, and in fact, in lactating women, urinary calcium levels are usually quite low despite their rapid bone loss. In lactation, serum calcium levels are normal, so PTH levels are normal. That means 125 levels should be normal and they are and intestinal calcium absorption then is also normal. And so a nursing mother eating normal amounts of calcium gets in about 150 mg of calcium a day, from the skeleton about another 200 or 250 comes in. So now you got about 400 coming in and 50 going out, so where does the rest go, it goes into milk. And so, as I had mentioned earlier, you lose around 300 or 400 mg a day of calcium in milk during lactation. So, I think the story is actually quite compelling. It’s fun for me to think about. We are doing a bunch of studies now to try and nail this down more definitively. But this explains the tremendous bone loss during lactation. So I think...if you think about it that way, then what is HHM? Well, HHM is really just a bad case of lactation. The levels of PTHrP are much higher in HHM instead of being 2 or 3 picomolar they are 20 picomolar. That gives you more net bone resorption but still no formation, that gives you severe hypercalcemia, that gives you severe hypercalciuria in spite of renal attempts to prevent it’s loses. And then finally, since people are hypercalcemic with HHM, they suppress PTH, they suppress 125. You might have guessed that PTHrP should have rescued that, but now you know that PTHrP is a crummy agonist for 125 production and so 125 levels don’t increase in HHM.
They decline in the absence of PTH and intestinal calcium absorption is zero. So I am over a little bit in time, I want to stop and thank, particularly Mara Horwitz who is shown here. Mara is an assistant professor at Pittsburgh who has done most of these infusion studies over the last several years.

Also, I want to acknowledge Mary Beth Tedesco who is our study coordinator, and IDDK for funding this and Susan Greenspan, Jane Cauley, David Roodman who are all at Pittsburgh and are great for bouncing ideas off of and Bob Near (ph) who is the chair of our DSMB. Thanks very much.

Dolores M. Shoback: Thank you very much Andy. That was really quite a tour de force. I would like to take questions and comments from the audience. Microphone number 2.

Inglis Fogleman: Thanks, Inglis Fogleman (ph), London. That was wonderful, thank you. Your hypothesis cannot explain the recovery of bone loss. I mean, you completely...you have explained everything beautifully, but it doesn’t quite follow how you regain all the bone that is lost that is...

Andrew F. Stewart: Yeah, that is a wonderful point. So here is what I think about that. So just for everybody else, I told you that you lose, in a human being, 10% of your skeleton while you are nursing and then within six months you get it all back. So why is that?

A lot of people are beginning to think that there is some other anabolic hormone. I think it’s all about this. If you continuously expose the skeleton to PTH, you arrest osteoblast development, that’s a good rethink for the reasons that I had mentioned.

But you accumulate all these fibroblasts, they are probably really pre-osteoblasts, and the moment you take PTHrP away when you stop nursing, look at these guys, they are all ready to go, they want to become osteoblasts in the next 24 hours and they are the thing that leads to increased mineralization.

Now that’s my theory, I haven’t proven that, but that’s exactly the kind of studies. What we are doing now is we are going to do two-week infusions of PTH and PTHrP and then stop it and also look at the recovery and formation markers...

Inglis Fogleman: But it could also be tested presumably in women who stop lactation, looking at biochemical markers, I mean...

Andrew F. Stewart: So that’s another thing we are doing right now. Mara has just gotten IRB approval to do exactly that study.
You would think that we would know that at this point, but in fact there are no studies. There are lots of studies looking at resorption markers during lactation, there are no studies looking at...in humans, at bone histology for obvious reasons. But when you look for biochemical markers of bone formation and lactation, it's usually total alkaline phosphatase or old studies with osteocalcin, etc., but you are using modern assays for formation, they are really not there, what the literature seems to say is that resorption markers definitely go up. They go up two or threefold. Formation markers don't do anything or maybe even go down a little bit.

Inglis Fogleman: But the key will be when the women stop lactation...
Andrew F. Stewart: Yes.
Inglis Fogleman: ...so look at the markers at that point.

Andrew F. Stewart: Exactly and you are talking it right, that's exactly what we want to do, thank you.

Dolores M. Shoback: Thank you, microphone 1.
Sophia Shalom: Sophia Shalom, (ph) Israel. We know that there are different effects of PTH, whether the exposure of bone forming cells is continuous or pulsatile. What is the evidence of during lactation the exposure to PTHrP is continuous? It might as well be pulsatile during the periods of breast-feeding and then interactive?

Andrew F. Stewart: So, that's a great question. So, here is the evidence, when people have looked, I am forgetting who off the top of my head, but MaryFran Sowers comes to mind.

I am not sure if she is the person who has done it, but people have looked at PTHrP levels between episodes of nursing and then during nursing, and it does go up, but it doesn't go up much. It goes from maybe around 2 picomolar to around 3 picomolar and it goes up over hours.

Also, if you think about the mammary gland doesn't have dense core secretory granules like an islet does for insulin or like a parathyroid does for PTH. So it actually does not have a mechanism to do pulsatile burst a neuroendocrine kinds of secretion.

That's not true for PTH, for parathyroid glands package it in the dense core secretory granules fire it out in spikes, and so it normally is secreted in a pulsatile fashion and I think that's why in hyperpara and in normal people, there is evidence of formation.

Dolores M. Shoback: Andy, one quick question. Your model doesn't explain preservation of cancellous bone in primary hyperpara unless I missed it. You see the negative effects on formation, the positive effects on resorption with your infusions, but in hyperpara, particularly the mild forms, you don't see.
Andrew F. Stewart: So the question is what...  
Dolores M. Shoback: The cancellous loss.  
Andrew F. Stewart: So, one question is how is PTH normally secreted? I think most people think in hyperpara, it is sort of secreted like this. If you look through the literature, there are lots and lots of papers that show that PTH secretion is pulsatile.

I found about, I think, 10 of them or 15 of them. I have never found a paper that says that it's not. The problem is that they are all associated with PTHs assays. We all know the problems of PTH assays, but I think the answer to Dolores's question is that in hyperpara, in fact there is an example here, in hyperpara I think, maybe not.

But it's been shown in hyperpara as well that PTH isn't really continuously secreted in pulses and that may be the thing that keeps formation going on normal hyperpara.

Dolores M. Shoback: But you have got resorption as well, because you have got the hypercalcemia, so you have got...

Andrew F. Stewart: You have got resorption because that's what PTH does and it doesn't matter if it's continuous or pulsatile. Alright, thanks very much.

Dolores M. Shoback: Thank you very much Andy. Our final presentation this afternoon is Dr. Shonni Silverberg from the Department of Medicine at Columbia University. Her topic is the 'Cardiovascular System in Primary Hyperparathyroidism: A Relic of the Past, or a Current Event?'

Shonni J. Silverberg: Thank you very much Dr. Shoback and Minisola. I am going to talk today about the cardiovascular system in primary hyperparathyroidism, which has been a subject of considerable controversy in the literature recently. That the cardiovascular system should be an end organ of the primary hyperparathyroid process should not be surprising because both cardinal manifestations of the disease have been associated with cardiovascular findings. Hypercalcemia has been associated with vascular and myocardial calcification with arrhythmias, hypertension, and left ventricular hypertrophy and PTH has being associated with LVH and also with vasodilatory effects that are independent of serum calcium, which have led to alterations in heart rate, coronary blood flow, peak pressure, and the rate of rise of LV pressure as well. We don't have time this afternoon to discuss cardiovascular features of PTHrP, but obviously at increased levels of PTH, it's not at all inconceivable that there should be consequences as well. The existing literature on cardiovascular manifestations of primary hyperparathyroidism is full of contradictions and what I hope to be able to show
you this afternoon is that most of the inconsistencies are really more apparent than real. The data in the studies have been collected using different cardiovascular parameters, looking at different parts of the cardiovascular system. Some of the studies have been well underpowered for looking at cardiovascular parameters and probably most importantly from my point of view, the cohorts of patients have represented primary hyperparathyroidism that is varied from the very, very mild to the extremely severe. I am going to discuss four different areas in terms of the cardiovascular manifestations of hyperparathyroidism, beginning with mortality. The Swedish epidemiologists who have done so much of the wonderful work in this area have shown very nicely that calcium even within the normal range is an independent predictor for cardiovascular mortality.

Individuals with serum calciums between 9.8 and 10.4, middle-aged Swedish men had about a 20% increased mortality rate. When the same authors looked at individuals with serum calciums that was elevated as compared to patients...normal people, not patients with serum calciums under 9.8, they actually found a mortality rate that was markedly increased and 60% of that increased mortality rate was due to cardiovascular death. There is no question that primary hyperparathyroidism when it was seen in a severe disease, classical primary hyperparathyroidism, was associated with marked increase in overall mortality and increases in cardiovascular mortality. What has been somewhat surprising is the fact that with the evolution of the disease to one that is biochemically much more mild, the studies from Scandinavia, which are the only large-scale epidemiologic studies in the world, continue to show increases in mortality.

Probably the largest study is this one from Nelson et al. showing 11,000 Swedish patients who underwent parathyroidectomy over a 30-year period. Overall mortality was increased, but it was notable that the increase in mortality disappeared in those who were operated on in later years.

There are many possible explanations; some of them are listed here. It’s possible that it’s due to early diagnosis because of the multi-channel screening tests. The cardiovascular mortality could have been reduced by the early intervention. There is no question that over a 30-year period, there were marked improvements in the treatment of cardiovascular diseases and finally, it is possible that the later patients just have less severe disease. About 4000 Swedish patients were looked at who were operated on in the later part of the last century, 1987 till 1994, and a search of death
registers did show an increase in cardiovascular mortality in both men and women, but no serum calcium was available on these patients. What was of interest in this particular study was that when one looked at the death risk over time that each year there was a gradual reduction in death risks so that men had about 15% annual percentage reduction in death risk and women had an 8% reduction in death risk each successive year of the study, and this was just due to the fact that the Swedish population was becoming more healthy...normal Swedish men and women are shown the hatch bars here.

Among the possible explanations for this finding could be a change in the referral pattern for parathyroidectomy, which the authors felt was not the case, improved treatment of cardiovascular disease again, or once again, decreased severity of the hyperparathyroidism.

The North American experience has been somewhat different and this study of 1000 patients operated on at the Mayo Clinic with biochemically mild disease showed absolutely no decrease in survival. The only epidemiologic study in the United States comes from Olmstead County, where all patients with primary hyperparathyroidism diagnosed between 1965 and 1992 were captured. The disease was biochemically very mild and in this group, there was no increase in all class mortality and indeed cardiovascular mortality was decreased relative to age and sex matched cohort.

It should be noted that even in this study, the survival in the highest quartile of serum calcium, which was between 11.2 and 16 mg/dL was significantly reduced as compared to the three higher quartiles of serum calcium levels. What about when you consider the level of serum calcium in the risk of death?

Well, the study of Hedback et al. looked at 900 patients and reported increased mortality in all groups. They related the risk of death independently to advancing age, to preoperative serum calcium levels, to longer time since surgery that was an inverse relationship with the further outcome surgery they have got that the less likely they were to have higher mortality and then finally to calendar year of surgery with the mortality risk being lowest in those who were operated on in later years. And I would posit that these two risk factors shown in yellow are related one to the other.

What I have done here is plot the calcium levels in the Hedback study over time, which are buried in their manuscript, and indeed although it’s plotted in two different ways, what you can see is that early in the study the mean serum calcium level was well over 12 and it declined in a stepwise fashion to about 11.5.

Looked at in another way, the same findings over 50% of patients had marked hypercalcemia early in the study, whereas by the end of study only about 25% of patients
had marked hypercalcemia. As I mentioned before, unfortunately many of the studies did not include serum calcium when they talked about risk of mortality.

This summarizes six studies from five groups showing the relative risk of cardiovascular death plotted against serum calcium levels. And when these three studies in which the 95% confidence intervals across one were eliminated from that graph, what you see are these three studies left, which gives an unmistakable impression that relative risk of cardiovascular death varies in a close to linear fashion with serum calcium levels. It is obviously essential to assess cardiovascular outcomes that are short of death. Obviously, mortality is the ultimate negative outcome, but since many of our patients opt against surgery, because they have been told by us that it is safe to do so, it's clear that we need to see if mild primary hyperparathyroidism is causing other problems in the cardiovascular system. Hypertension is well known to be increased in severe hypercalcemia and in MEN.

There is a statistical association with mild primary hyperparathyroidism, but the mechanism and the nature of the relationship is unclear, and with no improvement in blood pressure with parathyroidectomy, we really don’t exactly know where we stand with that parameter.

Cardiac abnormalities have also been assessed in many different investigations. Unfortunately, the disease that we would like to know about most, coronary artery disease, is one about which we know absolutely nothing that is applicable to today's population.

The necropsy study of Roberts and Waller from 1981 is still sided, unfortunately because the mean serum calcium in that study was 19.4. The presence of increased coronary calcifications really tells us nothing about the patients that we see today.

On the other hand, there have been studies more recently of cardiac calcifications still with pretty marked hypercalcemia and there is no question that myocardial and valvular calcifications do exist in these patients, although it is of interest to note that the relative percentage of such calcifications is very variable despite the fact that the serum calciums were around 12 in all of these patients. Again, one gets the sense that serum calcium is important in this structural abnormality and in the studies on the left from Niederle and Stefanelli there is no question that aortic and mitral valvular calcifications are markedly increased in the patients with hyperparathyroidism, shown in yellow as opposed to controls, while in the study from Dalberg et al. no such finding occurred. The study on the left-hand side had a mean serum calcium of 12.1 and on the other hand, Dalberg study had very mild hypercalcemia.
Left ventricular hypertrophy has been increased in many studies of primary hyperparathyroidism. It is clearly one of the strongest independent predictors of cardiovascular mortality and the pathogenesis remains somewhat unclear, although the only study correlating the left ventricular mass with

serum calcium was clearly underpowered with an end of only 20 and most studies suggesting a direct effect of PTH on the myocardium. This slide is somewhat complicated and included mainly to show the fact that left ventricular mass has been reported to be increased in primary hyperparathyroidism over a huge range of serum calciums from 12 down to 10.5, and in all of these studies there was no evidence of hypertension in the patient population as a whole to account for the increase in left ventricular mass. This again would be consistent with the suggestion that PTH is more important in the etiology of this finding, although obviously it's only by inference. To look at an even greater detail at the relationship between primary hyperparathyroidism and hypertension and the genesis of left ventricular hypertrophy, the study published in 1999 has been very helpful. Patients had significantly increased left ventricular hypertrophy as opposed to non-hyperparathyroid controls, and the same relationship held whether or not you looked at hypertensive patients as opposed to hypertensive non-hyperparathyroid controls or normotensive patients as opposed to normotensive controls. Furthermore, in this study, PTH was a stronger predictor of left ventricular mass index than was blood pressure, and in this study as well as in the studies of Stefanelli, there is evidence that left ventricular mass index decreases after successful parathyroidectomy without any change in blood pressure. With regard to the conduction and abnormalities and arrhythmias, again serum calcium of around 12, there was a decrease in the QT interval, which was inversely associated with the serum calcium levels. Again, with the calcium around 12 increase in left ventricular extra systolic beats that resolved with parathyroidectomy and yet when the serum calcium goes down somewhat, here with the mean serum calcium of 11.5, the QT interval is normal. And another study in which the mean serum calcium was also lower had no arrhythmia or AV block, giving the distinct impression that at higher levels of serum calcium, the findings were abnormal and this seems to go away somewhere at lower levels of calcium. Myocardial infarctions have been reported to be increased in patients with pretty severe hypercalcemia prior to and then for one year after parathyroidectomy. But what was of interest in this study was the fact that when you looked at the risk factors for death from the myocardial infarction, these had to do with factors that had nothing to do with
primary hyperparathyroidism. Age, male gender, preoperative cardiovascular disease, and preoperative cancers were associated with a marked increased risk of death, whereas ionized calcium, adenoma weight, and kidney stones were associated with no increase or actually a decreased risk of death from this cardiovascular problem. A much larger study showed decreased overall mortality after parathyroidectomy, but no decrease in cardiovascular events whether you looked at MI, angina, congestive failure, or arrhythmia. As the diseases become much more mild and more subtle, it has become more important to look at more subtle alterations in cardiovascular function, which may precede alterations in cardiovascular structure.

And there is actually an excellent model for this in atherosclerosis, where abnormal vascular function predates and is associated with subsequent development of overt structural abnormalities. And in this slide, we looked at diastolic function. This is a graph of a study that is currently up.

Jesse Fletcher has a poster even as we speak. This is just very preliminary data from the very small cohort that we have collected up to this time looking at diastolic dysfunction, which is found to be increased in patients with higher serum calcium levels.

For those of who you in the audience who are not cardiologists, a low E/A ratio is associated with increase in ventricular stiffness and decrease in ventricular compliance and as you can see that is what occurs at higher levels of serum calcium. Other cardiac functional abnormalities in small cohorts have been associated with PTH levels, myocardial perfusion defects, and circadian cardiac autonomic nerve dysfunction have both been associated with PTH levels and at least in the case of the latter, improve after parathyroidectomy. To go on to vascular abnormalities in this disorder, Dr.

Mishaela Rubin in our group actually will be publishing in next several weeks. The first evidence looking at carotid vasculature that there seems to be a structural association that extends the Swedish data on increased mortality in patients whose serum calciums are normal.

Looking at 1200 patients with serum calciums within the normal range, who had carotid ultrasound. On carotid ultrasound the presence or absence of carotid plaque was noted and then the thickness of that plaque was assessed. She found that those individuals who had plaque, had higher serum calcium levels than those who did not and found furthermore that those individuals in the highest quintile of serum calcium had significantly greater plaque thickness than did those in the lower quintiles of calcium. And finally that if you were in the highest quintile of serum calcium, there was a 64%
increase in the odds that you would also be in the highest quintile of plaque thickness. This data, one could hypothesize, should extend to patients with primary hyperparathyroidism and whom one might expect the excess in serum calcium to deposit in the arterial walls.

We are beginning to look at carotid plaque thickness but don’t have yet data to report. In terms of intimal and medial thickness of the carotid, there are some small studies that are suggestive. Fowler et al. have reported that in primary hyperparathyroidism, only those with cardiovascular risk factors had increased IMT related to controls and those without risk factors did not. But a close perusal of the manuscript shows that those individuals with cardiovascular risk factors also had much higher serum calcium levels than those who did not.

So it is impossible to know whether the increased IMT is attributable to the high serum calcium, the presence of the cardiovascular risk factors, or a combination of the two. Another small study with high serum calciums again reported an increased IMT and in the study that Jesse Fletcher is presenting in the poster, we have some very preliminary data with extremely low levels of elevation in surgically proven primary hyperparathyroidism suggesting that these patients have IMT in the 75th percentile and relative to age and sex matched cohort, and at least...although this data is preliminary from all three, there does seem to be a suggestion that serum calcium levels may be associated with a structural finding. With regard to vascular stiffness, Mishaela Rubin followed up on a study by Smith et al. and looked at patients with serum calcium mean of 10.8 with primary hyperparathyroidism, and this extremely busy slide exists here mainly to show that the presence of primary hyperparathyroidism was an independent risk factor for aortic stiffness joining many parameters that all of us recognize as cardiovascular risk factors and that furthermore aortic stiffness in this cohort was associated with PTH levels and not with serum calcium levels. There is significant controversy about vascular reactivity in the literature. However, I would posit once again that it may well be due to the fact that different parameters have been looked at.

There are abnormal levels of endothelial independent response whereas normal endothelial dependent responses and once again those stimuli to vascular reactivity that have been used in the different studies have been very different. So I think that we really can’t make much of it nor can we make that much out of the studies that have been reported on serum markers of endothelial dysfunction or inflammatory markers. These are markers that are elevated in atherosclerosis and in other cardiovascular diseases and have, in some cases, been reported to be increased in primary hyperparathyroidism,
but there is no association with calcium or PTH and no change with parathyroidectomy and it is clearly premature to suggest any causal relationship with primary hyperparathyroidism. So to summarize, cardiovascular mortalities is clearly increased in patients with significant hypercalcemii, but not clearly increased in mild disease.

Hypertension, again, similar conclusion, structural cardiovascular abnormalities clearly exist with LVH probably being the most consistent finding. Calcifications seem to be related to serum calcium levels. Conduction system abnormalities, arrhythmia, and diastolic dysfunction do exist,

particularly in relationship to elevated serum calcium, whereas some of the more subtle abnormalities may well be related to parathyroid hormone levels and we really can’t say much about vascular reactivity. I hope that I have shown you this afternoon that the discrepancies in the literature are really more apparent than real and mainly related to the severity of hyperparathyroidism. It is also important to look at different cardiovascular parameters because the diseases are often not the same throughout the cardiovascular system and finally, there have been camps, which have tried to unilaterally assign blame or responsibility for abnormalities to either calcium or parathyroid hormone. And it is clear, I think, that while mortality and most of the structural findings are probably related to levels of serum calcium, that LVH is probably not, and certain of the functional abnormalities may be related variously to calcium and/or PTH levels. So in answer to the question, are cardiovascular manifestations seen only in more severely affected patients, I think the answer is no, but we clearly need more data on mild disease to assess structural and functional abnormalities to look broadly in the vascular system; heart, large vessel and small vessel that may be affected differently, and we have to use methods that are sensitive enough to detect vascular stiffness and altered reactivity. This is extremely important, I think, because demonstration of significant or reversible cardiovascular abnormalities in asymptomatic primary hyperparathyroidism would lead to a revision of accepted guidelines for intervention and treatment. I would like to thank the program chairs, Dr. (inaudible) and Greenspan and Dr. Elizabeth Shane, the ASBMR President, for inviting me today.

I would like to acknowledge all the members of the Columbia Metabolic Bone Diseases Unit and, in particular single out Dr. Mishael Rubin without whom this talk would not have been possible. Thanks.
Dolores M. Shoback: Thank you very much Dr. Silverberg. I would like to open this talk to discussion and questions. Microphone 2.

Male Speaker 4: It’s me. So, Shonni that was great. Now, a couple questions, one is in Mishaela’s study that you mentioned, I am wondering, whether...were you looked at people with calciums in the normal or near normal range, I suspect that nutrition and albumin levels and ionized calcium might be relevant there, and I wonder if you have done any sort of adjustments or subgroup analysis. I mean, so if you are overnourished, your albumin, I could imagine, might be a little higher and your total calcium might be a little higher, so do you know ionized calcium...

Shonni J. Silverberg: Yeah, these are actually...we actually don’t have ionized calcium levels on any of them but we only included individuals in whom we also had a simultaneous albumin and the data are actually corrected for albumin, and I think that you are absolutely right. It was actually interesting because this was part of a larger study and there were normal people, as you might imagine, who did not have normal serum calcium levels when they were just as a part of the screening study and almost all of those people had calciums that were low, not high.

Dolores M. Shoback: Microphone 3.

Suzanne Jan de Beur: Yes, Suzanne Jan de Beur from Baltimore, very nice presentation. I have a question about EBCT. Are there any data in patients with primary hyperparathyroidism using EBCT, and do you think it will be a useful methodology?

Shonni J. Silverberg: No and yes, there are individual cases of primary hyperparathyroidism and I actually, not frequently but infrequently...somebody will come in to the office obviously carrying an EBCT result and say, could this possibly be because my calcium is 10.7 and unfortunately, we really don’t have data on that and the one thing that I didn’t have time to discuss this afternoon is the fact that we don’t really know the effect of calcium. There is a lot of controversy in the cardiology world on whether or not calcium in plaque is a good thing or a bad thing, whether it stabilizes plaque or it makes plaque shear and causes problems. I think that it...what would be important and what I actually propose doing, but the NIH didn’t like it very much, was to do longitudinally EBCT studies because there are so few data comparing the people of the age group and the gender of most of our patients in terms of what’s normal but to certainly to follow these people over time with EBCT would be very interesting to see if coronary calcium score got better with parathyroidectomy.

Suzanne Jan de Beur: Thank you.
Dolores M. Shoback: Microphone 4.  
Male Speaker 5: Shonni, thank you very much for the good review. Just a comment and a question. In the randomized trial that we conducted, there was no change either within or between groups in the echocardiograms after one year. So admittedly it’s a small sample size one-year followup, so that’s kind of discouraging in terms of whether or not parathyroidectomy will improve the cardiovascular abnormality, I am talking about the echocardiographic abnormality. We also looked at the diabetes as a covariant, and we have a poster here looking at whether that could explain and obviously in our large population of 700 patients, the diabetes prevalence was not increased. We were hoping that could explain after adjusting for the standardized incident ratio. So I wonder in your population is diabetes included in this analysis?

Shonni J. Silverberg: It’s included in the analysis though actually it’s interesting, for instance one of the motivations for Dr. Rubin’s undertaking the aortic stiffness study that she did, was that the earlier studies, looking at post wave velocity and the aortic stiffness, had not corrected for lipid abnormalities, diabetes, and obesity, which can also affect those things and we found evidence of increased aortic stiffness in the absence and taking all of those factors into account. I think that what we are probably going to see in patients who have disease as mild as the disease that they were looking at are abnormalities of reactivity and stiffness that may not be translated into echocardiographic abnormalities.  
Male Speaker 5: Sure, thank you.

Dolores M. Shoback: Question here Shonni.  
Male Speaker 6: You suggested that hypertension could not be linked to the disease because it persists after surgery. However, if the disease is longstanding, perhaps there are some anatomical changes that allow the continuation of increase in per patient.  
This is similar to what we have seen in a patient with primary hyperaldosteronism that after surgery continue to have increase in blood pressure level.

Shonni J. Silverberg: I actually didn’t mean to leave that impression, I think that what I meant was that decisions for management shouldn’t be based on the presence of hypertension because there really isn’t the expectation that it’s going to go away, but I think that we really don’t understand the relationship.

Dolores M. Shoback: Do you think that if you look cross-sectionally at people who have the degrees of...the very severe hypercalcemia and hyperpara today, their management, because we manage cardiovascular disease so much better, would be different or do you
thing that you can actually separate out a calcium effect as opposed to sort of better management today?

01:32:37
Shonni J. Silverberg: Yeah I mean...I can speculate I have absolutely no data on which to base this. I think that it seems to me that the patients that they are reporting on in Scandinavia are not patients who have...even the patients with the mean serum calcium of 12 don’t have all of the manifestations of classical primary hyperparathyroidism. So they don’t see a ton of osteitis fibrosa cystica. The things that have disappeared on this side of the pond are also not very common on that side of the pond.

01:33:08
And I think that in those patients they definitely see increased mortality and also increase in other consequences of cardiovascular calcification. So I think actually in patients who have significant hypercalcemia, we would see that here too.

01:33:23
Male Speaker 7: Just one quick question. Shonni, I mean, so this is all very interesting, and I guess another question that’s important is do you think this has relevance for the use of PTH for the treatment of osteoporosis and is anybody actually doing cardiac echos or carotid ultrasounds, for example, in following cohorts of PTH treated patients?

01:33:44
Shonni J. Silverberg: It’s a very interesting question. Inasmuch as some of these abnormalities seem to be related to calcium as opposed to PTH and since the serum calcium levels in most patients who get PTH as a therapeutic do not bump particularly, those particular manifestations would not be expected. On the other hand, the levels of PTH are very similar to the low levels of elevations that some patients get and certainly we think that some of the manifestations may be related to PTH.

01:34:19
That was another idea that the NIH didn’t like. We included it...we thought that that would be a great thing to look at, and to my knowledge, nobody is looking at that.

01:34:32
Dolores M. Shoback: Great. There are no further questions or discussion. Thank you very much. This ends the session.