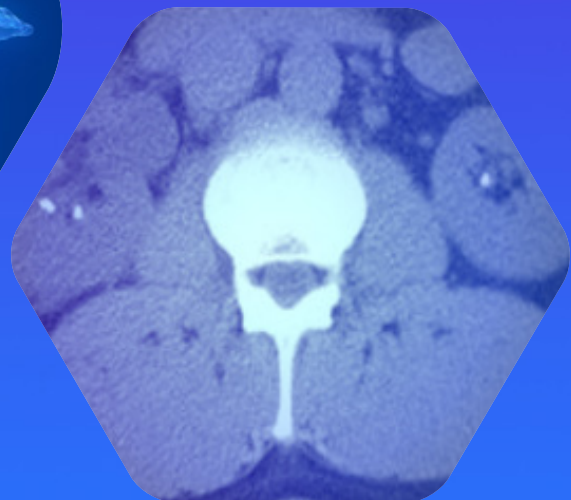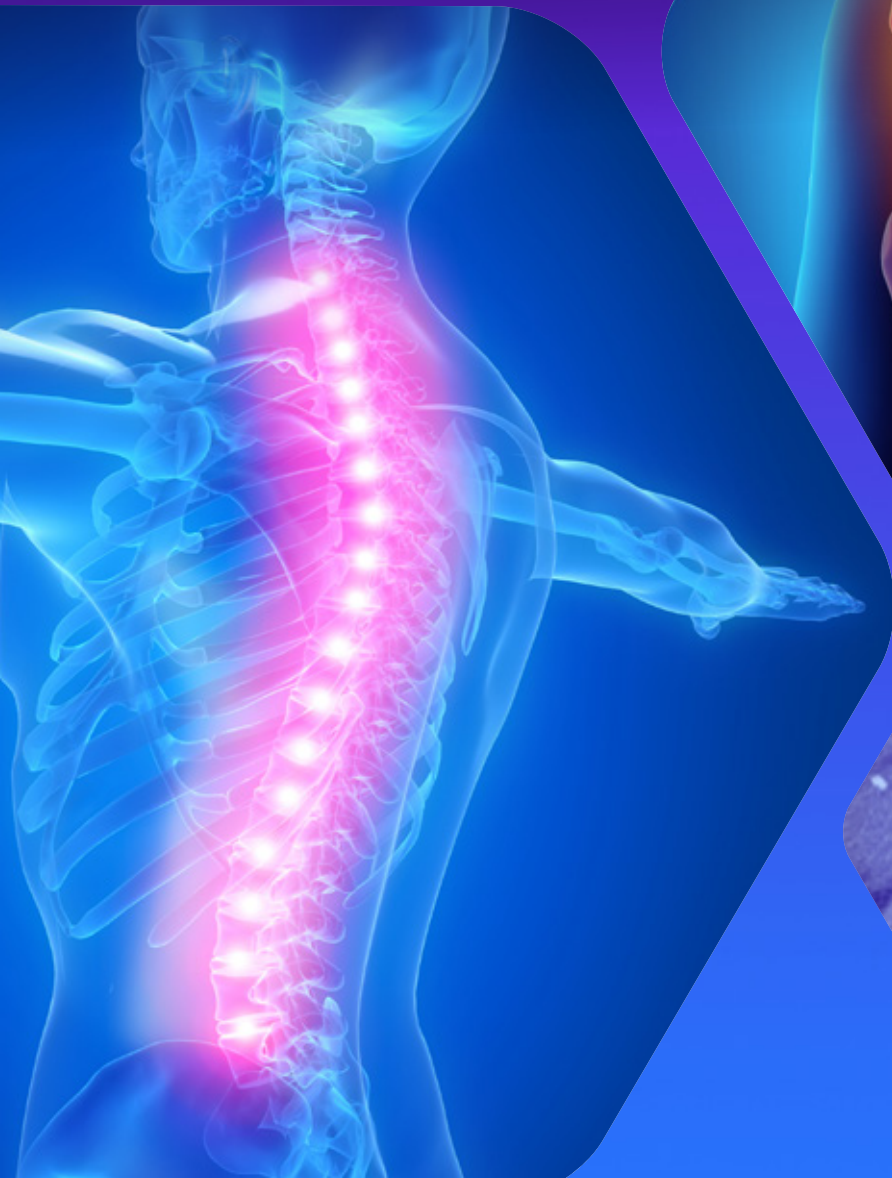European Society
of Endocrinology

ESE Clinical Update on Calcium and Bone 2022

A series of webinars held on 4–6 October 2022

Welcome

It is an exciting time to work in the field of calcium and bone. Studies on the underlying pathogenic mechanisms of classical bone diseases are advancing rapidly. Furthermore, the role of calcified tissue as an endocrine organ that interacts with many other body systems is now widely accepted, and makes the study of calcium and bone of greater relevance to many endocrinologists.

This report presents the content of three exciting webinars that examined the latest research in bone physiology and pathology in a range of areas.

The European Society of Endocrinology (ESE) was delighted to welcome experts in the field, who shared their knowledge and experience during three 2-hour webinar sessions that considered:

- **concepts in bone biology and their clinical impact,** such as the process of osteoclastogenesis, management of denosumab withdrawal after its use in osteoporosis, an insight into the actions of sclerostin in bone, and the clinical use of romosozumab

- **phosphate metabolism and conditions of hypophosphataemia,** including an up-to-date understanding of phosphate regulation, and the pathology and management of X-linked hypophosphataemia, tumour-induced osteomalacia and other causes of hypophosphataemia

- **the latest insights into hypoparathyroidism,** covering parathyroid hormone's actions in mineral metabolism in both bone and kidney, the causes of hypoparathyroidism, conventional treatment and its impact on patient-reported measures, and emerging therapies.

We are grateful to all who took part, including the attendees, who contributed important experience and many pertinent and relevant questions.

The content of the webinars is available to attendees at **www.eseondemand.org**.

Peter Kamenický, Martina Rauner, Athanasios Anastasilakis, Maria Luisa Brandi, Annegreet Veldhuis-Vlug, Katharina Jähn-Rickert, Jean-Philippe Bertocchio and **Neil Gittoes**
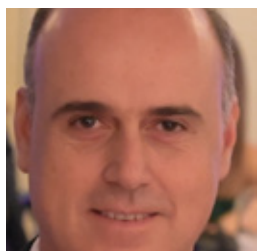Scientific Programme Committee

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Chairs and Speakers

ESE thanks all faculty members for their valuable contributions to the ESE Clinical Update on Bone and Calcium 2022.

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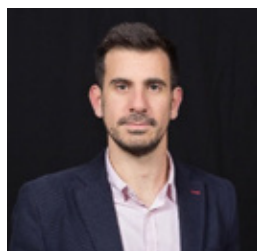
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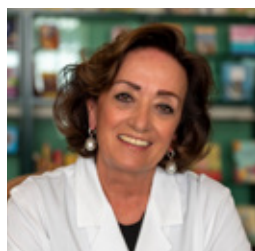
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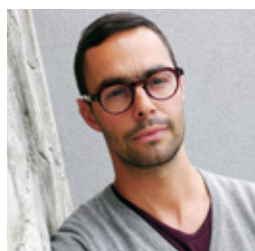
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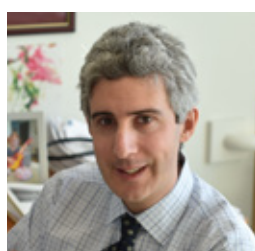
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New concepts in bone biology and their clinical impact

Chair: **Athanasios Anastasilakis** *(Greece)*

New concepts in osteoclastogenesis

Kent Søe *Odense, Denmark*

Osteoclasts are heterogeneous bone-resorbing cells that play a key role in bone remodelling. They are known to derive from monocytes through the process of osteoclastogenesis. Pre-osteoclasts differentiate into osteoclasts when exposed to macrophage colony-stimulating factor and RANK ligand (RANKL). Cytokines and the microenvironment can elevate or reduce osteoclastogenesis, in turn affecting bone resorption.

However, three 'hallmark' papers suggest that osteoclast development may not be so linear. These three papers propose new ways of looking at the source and lifespan of osteoclasts and their role in inflammation. Our group has also observed the effects of gender, ageing, menopause and lifestyle on osteoclasts.

Insights into developmental origin
The first of these three papers, entitled 'Developmental origin, functional maintenance and genetic rescue of osteoclasts', challenges the understanding that fetal osteoclasts form through haematopoietic stem cells (HSCs).[1] In an animal model, fetal osteoclasts were found to arise not from HSC lineage, but from the erythromyeloid progenitors (EMPs) in ossification centres. Osteoclasts present at birth were found to persist and remain viable for up to 6 months. Fusion of HSCs with pre-existing osteoclasts generates a heterotypic osteoclast with a mixture of nuclei. As the nuclei are replaced one by one over time, eventually all osteoclasts show HSC-like characteristics.

The fetal origin of osteoclasts in humans is largely unexplored, for obvious ethical reasons. Whether these also originate from EMPs is therefore unknown at present. Fetal and postnatal osteoclasts do appear to differ from adult osteoclasts, but little is known about this. It is also unclear whether osteoclasts are long-lived in humans and whether a pool of osteoclasts can ensure bone turnover for an individual's lifetime.

Another question concerns quiescent bone surfaces. Humans have many bone surfaces that are not turned over for many years, so how can existing osteoclasts move to those surfaces? If osteoclasts of different origins are also long-lived, this would have implications for bone physiology and pathophysiology in humans. This may have therapeutic importance, as shown by the partial rescue of osteopetrosis through fusion of osteoclasts with donor cells.[1] More data are needed.

The influence of inflammation
The second paper, 'Dissecting the phenotypic and functional heterogeneity of mouse inflammatory osteoclasts by the expression of *CX3CR1*', demonstrates that osteoclasts formed under inflammatory conditions may have multiple purposes.[2]

The authors had previously shown that, in inflammatory conditions, the pool of pre-osteoclasts can change so that the osteoclasts form from different types of precursors, and the resulting osteoclasts have a different phenotype.[2] CX3CR1 was identified as a marker of inflammatory osteoclasts (iOCs). In this paper, they characterise iOCs and show that they increase during inflammation.

In inflammatory conditions, osteoclasts can be derived from dendritic cells and form osteoclasts that are CX3CR1– and CX3CR1+, which play a role in bone loss. The study shows that those that are CX3CR1– may effectively resorb bone, while also activating inflammatory T cells. The CX3CR1+ iOCs may not resorb bone as well, and may suppress inflammatory T cells. However, this has not yet been tested on a bone substrate (only on hydroxyapatite-coated glass substrates).

In humans, iOCs are poorly investigated and understood. There has been some relevant research in rheumatoid arthritis at the precursor level, but less is known about how the resulting osteoclasts behave. Inflammatory osteoclasts may contribute to several diseases where inflammation plays a role, such as osteoporosis, rheumatoid arthritis and cancer-induced bone disease. There is a lack of suitable markers to identify osteoclasts in humans, which limits further exploration. Whether iOCs respond differently to drugs would be an interesting question to explore.

Recycling of osteoclasts via osteomorphs
The final paper, 'Osteoclasts recycle via osteomorphs during RANKL-stimulated bone resorption', has attracted considerable debate, highlighting the level of interest (and relatively small body of knowledge) in this topic. Osteoclasts are known to undergo fission *in vitro* and *in vivo*. This paper reports that fission of existing osteoclasts is observed only in the presence of RANKL.[3] Re-fusion of these smaller osteoclasts, known as osteomorphs, was also found to be dependent on RANKL. When mice were treated with osteoprotegerin–Fc fusion protein (OPG-Fc), this prevented re-fusion of existing osteomorphs. *In vivo*, 2-week treatment with OPG-Fc resulted in an accumulation of osteomorphs. When OPG-Fc was removed, the osteomorphs fused back into functional osteoclasts.

Regarding the earlier question of whether osteoclasts could be long-lived in humans, who have much larger bones and longer lifespans than mice, one hypothesis is that osteomorphs may play a role in recycling osteoclasts from an original pool. Osteomorphs may also play a role in transporting existing osteoclasts to new sites where there is no current bone turnover.

'These three papers propose new ways of looking at the source and lifespan of osteoclasts and their role in inflammation.'

It is not known whether this recycling may be a challenge for drug efficiency. It is possible that, when osteoclasts are put under stress, they respond by undergoing fission and thereby escaping inhibition, then reform when the environment improves. It is also possible that osteomorphs play a role in the rebound observed after the termination of denosumab, an antibody to RANKL with similar effects to the OPG-Fc treatment administered in this study.

Osteomorphs only form in the presence of RANKL, and therefore the osteomorphs that form when denosumab treatment begins can only form before RANKL levels begin to drop. It is not clear whether osteomorphs can accumulate and persist long enough to re-fuse after years of denosumab treatment. Stringent markers are required to investigate osteomorph behaviour in humans.

Several projects are considering these questions.

Osteoclasts absorb bone in two ways
Our group's own research has shown that osteoclasts make pits through two modes of resorption:

- by remaining immobile while making the pit and then moving
- in 'trench mode', where they glide over the bone surface during resorption.[4]

In an investigation of the resorptive behaviour of osteoclasts generated from 28 different healthy osteoclast donors, no difference was found in overall osteoclast activity between male and female subjects.[5] However, the type of resorption varied considerably, with osteoclasts in males making more trenches than those of females. It has since been shown that cathepsin K gene expression affects whether osteoclasts make trenches or not. Cathepsin K levels tend to be higher in the osteoclasts of males than in those of females.

Another investigation looked at resorption and bone biomarker levels in healthy donors, to further assess differences in osteoclast behaviour.[6] With increasing age, osteoclasts resorbed more aggressively *in vitro*. The higher the level of bone formation marker P1NP (procollagen type 1 N-terminal peptide) *in vivo*, the less the osteoclast resorbed *in vitro*. Trench osteoclasts were found to correlate with *in vivo* characteristics, while pit osteoclasts did not.

Osteoclasts seem to have different intrinsic properties to resorb bone, depending on age (and years since menopause) and the bone formation marker levels *in vivo*. This could contribute to understanding of individual differences in age-induced bone loss.

Bisphosphonate sensitivity
The zoledronic acid sensitivity of the osteoclasts was also tested. Depending on the donor, the osteoclasts showed more than 200-fold variance in sensitivity. Smoking habits explained some of the variation: current smokers had osteoclasts that were 20-fold less sensitive than those of people who did not smoke.

An epigenome-wide association study based on DNA from the same donors has found a smoking-dependent association of DNA methylation with zoledronic acid sensitivity: 59 CpG methylation sites were identified, reflecting 37 genes. When compared with existing genome-wide association study analysis in the UK Biobank, looking for signals associated with bone or body size-related traits, there was found to be an overlap. Current research focuses on whether these genes could be used to predict zoledronic acid sensitivity in patients.

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How to handle discontinuation of denosumab

Elena Tsourdi *Dresden, Germany*

Denosumab is a monoclonal antibody against RANK ligand (RANKL), which is the key mediator of osteoclast formation, function and survival. Denosumab was approved for use in clinical practice following the 10-year extended FREEDOM Trial.[1] For the first 3 years of the trial, women with postmenopausal osteoporosis were treated either with 60mg denosumab every 6 months or with a placebo. For the next 7 years, all patients received denosumab. The trial primarily investigated the long term safety and efficacy of denosumab.

Bone mineral density (BMD) in the lumbar spine increased by 21.7% in those receiving denosumab for the full period, compared with 16.5% in the placebo–denosumab crossover group. Cumulative total hip BMD increased by 9.2% in the long term denosumab group; it dipped in the crossover group in the first 3 years before increasing by 7.4% overall. The yearly incidence of new vertebral fractures was lower in the long term denosumab group than in the crossover group. Serious adverse effects were very low in both groups. These long term data highlighted the efficacy and tolerability of denosumab over 10 years.

Denosumab discontinuation
Unlike bisphosphonate therapy, treatment with denosumab is reversible. This means that, when the drug is discontinued, there is a rapid increase in bone turnover, and bone mass gained during treatment is lost. This is estimated to occur within the first 12–24 months after discontinuation.

A 2011 study of women with postmenopausal osteoporosis showed that the BMD of the lumbar spine, radius and total hip rapidly decreased following discontinuation of denosumab.[2] These patients maintained more BMD than the placebo group, but lost the majority of gains made during denosumab treatment, because of the 'overshoot' in bone turnover. Serum concentrations of the bone resorption marker CTX (C-terminal telopeptide of type 1 collagen) and bone formation marker P1NP (procollagen type 1 N-terminal peptide) also increased in the first 3–6 months after discontinuation, and returned to baseline by month 48.

Bone loss after denosumab discontinuation is more accentuated in cases with:

- higher baseline CTX levels
- younger age
- longer treatment duration with denosumab
- lower body mass index
- no prior antiresorptive treatment
- previous history of fracture.

Several case reports have described unexpected fragility fractures in patients discontinuing denosumab, with a majority being multiple vertebral fractures in women.

A post-hoc analysis of the FREEDOM Trial and extension investigated fractures in patients discontinuing denosumab.[3] The vertebral fracture rate was lower in the long term denosumab group, both during and after treatment, compared with those who switched from denosumab to a placebo. However, the rate of multiple vertebral fractures was higher in the denosumab group after discontinuation, compared with the placebo group. The study found that prior vertebral fracture was the strongest predictor for multiple fractures after discontinuing denosumab.
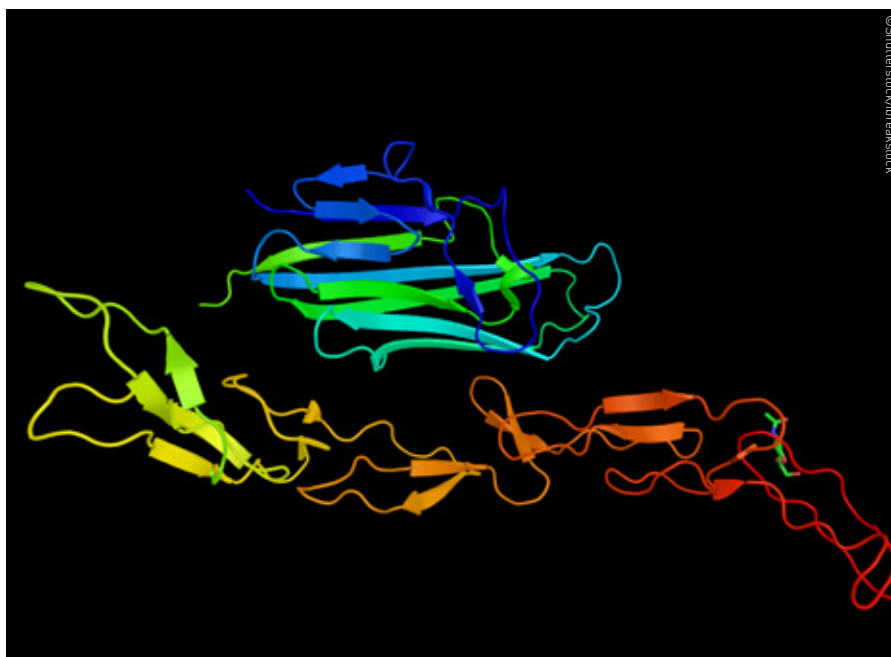
Figure. RANKL, the key mediator of osteoclast formation, function and survival, and the target of the drug denosumab in the treatment of osteoporosis.

Treatment after denosumab

Treatment to preserve bone after short term denosumab therapy (defined as up to 2.5 years) includes the bisphosphonate alendronate. Treatment with denosumab for 12 months followed by alendronate has been shown to maintain BMD at both the lumbar spine and the hip.

Raloxifene is a weaker anti-resorptive treatment. A 2022 study found that, when raloxifene was given for 12 months after discontinuing denosumab, postmenopausal patients had a significantly lower BMD at all sites compared with 6 months after their last denosumab injection. There was also a rebound in bone turnover markers, although this small observational study was not sufficient to investigate fracture incidence. This suggests that raloxifene is not potent enough to retain BMD gains.

Zoledronate is an alternative, potent post-denosumab treatment option. In a randomised controlled trial, discontinuation of denosumab after 2.2 years of treatment led to osteopenia in postmenopausal women with osteoporosis.[4] Patients were randomised for treatment with one infusion of zoledronate or two more denosumab injections, and followed for another 12 months. At 24 months, BMD had not changed from baseline in the zoledronate group, but had significantly decreased in the denosumab group. Treatment with zoledronate 6 months after the last denosumab injection prevented bone loss for a further 2 years.

A recent randomised controlled phase 4 trial looked at patients treated with a combination of teriparatide and denosumab for 9 months, followed by denosumab alone for 6 months, then one treatment with zoledronic acid at 12 months. BMD was maintained at all sites for at least 12 months after zoledronic acid was administered, but had decreased by month 27.

These studies suggest that zoledronic acid can counteract the overshoot in bone turnover in patients who received short term denosumab treatment.

However, this does not seem to be the case in patients who have been treated with denosumab for longer periods. In the ZOLARMAB Study, patients who received denosumab for 4.5 years were given zoledronic acid 6 or 9 months after their last denosumab injection or as part of an observational group (individuals whose bone turnover markers increased significantly, whose BMD loss was more than 5% or who sustained fragility fractures). After a year, BMD had decreased in all groups. Bone loss was higher in those given zoledronic acid 9 months after their last denosumab treatment and in the observational group. Therefore, one zoledronic acid infusion cannot fully compensate for bone loss after long term treatment with denosumab. This was confirmed in another 8-year observational study.

Clinical recommendations

Ceasing denosumab may be an option for patients who do not have a high risk of fracture, but it should be followed by a course of bisphosphonate treatment. Bone loss should be expected if patients stop denosumab after long term treatment. To counteract potential bone loss, it makes sense to set a higher BMD target before considering switching to bisphosphonates. A post-hoc analysis of the FREEDOM Trial proposed a treat-to-target approach. This suggested a BMD T-score threshold of −1.5.

The European Calcified Tissue Society made detailed recommendations in its 2020 position paper.[5] To summarise:

• Young patients with a low risk of fracture should not be given denosumab.

• Following short term treatment with denosumab (up to 2.5 years), oral bisphosphonates such as alendronate could be considered for 1–2 years after the last denosumab injection, if there are no contraindications. Alternatively, a 5-mg zoledronate infusion could be considered, 6 months after last denosumab injection.

• Following long term treatment with denosumab (more than 2.5 years), it may be better to switch to 5mg zoledronate around 6 months after the last denosumab injection. Bone turnover markers would need to be monitored closely at 3, 6 and 12 months, potentially leading to a repeat infusion of zoledronate 6 months after the first dose. If zoledronate is not an option, treat with oral bisphosphonates for 12–24 months and monitor bone turnover markers and BMD.

If vertebral fractures occur in the first 2 years following denosumab discontinuation, vertebroplasty and monotherapy with teriparatide should be avoided. Bone loss is likely. In these cases, reinitiate denosumab, treat with zoledronate, or try a combination of denosumab and teriparatide.

Conclusions

Denosumab is a very potent drug: BMD continues to increase for up to 10 years. Fracture risks at all sites are reduced over that period, and the benefit–risk ratio is positive in patients with osteoporosis who have a high risk of fracture.

Discontinuation results in a rebound activation of bone resorption, bone loss and an increased risk of vertebral fracture. The optimal transition from denosumab to bisphosphonates is unclear. Bisphosphonates will reduce, but not completely prevent, the increase in bone turnover and loss of BMD. When considering treatment discontinuation, aim for a high enough BMD to allow for a minor loss.

Transition from denosumab to teriparatide is associated with very high bone turnover and temporary bone loss, therefore dual therapy using denosumab and teriparatide may be appropriate.

During Q&A

It was asked if other bisphosphonates besides zoledronate or alendronate could be used to prevent bone loss. It was confirmed that these two are the preferred options. Most patients have denosumab as a second-line treatment after years of bisphosphonate therapy, which may lessen the effects of denosumab discontinuation, including risk of vertebral fractures.

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Osteocytes and sclerostin

Katharina Jähn-Rickert *Hamburg, Germany*

To date, there are limited data regarding which osteoblasts differentiate to become osteocytes. Some osteoblasts die via apoptosis, some remain on the bone surface and others embed themselves in the mineralised bone matrix.

The change from osteoblast to osteocyte begins with a cuboidal active cell with no cell processes. Dendrites form on one side initially, which become embedded in the bone matrix. As the osteocyte matures, it develops an extensive dendrite formation across the entire surface and becomes fully embedded. Osteocyte differentiation can be tracked using markers including DMP1 (dentin matrix acidic phosphoprotein-1), green fluorescent protein, alkaline phosphatase, E11 and others.

A review of the lacuna–canalicular network, by which osteocytes connect to one another, showed that a healthy human skeleton has around 42 billion osteocytes, with 89 dendrites per osteocyte.[1] The combined dendrite length is around 175 000km, with a lacuna–canalicular surface area of 215m². Around 7% of osteocytes are replaced each year.

Osteocytes and bone homeostasis

Osteocytes help to maintain bone homeostasis in several ways. One of the first means discovered was mechanosensation. In early *in vitro* studies using chicken samples, osteocytes demonstrated higher stimulation in response to hydrostatic compression compared with osteoblasts and fibroblasts, releasing prostaglandin E2. Osteocytes appear to be sensitive to a pulsating fluid flow through the canalicular system, causing the release of nitric oxide, which prompts β-catenin to move to the cell nucleus.

A mouse model comparing loaded and unloaded bone showed that mechanical loading rapidly activated β-catenin signalling in osteocytes for up to 24 hours. Not every osteocyte was stimulated, suggesting both positional and temporal regulation. Removing one allele of β-catenin blunted the anabolic loading response, measured in changes to cortical thickness or an increase in bone formation in the trabecular bone.[2]

A 2008 study of ulnar loading in mice found a downregulation of sclerostin and Dickkopf-1 expression after 24 hours, varying according to the level of strain placed on the bone. Again, there was a temporal change in response, with a return to baseline after 48–72 hours.

Sclerostin is an inhibitor of the Wnt signalling pathway. Binding to two Wnt receptors (low-density lipoprotein receptor-related protein-4 (LRP4) and LRP5/6), it blocks the binding of Wnt ligands that would normally stimulate this pathway. This prevents β-catenin from reaching the nucleus and inhibits Wnt signalling (see Figure below).[3]

Anti-anabolic actions of sclerostin

Sclerostin has anti-anabolic actions: it reduces the osteoblast surface and the osteoid produced by osteoblasts, and it also acts on mineralisation, resulting in a lower bone formation rate. Sclerostin also impairs osteocyte differentiation.

Catabolic actions of sclerostin

In addition, sclerostin has catabolic actions. Unloaded cells show a higher expression of sclerostin. This leads to upregulation of RANK ligand (RANKL), thus increasing the number of osteoclasts. Downregulating RANKL blunts the effect.

There are some data to show that sclerostin induces osteoclasts and osteoclast bone resorption via the release of RANKL or via the downregulation of osteoprotegerin (OPG).

When Wnt signalling is blocked, bone resorption increases. Studies show that the deletion of β-catenin in osteoblasts leads to lower bone mass, due to increased bone resorption markers and lower OPG expression.[4]

Osteocytic osteolysis could also play a role in the catabolic actions of sclerostin. Osteocytes have been shown to remodel their own surrounding lacunar matrix. For example, a mouse model showed that, during lactation, there is an enlargement of osteocyte lacuna which indicates demineralisation of the surrounding perilacunar matrix. This seemed to be reversible post-lactation. An upregulation in osteoclast resorptive genes could also be seen, caused by acidification – and thus demineralisation – of the environment.

Osteocytic osteolysis may also play a role in microgravity-induced bone loss.

In vitro cultures with recombinant sclerostin showed that sclerostin could upregulate the resorptive phenotype of osteocytes, suggesting a role in osteocytic osteolysis.

Conclusions

Mechanical stimulation affects how sclerostin acts on the Wnt signalling pathway. This blocks bone formation, induces resorption by osteoclasts or induces an osteocytic osteolysed phenotype.

Other stimulators can also increase sclerostin expression. Some examples include high glucose levels in diabetes, glucocorticoids and secondary osteoporosis, parathyroid hormone, and pathologies such as multiple myeloma.

During Q&A

It was asked if there is any evidence that sclerostin plays a role in postmenopausal osteoporosis. While there are no conclusive data, the reduction in bone formation and increase in bone resorption suggest a possible role for sclerostin. Further research into the formation of the osteoporosis phenotype (and specifically the role of sclerostin expression in osteocyte populations) would be interesting.

It was also noted that paediatric patients with immobilising neurological conditions often lose mechanical stimulation in the skeleton. While sclerostin plays a role in bone loss and immobilisation, it is not known how this affects paediatric patients specifically.

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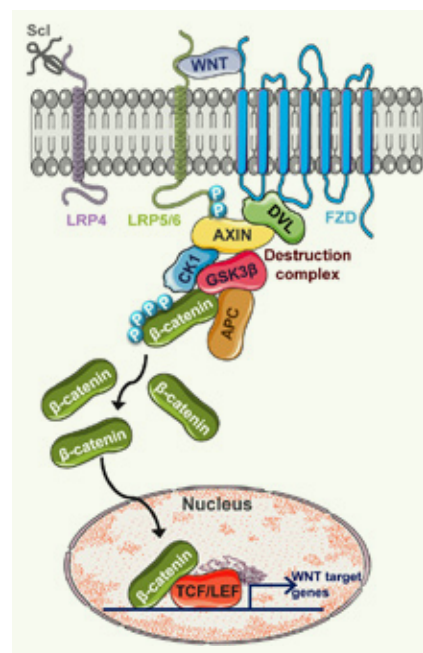4. Glass *et al.* 2005 *Developmental Cell* **8** 751–764.

Figure. Wnt ligand binds LRP5/6 and frizzled (FZD) family receptors, leading to accumulation of β-catenin, which translocates to the nucleus, triggering transcription of Wnt target genes. Sclerostin (Scl) inhibits this process by binding to LRP4 and LRP5/6. Reproduced by permission from Holdsworth *et al.*[3]

Romosozumab: opportunities and challenges

Serge Ferrari *Geneva, Switzerland*

Despite being a disease that develops over a period of 30 years, osteoporosis becomes an emergency in patients where fracture cascade is imminent. Early treatment is essential. Fracture risk increases with age, previous fracture and recency of fracture.

Anti-fracture efficacy of zoledronate
The standard of care for hip fracture patients is an infusion of zoledronic acid. A 2017 study demonstrated that zoledronate led to a reduction in secondary clinical non-vertebral fractures, hip fractures and mortality.[1]

However, no notable effect occurs until at least 16–18 months after the initial hip fracture. This means that patients are not protected by standard therapies during the time at which they are at greatest risk of refracture. The challenge is to find therapies that will restore bone strength more quickly than standard care.

Romosozumab: a new therapy
Romosozumab is a monoclonal antibody that targets and blocks sclerostin, which is an inhibitor of bone formation and promoter of bone resorption. This drug works extremely quickly. Bone biopsies analysed in the FRAME Study of postmenopausal women showed a 300% increase in bone formation rate of both trabecular bone and cortical bone within just 2 months of romosozumab being administered.[2]

Within 2 weeks of treatment, there was a 150% increase in the bone turnover marker P1NP (procollagen type 1 N-terminal peptide), and a 50% reduction in CTX (C-terminal telopeptide of type 1 collagen). However, this gradually returned to baseline levels within 6–9 months.[3]

Mechanism of action of bone-forming drugs
Commonly used bone anabolic agents, such as teriparatide and abaloparatide, stimulate bone formation through remodelling. Romosozumab has dual actions, stimulating bone modelling from scratch but also inhibiting bone resorption. This translates into a higher bone density gain.

Romosozumab has been shown to promote new bone mineralisation and prevent bone degradation at the same time. Another FRAME analysis compared fracture risk in patients treated with romosozumab and a placebo over a 12-month period. Because romosozumab's effect is reversible, patients were given romosozumab or a placebo for 12 months, then all were given denosumab for a further 12 months. In that period, lumbar spine bone mineral density (BMD) increased by 17.6% in the romosozumab group, compared with a 5% increase in the placebo group. Total hip BMD increased by 8.8% and 2.9% respectively. This represents a significant gain in patients with very low BMD and high fracture risk.[3]

However, the control group was not a high-risk population and possibly below the threshold for treatment in many countries. An analysis of a subset of patients in the FRAME Study considered the effects in a higher risk population. Again, patients treated with the romosozumab–denosumab sequence had a greater reduction in clinical and non-vertebral fractures in the first year, compared with those on the placebo–denosumab sequence.[4]

'Bone biopsies showed a 300% increase in bone formation rate within just 2 months of romosozumab being administered.'

Analysis using the FRAX® fracture risk assessment tool showed that the higher the patient's baseline risk, the greater the reduction of clinical and non-vertebral fractures when treated with romosozumab compared with placebo. This showed that targeting patients at higher risk could result in even greater benefits.

ARCH Trial
In the ARCH Trial (a phase 3 study in postmenopausal women with osteoporosis at high risk of fracture), romosozumab was compared with alendronate.[5] Patients were at higher risk than in the FRAME Study, with T-scores of −3 and a history of fractures. They were given either romosozumab or alendronate for 12 months, followed by alendronate for a further 24 months.

Again, romosozumab offered a very large gain, which was maintained when treatment switched to alendronate. Patients given alendronate alone maintained bone density, but without the initial gain offered by romosozumab. Romosozumab also appeared to reduce fracture risk by 37% after 1 year, and by 50% after 2 years, compared with the alendronate-only sequence. Risk reductions were seen across every fracture endpoint studied.

Romosozumab appears to be the most effective strategy when targeting patients at imminent risk of refracture, compared with alendronate, denosumab and teriparatide.

Adverse effects of romosozumab
Unfortunately, patients in the ARCH Trial who were treated with romosozumab then alendronate experienced more severe cardiovascular events than those treated with alendronate alone. This risk was not seen in the FRAME Study. The US Food and Drug Administration concluded that the estimated hazard of major adverse cardiovascular events is highest on romosozumab and lowest on alendronate. It is not clear whether the effect is due to chance, romosozumab risk, or a protective effect offered by alendronate. The treatment is contraindicated in patients with previous myocardial infarction or stroke.

Another challenge is that romosozumab is most effective when used as a first line treatment. It is also a more expensive treatment in most countries than all other drugs.

Conclusions
To summarise, romosozumab is a promising treatment option, offering a large and rapid gain in bone density, particularly when followed with denosumab. BMD gains are greater than any seen with other treatments, with an immediate reduction in fracture risk. This makes it a potentially useful treatment for patients at imminent risk.

It is most efficient in patients with very high fracture risk. However, those patients are likely to be older and therefore also have a higher cardiovascular risk.

During Q&A
It was noted that many patients in Japan have completed a year of romosozumab therapy. Observational data do not suggest an increased risk of cardiovascular events so far.

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Phosphate metabolism and hypophosphataemic conditions revisited

Chairs: **Martina Rauner** *(Germany)*, **Jean-Philippe Bertocchio** *(France)* & **Peter Kamenický** *(France)*

Phosphate physiology

Petra Simic *Boston, MA, USA*

Phosphate plays a vital role in bone metabolism, energy metabolism, phospholipids, nucleic acid and nucleotides, enzyme activity and the respiratory chain. Around 85% of the phosphate in the human body is found in bone and 15% in soft tissue. It weighs around 600g in total. Much more is stored than is needed.

Both low and high phosphate levels can have detrimental effects, leading to acute and chronic conditions. For example, in hypophosphataemia, patients can experience neuromuscular symptoms, including muscle weakness, tremors, paraesthesia, bone pain and seizures. Haematologic symptoms can include tissue hypoxia and bleeding. Cardiopulmonary symptoms may include weak pulse, hyperventilation and respiratory weakness. Patients may also be susceptible to anorexia and dysphagia.

Acute hyperphosphataemia can cause tetany (as a result of decreased calcium levels), tachycardia, nausea and diarrhoea. Chronic hyperphosphataemia can lead to ectopic calcification. Patients with chronic kidney disease (CKD) often have this type of soft tissue and vascular calcification. As glomerular filtration rate (GFR) reduces, patients are more likely to develop hyperphosphataemia. Around 30% of patients with CKD 4 and 50% of patients with CKD 5 have high phosphate levels. This is important, because phosphate correlates with survival: higher phosphate levels correlate with higher mortality in patients with CKD, while lower phosphate prolongs survival.

Regulation of calcium and phosphate homeostasis

Phosphate is regulated in multiple ways, including by fibroblast growth factor 23 (FGF23) from bone and bone marrow, by parathyroid hormone (PTH), and by vitamin D from the kidney (see Figure below).

FGF23 reduces phosphate reabsorption in the kidney via the proximal tubules, increasing phosphate excretion in urine and decreasing serum phosphate levels. It also decreases 1α-hydroxylase activity and calcitriol and sodium phosphate levels in the gut, leading to decreased phosphate absorption from the bowel. This reduces serum phosphate.[1]

Diseases associated with FGF23 deficiency, such as familial tumoural calcinosis, present with high levels of phosphate and vitamin D, causing extraskeletal calcification. By contrast, diseases such as rickets and osteomalacia are associated with low levels of phosphate and vitamin D, caused by an excess of FGF23.

FGF23 primarily signals via FGF-receptor 1 and Klotho co-receptors. In the case of cardiac myocytes, it signals via FGF-receptor 4. Apart from regulating phosphate, the downstream effects of excess FGF23 include bone mineralisation, reduction of leukocyte recruitment and response to infection. FGF23 also causes cardiac hypertrophy, vascular stiffness and hypertension.

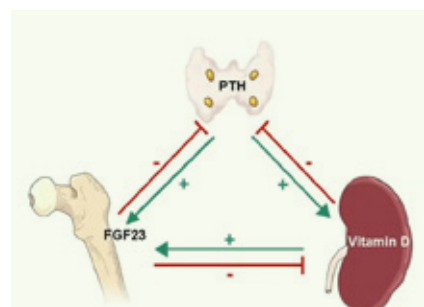Dietary phosphate increases FGF23 levels, which then drop with phosphate depletion.

Figure. Feedback loops regulating phosphate homeostasis by means of FGF23 from bone and bone marrow, PTH from the parathyroid glands and vitamin D from the kidney.

FGF23 and kidney disease

Kidney failure, anaemia and inflammation also affect FGF23. Patients who develop acute kidney injury (AKI) following cardiac surgery have been found to have increased levels of FGF23.

As with phosphate, FGF23 levels correlate with mortality in CKD. This is thought to be because FGF23 causes left ventricular hypertrophy and an increase in left ventricular mass. In kidney injury, there is a reduction in GFR and phosphate retention, which leads to elevated FGF23 levels and hyperphosphataemia. This affects vascular calcification and hypertension, which contribute to cardiovascular disease and mortality.[2]

Regulating FGF23 to improve disease outcomes

It was hypothesised that blocking FGF23 with neutralising antibodies could improve the risk of mortality. However, a mouse model showed that while FGF23 neutralisation improved CKD-associated hyperparathyroidism, it led to increased mortality rates. This was because phosphate levels increased even more, causing higher levels of aortical calcification.[3]

Currently, strategies to target hyperphosphataemia in patients with CKD focus on reducing phosphate intake through diet and the use of phosphate binders. These have a limited effect on FGF23 levels and mortality. A new trial is investigating the use of the sodium phosphate channel inhibitor ferric citrate, with promising results.[2] A further study has shown that inhibition of the type II sodium-phosphate co-transporter (NaPT2a) promotes phosphate excretion in urine and decreases phosphate in serum in a dose-dependent manner, but with a relatively short term effect.[4]

Several trials are investigating different aspects of how CKD and AKI could modulate FGF23, beyond phosphate pathways. These have shown effects relating to inflammation, low iron, anaemia and metabolism.[5]

Glycerol-3-phosphate and FGF23

The glycerol-3-phosphate (G3P) molecule is an FGF23 regulator derived from the injured kidney. G3P is a side product of glycolysis

'Around 85% of the phosphate in the human body is found in bone and 15% in soft tissue.'

and a starting material for *de novo* synthesis of triglycerides. In a 2020 study, renal venous plasma collected from patients undergoing cardiac catheterisation showed a correlation between G3P and arterial FGF23.[6] When G3P was injected into mice, FGF23 levels increased, along with an increase in urinary phosphate excretion and a decrease in serum phosphate.

G3P is a precursor of lysophosphatidic acid (LPA), which promotes osteoblast differentiation. LPA was also found to increase FGF23 in mice.

The researchers subsequently looked at glycerol phosphate acyltransferase (GPAT), the enzyme that converts G3P into LPA. Inhibiting GPAT prevented the expected increase in FGF23 in response to LPA, confirming that G3P stimulates FGF23 production through GPAT-mediated LPA production. LPA itself stimulates FGF23 production via the LPA receptor 1 (LPAR1).

An ischaemia reperfusion model was used to investigate G3P regulation in kidney injury in mice. Kidney injury led to an increase in glycolytic products, including G3P, in the kidney cortex and in the circulation. Inhibiting LPA production using ischaemic reperfusion blocked the increase in FGF23 after AKI. This suggests that LPA may be a potential target to help regulate FGF23 levels in kidney injury.

A case–control study of patients who underwent cardiac surgery compared G3P levels in patients who developed AKI and those who did not. G3P was elevated in patients who developed AKI immediately after surgery. There was a correlation between G3P and FGF23 in patients who developed AKI, but not in those without AKI. No correlation was seen between FGF23 and PTH, phosphate or vitamin D.

Current research is examining how to modulate the pathway from G3P to FGF23 via LPA and LPAR1 and develop novel phosphate regulators.

During Q&A
It was confirmed that the relationship between G3P and phosphate works both ways: phosphate also influences G3P synthesis. Some data are available to show that phosphate in kidney tubules uses the NaPT2a to enter the glycolytic pathway and affect FGF23. If the glycolytic pathway is blocked using G3P, then FGF23 does not increase. Because of this variation, glycolysis may be a sensor for phosphate in the kidney.

In mice, G3P appears to peak within 10 minutes of acute kidney injury, and reduces within 30 minutes, suggesting a short half-life. In humans, this is likely to be hours.

FGF23 increases sodium reabsorption directly via the distal tubules and in the renin–angiotensin system.

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X-linked hypophosphataemia in adults

Anne-Lise Lecoq *Paris, France*

X-linked hypophosphataemia (XLH) is a genetic disease caused by a mutation of the *PHEX* gene, which results in increased concentrations of circulating fibroblast growth factor 23 (FGF23). This leads to defects in calcitriol production and chronic hypophosphataemia.

In children, the most common clinical presentation is rickets, with impaired growth and leg bowing. Standard treatment is oral phosphate salts and active vitamin D supplements. Adults with XLH remain smaller than the general population, though disease management is improving. In severe phenotypes, children can also present with neurological complications such as craniosynostosis, Chiari 1 malformation and syringomyelia.[1] They may also present with hearing impairment, altered dental mineralisation and tooth abscesses.

In adults, osteoarticular manifestations are common, due to osteomalacia. This is a consequence of chronic hypophosphataemia and defects in calcitriol production, characterised by low bone matrix mineralisation. Standard treatment and burosumab therapy can improve bone mineralisation.

Densitometry is not very effective in diagnosing osteomalacia, since bone density is most often normal in adult patients with XLH: they do not tend to have osteoporosis. In some cases, bone mineral density may be elevated at the lumbar spine, but this may be due to increased bone volume, which compensates for low bone matrix mineralisation.

Patients may present with fractures and pseudofractures due to osteomalacia, and osteoarthritis of the hip and knee. Bone scintigraphy is useful to identify pseudofractures.

Another typical osteoarticular manifestation is enthesopathy (inflammation at the point where tendons join bones), often seen in the spine, hip or Achilles tendon. A retrospective study of the prevalence of enthesopathy in patients with XLH found an association with age, dental complications and pseudofractures, suggesting that enthesopathy may be a marker of XLH severity.[2]

Pain and decreased physical function
Patients with XLH may also present with muscle weakness in lower limbs, bone pain and joint pain. They score poorly on the WOMAC scale for pain, stiffness and physical function, compared with the general population. Fatigue levels are higher and overall quality of life is affected.

Dental manifestations in adults include spontaneous dental abscesses, taurodontism and dentin structure odontomalacia.

Some patients present with hearing loss. Conductive hearing loss is associated with impaired ossicular mineralisation, and possibly also with alterations in phosphate concentration in the endolymph and perilymph.

Endocrine manifestations
Our research focuses on the prevalence of hyperparathyroidism in XLH.[3] We compared the phosphocalcic status of 68 patients with XLH with that of controls matched for age, sex and vitamin D status. As expected, the results showed hypophosphataemia and increased FGF23 concentration in the subjects with XLH. Interestingly, serum creatinine was lower in XLH, which suggests that estimated glomerular filtration rate may not be the most appropriate tool to evaluate kidney function

in these patients. The patients also had higher concentrations of parathyroid hormone (PTH) compared with the control group. Around 25% of patients had PTH levels above the normal range.

Around 10% of the cohort had hypercalcaemic hyperparathyroidism. These patients had elevated calcium and PTH concentrations, decreased phosphate concentration, and a higher urinary ratio of calcium and creatinine. All parameters shifted to within the normal range following parathyroid surgery. Surgery is an effective way to normalise hyperparathyroidism in patients with XLH, with a long term effect.

Histological results revealed that some patients had parathyroid hyperplasia or chief cell adenoma and, less frequently, adenomatous hyperplasia or oncocytic parathyroid adenoma. Patients with hypercalcaemic hyperparathyroidism did not appear to have a higher rate of renal complications, even though the sample was too small for a formal conclusion.

Therapeutic options for hypercalcaemic hyperparathyroidism include paricalcitol and cinacalcet. The effect of burosumab on PTH concentrations is yet to be confirmed in a large sample of patients.

Pathophysiology
Research has shown an unexpected positive relationship between serum calcium and serum PTH in multiple samples from patients with XLH, illustrating a clear disruption of the physiological regulation of PTH secretion in these individuals.[3] This could be the effect of a reduction in calcitriol production, the impact of oral phosphate salts, or the result of a loss of *PHEX* in the parathyroid glands, or excess FGF23.

Obesity and overweight
The endocrine manifestations of XLH include overweight and obesity. Research to evaluate the prevalence of obesity in a cohort of 113 adult patients with XLH found that half of the cohort were overweight or obese: 21% had a body mass index (BMI) above 30, and 5% had a BMI above 40.

Dual-energy X-ray absorptiometry scans showed that both men and women had excess fat mass, including younger patients.

Obesity in patients with XLH may be considered metabolically healthy obesity. A study of metabolic complications found that these patients had better fasting glucose levels than matched controls. Amongst patients with XLH, 10% had impaired glucose tolerance, 18% demonstrated insulin resistance and only

4% had type 2 diabetes. Just 7% of patients presented with metabolic syndrome. This raises the question of whether an excess of FGF23 plays a protective role regarding metabolic complications.

Unresolved questions
High levels of FGF23 are associated with left ventricular hypertrophy in patients with chronic kidney disease. This raises questions about the risk of high blood pressure and left ventricular hypertrophy in patients with XLH. An animal model suggests this to be the case, but it has not yet been confirmed in human subjects.

There are open questions about the management of preconception and pregnancy in women with XLH. Specifically, standard treatment with oral phosphate salts appears sensible, given that the fetus requires high bone mineralisation, but this is open to discussion. Secondly, the possibility of pre-implantation diagnosis in individuals with XLH has to be discussed with geneticists, with a multidisciplinary approach.

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Tumour-induced osteomalacia and other causes of hypophosphataemia

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Tumour-induced osteomalacia (TIO), also known as oncogenic osteomalacia, is caused by phosphaturic mesenchymal tumours that are composed of spindle-shaped mesenchymal cells and giant cells. These cells are found in the extracellular matrix, which can differentiate into woven and lamellar bone. Immunohistochemical stains show that tumours associated with TIO secrete fibroblast growth factor 23 (FGF23).

Around 80% of these highly vascular mesenchymal tumours are benign, while 20% recur, of which 20% are malignant.

A 2016 study clarified the genetics of TIO.[1] Fluorescence *in situ* hybridisation analyses confirmed that there are gene rearrangements to give *FN1-FGFR1* and/or *FN1-FGF1* fusion genes in more than 40% of cases. Both genes are thought to activate a cellular signalling cascade by binding to extracellular matrix proteins, which allows for cross-linking of FGF receptors. The remaining 60% of tumours that do not have these rearrangements may have a similar mechanism underlying FGF signalling. These tumours express high levels of co-receptor Klotho. Activation of this pathway not only stimulates proliferation of these tumour cells, but also production of FGF23 by these tumours.

Other classical regulators of FGF23 have not been evaluated in the tumours associated with TIO in detail.

Clinical presentation
TIO is a paraneoplastic syndrome, causing FGF23-dependent hypophosphataemia. Tumours are often small and can go unrecognised for decades. Therefore, it

is important to check phosphate levels in patients who present with severe myopathy and osteomalacia.

A systematic review in 2022 analysed 468 articles covering 895 cases of TIO.[2] The median age of presentation was 46 years, with a range from 9 months to 90 years of age. A slight majority of cases (58.3%) were in men. Out of 754 surgically treated cases, 279 had follow up for a minimum of 6 months. Of these patients, 14.2% reported disease recurrence. Among recurrent tumours, 20% were malignant.

Biochemical severity correlates with tumour size. FGF23 levels are highest in tumours greater than 5cm. Most tumours are found in the tendons of the lower limbs (46.4%), followed by the head and neck (25.7%) and the pelvic area (10.3%) (see Figure on page 13).

Diagnostic evaluation
It is important to examine the family history of inherited phosphate disorders in patients who present with hypophosphataemia. If there is a family history, genetic disorders of impaired tubular reabsorption should be considered before measuring FGF23 levels. If there is no family history of phosphate

disorders, clinicians should aim to exclude common causes of hypophosphataemia, such as vitamin D deficiency, malabsorption, hyperparathyroidism and medications that cause hypophosphataemia, and correct these before determining FGF23 levels.[3]

If FGF23 levels are in the normal range, clinicians should exclude hereditary forms of FGF23-independent renal phosphate wasting and Fanconi syndrome. If FGF23 levels are high, clinicians should exclude hereditary forms of FGF23-dependent renal phosphate wasting, McCune–Albright syndrome and fibrous dysplasia. Following biochemical diagnosis, the next step is to search for and remove any occult tumour.

One can differentiate between genetic disorders of phosphate homeostasis based on whether they affect extracellular or intracellular phosphate homeostasis. The extracellular category can be further classified as hypophosphataemic or hyperphosphataemic disorders that are parathyroid hormone (PTH)-dependent, FGF23-dependent, or PTH-/FGF23-independent (see Table above). The FGF23-dependent disorders should be considered in the diagnosis of TIO. Disorders of intracellular phosphate homeostasis are usually normophosphataemic.[4]

The preferred way to localise tumours is with functional imaging, such as ^{111}In-DTPA-octreotide SPECT-CT, ^{68}Ga-DOTATATE PET-CT or ^{18}F–FDG PET-CT. Once localised, anatomical imaging can be helpful for surgical planning. Venous sampling can be useful if lesions are not identified or multiple lesions are suspected.

Therapy in TIO
Medical therapy consists primarily of surgical removal of the lesion. Around 4% of cases will also involve chemotherapy or radiotherapy. In preparation for surgery, or in cases where the lesion is not localised,
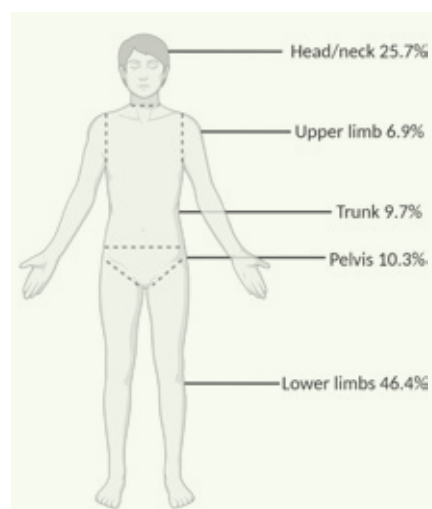
Table. Human genetic disorders associated with hypophosphataemia.[4]	
Disorder	**Mechanism**
X-linked hypophosphataemia	FGF23-dependent
Autosomal dominant hypophosphataemic rickets	FGF23-dependent
Autosomal recessive hypophosphataemic rickets types 1, 2, and 3	FGF23-dependent
Hereditary hypophosphataemic rickets with hypercalciuria	Proximal tubular phosphate wasting, FGF23-independent
Vitamin D-resistant rickets type 1A	$1,25(OH)_2D$ deficiency, FGF23-independent
Hereditary $1,25(OH)_2$–vitamin D-resistant rickets	$1,25(OH)_2D$ resistance, FGF23-independent
Familial hypocalciuric hypercalcaemia/neonatal severe hyperparathyroidism	PTH excess, FGF23-independent
Jansen disease	Constitutively active PTHR1, FGF23-dependent
PTHR1, PTH receptor 1.	

oral phosphate supplementation and calcitriol may be administered. Cinacalcet can help to reduce the requirement for phosphate supplementation if secondary hyperparathyroidism is present.

Burosumab, an FGF23-neutralising antibody, has also recently been approved for treatment of TIO. A prospective trial of 14 patients with TIO showed that burosumab completely normalised blood phosphate levels within just a few months, along with improvement of active vitamin D synthesis and normalisation of elevated alkaline phosphatase levels.

Bone biopsies at week 48 showed improved bone volume, leading to total or partial healing of metabolic bone disease in about 60% of patients. Patient-reported outcome measures and physical functioning scores also improved. Burosumab was extremely well tolerated, with an absence of severe adverse events.

However, the tumours associated with TIO progressed in 5 out of the 14 patients. For this reason, alternative experimental approaches are under consideration.[5] These include:

- blocking FGF23 action at the receptor level (e.g. BJG398/infigratinib)

- mitogen-activated protein kinase kinase 1/2 (MEK1/2) inhibitors (trametinib)

- recombinant phosphate-regulating neutral endopeptidase, X-linked (*PHEX*)

- modulation of ASARM (acidic serine–aspartate-rich matrix extracellular phosphoglycoprotein-associated motif) peptides.

In an open label, single centre, phase 2 trial, infigratinib was shown to reduce FGF23 and normalise blood phosphate levels in patients with TIO, after a single dose. Tumours were found to be metabolically less active 6 months later. However, the effects disappeared after treatment ended and hypophosphataemia recurred. Furthermore, patients experienced several severe adverse events which required dose reduction, and even discontinuation of the drug in one subject.[6]

The authors concluded that infigratinib effectively ameliorated biochemical findings

in TIO but, in contrast to standard therapy, also decreased tumoural FGF23 production. The fact that the effect reverses after drug cessation suggests that the drug is not tumoricidal with the tested regimen. A longer course of therapy at higher doses may induce persistent remission, but would be limited by treatment-related adverse events. For these reasons, long term therapy should not be advocated, except perhaps in cases of metastatic disease.

Two untested agents
Two promising agents that have not yet been tested in TIO are trametinib and a small molecule inhibitor of FGF23 signalling (ZINC12409120).

Trametinib, a MEK1/2 inhibitor, was given to a child with cutaneous skeletal hypophosphataemia syndrome and chylothorax. The drug successfully normalised phosphate metabolism, inducing a regression of recurrent chylothorax and mitigating skin manifestations. Minor adverse events included a transient increase in muscular creatine phosphokinase and serum potassium levels.

ZINC12409120 was shown via *in silico* docking to block the interaction between Klotho and FGF23, making it a candidate for lead optimisation. This compound could be particularly helpful in treating TIO bone tumours where genetic rearrangement of *FN1-FGFR1* and/or *FN1-FGF1* is not identified, but which are known to express high levels of Klotho.

During Q&A
It was confirmed that TIO is typically caused by phosphaturic mesenchymal tumours, but FGF23 can be produced by several other benign or malignant lesions.

Figure. Typical distribution of tumours in TIO.
- Head/neck 25.7%
- Upper limb 6.9%
- Trunk 9.7%
- Pelvis 10.3%
- Lower limbs 46.4%

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New therapies for hypophosphataemia due to FGF23 excess

Volha Zhukouskaya *Paris, France*

As a phosphate regulator, fibroblast growth factor 23 (FGF23) plays a significant role in hypophosphataemic disease. Cases that are related to FGF23 may be inherited or acquired.

Around 90% of patients with inherited hypophosphataemia have X-linked hypophosphataemia (XLH) and a minority have other forms of rickets or McCune–Albright syndrome. Most patients with acquired hypophosphataemia have tumour-induced osteomalacia (TIO), while a minority of cases are caused by cutaneous skeletal hypophosphataemia syndrome (CSHS) or intravenous iron preparations.

FGF23 is expressed in bone cells or produced locally in mesenchymal tumours in bone or soft tissue. FGF23 binds the FGF receptor 1 (FGFR1) in the kidney, resulting in reduced expression of the type II sodium–phosphate co-transporter. Excess FGF23 leads to a reduction in renal phosphate reabsorption and low renal synthesis of calcitriol, in turn resulting in hypophosphataemia and low blood levels of calcitriol. Clinically, this inhibits bone mineralisation.

Therapeutic strategies
For more than 50 years, hypophosphataemia has been treated with phosphate supplementation and active vitamin D analogues. This approach substitutes what is missing, rather than counteracting FGF23 expression. Treatment is administered three or four times per day. Side effects include gastrointestinal disturbance, hyperparathyroidism and kidney stones. Adherence can be poor, especially in adolescents. This treatment also fails to correct the hallmark of the disease, osteomalacia.[1]

Pathogenetic treatment has been available since 2014 in the form of burosumab. This is a monoclonal antibody which captures high levels of FGF23 and restores phosphate metabolism.

Burosumab and XLH
Burosumab has been evaluated in several clinical trials in children and adults. In three phase 2 trials in children, burosumab was shown to restore phosphate reabsorption, maintain serum phosphate levels and rescue endogenous synthesis of active vitamin D. It reduces disease activity and significantly improves radiographic signs of rickets. There is a significant decrease in alkaline phosphate and an improvement in musculoskeletal function. Burosumab also improves overall physical function and pain.

In a phase 3 trial, burosumab showed significantly higher potency to reduce disease activity and restore physical function in children with XLH, compared with conventional treatment.[2] After 64 weeks, radiographic signs of rickets were reduced by half. Performance in a 6-minute walk test increased by 9%, compared with 2% for conventional therapy.

In adults, burosumab has been shown to restore phosphate metabolism. In one study, it improved fracture healing by 63.1% after 48 weeks of treatment, compared with 35.2% with a placebo.[3] Burosumab produces much quicker improvements in stiffness, pain and physical function compared with a placebo.

In Europe, these positive results prompted approval of burosumab to treat XLH in adults and in children with signs of bone disease on X-rays. Adults may receive one subcutaneous injection per month, while children and adolescents may be given one dose every 2 weeks.

Side effects may be present but it is well-tolerated. For adults, the most common side effects are back pain, headache, tooth infection, restless legs syndrome and muscle spasms. For children, they include injection site reactions, cough, headache, pyrexia, pain in extremities and vomiting.

Burosumab and TIO
A phase 2 trial of burosumab in patients with TIO showed that the drug could restore and maintain serum phosphate levels for 144 weeks.[4] Fracture healing improved by 33% over the same period. Reduction in osteomalacia and osteoid thickness, pain and fatigue was also observed, along with an improvement in physical function.

Burosumab and other medical conditions
There have been no clinical trials involving burosumab and other medical conditions. However, two clinical case reports have shown promising results.

• The first was a child with CSHS (a condition in which elevated levels of FGF23 cause rickets that are not amenable to causal therapy).

Treatment with burosumab over 42 months showed convincing efficacy and safety, without any loss of effect or dose increase.[5]

• The second was a child with fibrous dysplasia/McCune–Albright syndrome. Burosumab treatment normalised serum phosphate and improved alkaline phosphate levels. After 17 months of therapy, bone pain and strength had improved and the patient had no fractures. No additional surgeries were needed and the patient experienced no adverse effects.

Gene therapy
Conventional treatment has adverse side effects, but burosumab is a high-cost lifelong therapy. Gene therapy may offer an alternative.

A gene therapy approach for XLH uses adeno-associated virus vectors to inhibit FGF23 signalling. It is not possible to target the kidney or bone directly, due to their tight microstructure, so this approach targets the liver. It uses C-terminal tail FGF23 because it can bind to FGFR1 but is itself biologically inactive. This blocks the FGF23 pathway and restores phosphate metabolism.

A proof of concept study published in 2021 showed that, in mice with XLH, this approach led to improved bone microarchitecture, osteoid reduction and better bone growth. It also rescued sacroiliac joint abnormalities.[6]

Next steps towards clinical development involve longer term studies to see if the effects persist and to monitor any side effects. These may also highlight any potential risk of overcorrection, resulting in hyperphosphataemia. It may be possible to test the approach for other hypophosphataemic diseases, such as TIO or McCune–Albright syndrome.

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Evolving insights in hypoparathyroidism

Chairs: **Maria Luisa Brandi** *(Italy)* & **Neil Gittoes** *(UK)*

PTH actions in bone and mineral metabolism

Marc Wein *Boston, MA, USA*

The primary function of parathyroid hormone (PTH) is to maintain normal blood calcium levels. When serum calcium dips, PTH is secreted by the parathyroid gland. PTH restores normocalcaemia by increasing bone resorption and through renal activation of vitamin D and renal calcium absorption.

Skeletal actions of PTH
Previously, bone remodelling was understood to be a balance between bone formation by cells of a mesenchymal lineage, known as osteoblasts, and bone resorption by cells of a haematopoietic lineage, called osteoclasts. Now, a third cell type is known to be involved in bone remodelling – the osteocyte. Osteocytes are the most abundant cells in bone, living deep within cortical bone tissue.

Osteocytes are the 'brains' of the bone, sensing hormonal and mechanical cues to influence bone remodelling. They are an important cellular source of RANK ligand (RANKL), which is a key factor that drives osteoclast differentiation. The osteoporosis therapy denosumab works by inhibiting this pathway. Osteocytes also make sclerostin, which inhibits the Wnt signalling pathway, in turn limiting bone formation by osteoblasts. This process is the target of the osteoporosis drug romosozumab.

PTH stimulates both bone formation and bone resorption, by turning off the expression of sclerostin and turning on the expression of RANKL. Continuous hyperparathyroidism causes a reduction in bone mass despite higher bone formation. Osteoporosis treatment with teriparatide exploits this process by inducing intermittent hyperparathyroidism, resulting in a net increase in bone mass.

PTH signalling pathways within osteocytes
Downstream of the PTH receptor, signalling mechanisms involve cAMP and the transcription factors cAMP response element-binding protein (CREB) and myocyte enhancer factor-2C (MEF2C). Until recently, little was known about their relationship.

Over the last 6 years, salt-inducible kinases (SIK) have been identified as key mediators of the intracellular action of PTH.[1] These are widely expressed kinases of the AMP-activated protein kinase family. They are referred to as salt-inducible, because SIK1 is induced via dietary salt intake.

SIK2 and SIK3 are not regulated by salt. They are expressed constitutively, induced by the upstream liver kinase B1 and suppressed by cAMP and protein kinase A (PKA). PTH signalling activates PKA, which phosphorylates SIK2 and SIK3. When these kinases are thus inhibited, their substrates become dephosphorylated and move into the nucleus, where they can regulate gene expression. Dephosphorylation of the SIK2 substrate CRTC2 (CREB-regulated transcription coactivator 2) stimulates RANKL via CREB, while dephosphorylation of the SIK3 substrates histone deacetylases 4 and 5 turns off MEF2C-driven sclerostin expression.

Development of SIK inhibitors
Since PTH signalling inhibits SIK activity, it was hypothesised that SIK deletion in bone should mimic constitutively active PTH receptor expression. In a study of SIK mutant mice, single SIK deletions showed no significant skeletal phenotypes. However, mice with deletion of both SIK2 and SIK3 showed a dramatic expansion of trabecular bone that was not seen when SIK1/SIK2 and SIK1/SIK3 are deleted. This indicates that SIK2 and SIK3 are key isoforms in mediating this skeletal activity.[2]

This led to a second hypothesis that small molecule SIK2 and SIK3 inhibitors would show PTH-like effects, which was confirmed in mice. This suggested that SIK inhibitors could be a potential mechanism for osteoporosis and hypoparathyroidism therapy.

However, developing inhibitors that target specific kinases is challenging. Kinome profiling revealed that multiple tyrosine kinases were also turned off by the SIK2/SIK3 inhibitor, which could explain toxicities seen in animals treated with this agent.[3]

Using a SIK2 homology model, *in silico* screening and iterative chemotyping, a more specific lead compound, SK-124, was developed. This has shown potent cell-based target engagement.

SK-124 was administered orally to mice, resulting in modest increases in calcium levels and trabecular bone mass, due to PTH-like effects. The compound also lowers sclerostin levels and stimulates bone formation. This suggests that SIK inhibitors could represent a new orally available bone anabolic agent to stimulate bone formation and increase calcium levels.[4]

PTH action in the kidney
PTH signalling in the proximal tubule increases $1,25(OH)_2$-vitamin D synthesis, and decreases phosphate reabsorption. In the distal tubule, PTH increases calcium reabsorption. Because SIKs are so important for the skeletal actions of PTH, it was hypothesised that they may also participate in renal actions.

Again, SIK2/SIK3 inhibitors were found to increase active vitamin D in mice. Kidney-specific SIK gene deletion resulted in increased $1,25(OH)_2$-vitamin D and mild hypercalcaemia associated with suppressed PTH. Interestingly, these mice did not show changes in blood phosphate, suggesting that SIKs participate in $1,25(OH)_2$-vitamin D generation but do not affect PTH regulation of blood phosphate in the proximal tubule. This could be described as a 'pseudo-hyperparathyroidism' phenotype.[5]

Thus, we now have a working model where PTH signalling in the proximal tubule regulates the phosphorylation of CRTC2 protein, which works with CREB to turn on the expression of *CYP27B1* and generate $1,25(OH)_2$-vitamin D.

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PTH actions in kidney and mineral metabolism

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The normal range for blood calcium is 2.10–2.55mmol/l in adults and slightly higher in infants and children. Its concentration is measured in fasting subjects. This reference range applies to the general population, but what is 'normal' for an individual will fall within a much narrower range. Sometimes serum calcium concentration is maintained despite high levels of urinary calcium. This is usually because the amount of calcium released by bone matches the amount lost in urine.

This is an efficient system: when calcium levels drop, this triggers the secretion of parathyroid hormone (PTH), which prompts the immediate release of calcium from bone and acts on the renal tubules to promote reabsorption of calcium.

Renal calcium handling in hypoparathyroidism

The kidney is central to maintaining calcium within the normal range. This is especially evident in patients with hypoparathyroidism (hypoPT). Such patients, who have low levels of PTH, are less able to reabsorb calcium normally within the kidney. In contrast, patients with high levels of PTH reabsorb more calcium than a healthy individual.

PTH receptor 1 (PTH1R) is expressed in the proximal tubule, cortical thick ascending limb, distal convoluted tubule and connecting tubules within the kidney. Calcium is reabsorbed at each of these sites, to varying degrees. While there is massive calcium reabsorption in the proximal tubule, it is not regulated by PTH.

In the cortical thick ascending limb, cells contain both PTH1R and the calcium sensing receptor, which act in opposition. Activation of PTH1R increases calcium reabsorption, while activation of the calcium sensing receptor inhibits calcium reabsorption.[1] The permeability of the paracellular pathway is determined at the tight junctions, which express small proteins known as claudins. Claudins can form pores or barriers. Claudins 16 and 19 interact to form a pore that is permeable to calcium, though it is not known how this is regulated.[2]

In the distal convoluted tubule, cells can reabsorb calcium transcellularly, thanks to a calcium-specific channel in the apical membrane that acts as a gatekeeper.[1] A binding protein of 28kDa ferries the calcium across the cell to the basolateral membrane, where it exits via the calcium–sodium exchanger. These cells also express PTH1R, which allows more calcium to enter the cell and promotes the movement of calcium between membranes.

Effects of PTH in the proximal tubule

PTH plays a role in vitamin D metabolism. Following hydroxylation of vitamin D to 25(OH)-vitamin D in the liver, it can be converted in the kidney to either the very active hormone $1,25(OH)_2$-vitamin D or the inactive hormone $24,25(OH)_2$-vitamin D. PTH strongly promotes the creation of $1,25(OH)_2$-vitamin D. Extracellular calcium/phosphate and fibroblast growth factor 23 (FGF23) inhibit this process, and promote the formation of $24,25(OH)_2$-vitamin D. Depleted PTH therefore reduces the ability to activate vitamin D.

The proximal tubule is the main location for phosphate reabsorption, and PTH also affects this. Sodium–phosphate co-transporters 2A and 2C are expressed at the apical membrane. PTH increases endocytosis of both receptors, so fewer co-transporters are available for phosphate reabsorption, thus reducing blood phosphate.[3]

FGF23 also has a role in phosphate homeostasis. It is synthesised in bone upon phosphate loading, mainly by osteocytes. Like PTH, it promotes retrieval of sodium–phosphate co-transporters from the apical membrane, so decreasing phosphate reabsorption. In contrast to PTH, FGF23 inhibits the activation of vitamin D.
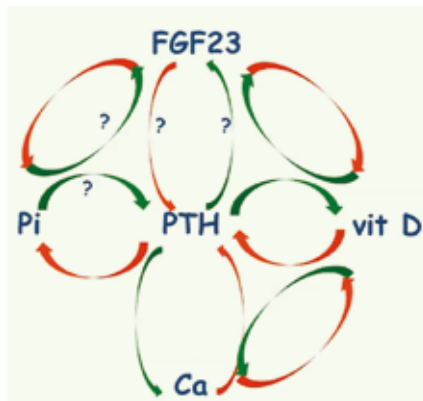

Figure. Known and possible feedback loops (green=stimulation, red=inhibition) involved in the normal hormonal control of calcium and phosphate homeostasis; vit D, $1,25(OH)_2$-vitamin D.

Calcium and phosphate homeostasis

Calcium and phosphate homeostasis is maintained through a series of hormonal feedback loops, as shown in the Figure below.

PTH increases blood calcium by promoting calcium release from bone and promoting calcium reabsorption in the kidney. Calcium inhibits PTH release via a negative feedback loop. PTH promotes activation of vitamin D, which in turn has a negative effect on PTH synthesis and release. Active vitamin D increases blood calcium via renal, bone and gut actions. Calcium inhibits the activation of vitamin D. PTH decreases blood phosphate by decreasing the kidney's ability to reabsorb phosphate. Phosphate increases FGF23, which inhibits phosphate release. It is possible that there is also a feedback loop between FGF23 and active vitamin D.

In untreated hypoPT, there is insufficient PTH to promote calcium release or to activate vitamin D. The effect on phosphate reabsorption is lost and phosphate increases.

Conventional treatment of hypoPT involves replacement calcium and active vitamin D. Despite the continuing absence of PTH, somewhat normal calcium levels can be restored. However, this is pharmacology and not homeostasis. Data presented at the Endocrine Society Annual Meeting in March 2021 showed high variability in serum calcium measurements, with patients rarely within the normal range.[4] This is consistent with findings from a 2012 study.[5]

Conclusions

Multiple mediators, including PTH, vitamin D and FGF23, are involved in renal functions that contribute to calcium and phosphate homeostasis. The loss of PTH in patients with untreated hypoPT disrupts normal relationships among calcium, phosphate and further hormonal factors. Conventional treatment is associated with significant fluctuations in serum calcium.

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Causes of hypoparathyroidism

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Hypoparathyroidism (hypoPT) is characterised by low plasma calcium levels caused by low levels of parathyroid hormone (PTH). HypoPT is normally categorised as post-surgical or non-surgical.

Post-surgical hypoPT
Around 80–90% of cases of hypoPT are post-surgical. Of these, 80–90% follow a total thyroidectomy, 10–15% follow a parathyroidectomy and the remainder follow other neck surgeries.

Diagnosis of post-surgical cases is often obvious. Following neck surgery, patients often develop symptoms of hypocalcaemia, low plasma calcium levels and low levels of PTH. A recent systematic review showed that if PTH levels remain above 10pg/ml in the first 12–24 hours after surgery, development of chronic hypoPT is very unlikely.[1]

When making a diagnosis of post-surgical hypoPT, it is important to note the starting plasma calcium levels. Low PTH levels may be wrongly attributed to suppression of the remaining parathyroid glands after longstanding hypercalcaemia when, in fact, the patient is hypocalcaemic.

Some patients are in the 'grey zone', with low–normal calcium levels and/or low–normal PTH levels. Patients with partial hypoPT do well most of the time, but may experience intermittent hypocalcaemia, for example, due to physical stress (this may also be found in non-surgical hypoPT, if parathyroid gland reserves are reduced). Hypocalcaemia may become chronic in the presence of co-morbidities, such as malabsorption or renal insufficiency. Rarely, hypoPT occurs several years after surgery.

HypoPT may also be transient. After surgery, the chances of regaining parathyroid function are best within the first month. Chronic or permanent hypoPT is defined as lasting for at least 12 months after surgery, according to the most recent guidelines.[1]

Recovery is always possible. Some patients may regain sufficient PTH synthesis several years after surgery. PTH levels should therefore be measured once a year. If PTH levels are rising measurably, the dose of calcium/alfacalcidol can be reduced.

Non-surgical hypoPT
Around 10–20% of cases of hypoPT are non-surgical, with a variety of causes. Genetic causes include isolated hypoPT or syndromic disease. HypoPT can also be caused by inherited or acquired autoimmune disease. Radiation, metastatic disease or mineral deposits can destroy the organs. Functional hypoPT occurs if there is a magnesium deficiency or excess, or if a baby is born to a mother with hypercalcaemia. Non-surgical hypoPT can also be transient (e.g. in acute illness) or idiopathic.

A small number of people have pseudo- or pseudopseudohypoPT due to PTH resistance.

Non-surgical hypoPT can be divided into isolated hypoPT or hypoPT with associated features. Autosomal dominant hypocalcaemia is one example of isolated hypoPT; it is one of the most common forms of non-surgical hypoPT. HypoPT with associated features can include autosomal dominant, autosomal recessive, mitochondrial or autoimmune diseases.

DiGeorge syndrome
This is an autosomal dominant disorder, caused by 22q11 deletion, remembered through the mnemonic CATCH-22 as it is characterised by:
- cardiac defects
- abnormal facial features
- thymic hypoplasia
- cleft palate
- hypocalcaemia.

It may be spontaneous, or autosomal inherited. It features aplasia or hypoplasia of the parathyroid glands. Not all patients have hypocalcaemia at birth, but may develop it later.

Autoimmune hypoPT
Autoimmune hypoPT can be isolated or part of autoimmune polyendocrine syndrome type 1, also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED). Parathyroid damage is caused by circulating antibodies and infiltration by lymphocytes. APECED can be caused by variants in the autoimmune regulator (*AIRE*) gene, for which there is a genetic test.

The syndrome has three major features: adrenal insufficiency, mucocutaneous candidiasis and hypoPT. A diagnosis can be made if at least two are present. Minor features include enamel hypoplasia,

pancreatitis, thyroiditis, gonadal failure and pernicious anaemia, among others. The term 'autoimmune hypoPT' should be used with caution if patients don't have a confirmed APECED diagnosis, as no specific tests are available.

Autosomal dominant hypocalcaemia
This results from a gain-of-function mutation in the calcium-sensing receptor (CaSR), expressed in several tissues (including the parathyroid glands, kidney and bone). Normally, a decrease in plasma calcium levels causes an increase in PTH secretion and vice versa. In autosomal dominant hypocalcaemia, PTH secretion will be reduced at lower calcium concentrations. This oversensitivity to plasma calcium causes hypocalcaemia with low PTH, similar to hypoPT. Urinary calcium will be higher than normal, so patients should not be treated with high doses of calcium.

The clinical phenotype is highly variable. Some patients have almost no symptoms, while others have severe hypocalcaemia and tetany. Some patients have Bartter syndrome type 5 with hypocalcaemia and metabolic alkalosis.

Encaleret is a treatment specifically targeting the CaSR, currently in development.

PseudohypoPT
PseudohypoPT presents with hypocalcaemia accompanied by grossly elevated PTH levels, due to PTH resistance. It is caused by inactivating genetic variants or epigenetic alterations in the *GNAS* gene, resulting in insensitivity to hormone actions, including PTH. Genetic diagnosis is difficult because the disease is sometimes caused by changes in methylation in *GNAS*.

Clinical characteristics can vary significantly, even among patients with the same genetic alteration. Symptoms become evident in mid- to late childhood and often go unnoticed in young children.

Despite chronically elevated PTH levels, patients do not seem to be at specific risk of osteoporosis. In the kidney, PTH resistance is pronounced in the proximal tubule, causing hyperphosphataemia (low urinary phosphate), but less pronounced in the distal tubule, so patients are not prone to hypercalciuria.

A differential diagnosis is secondary hypoPT.

Importance of an exact diagnosis
Treatment of post-surgical and non-surgical hypoPT is similar, so an exact diagnosis is not essential. However, a precise diagnosis in non-surgical hypoPT enables genetic counselling, awareness of associated disease features, exclusion of potential alternative diagnoses, and adjustment of treatment.

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Conventional therapy, complications and patient-reported outcome measures

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Conventional treatment for hypoparathyroidism (hypoPT) includes activated vitamin D analogues and calcium supplements in divided doses, or high doses of cholecalciferol, or vitamin D supplementation at lower doses, with magnesium supplementation if needed.

Parathyroid hormone (PTH) levels vary throughout the day, with lower levels in the morning and higher levels at night. Levels also change during effort, increasing by 70% during exercise. PTH causes an increase in serum calcium levels and a decrease in phosphate levels. In contrast, activated vitamin D causes serum calcium and phosphate levels to rise.

Vitamin D medications include cholecalciferol, ergocalciferol, alfacalcidol, calcitriol and dihydrotachysterol. They vary by relative potency, by start and duration of effect and, therefore, by daily dose. A randomised controlled trial comparing alfacalcidol and calcitriol in patients with hypoPT found that, at optimum calcium control, both led to high levels of serum phosphate, hypercalciuria and circulating activated vitamin D. In another study, calcitriol led to a faster and greater increase in activated vitamin D levels before dropping, compared with alfacalcidol. This may explain why calcitriol can cause renal issues.

Effects of calcium supplementation
Calcium is better absorbed in lower doses and with meals. Resorption decreases with age. Several studies have investigated the effects of different calcium sources.

One online survey asked 330 patients with hypoPT about their current and past replacement regimen in relation to calcium salts, unhydroxylated and activated vitamin D, and their experience of adverse effects. Calcium salts were associated with kidney stones, low and high calcium levels needing hospital admission, constipation and

heartburn. The authors concluded that a 'no calcium' regimen could be considered for those experiencing adverse effects from calcium supplements, if confounding factors such as hypervitaminosis D and proton pump inhibitor-induced hypomagnesaemia were eliminated.[1]

A study of 18 healthy adult men looked at acute changes in serum calcium and circulating levels of PTH induced by oral intake of five different calcium salts. The authors concluded that calcium citrate is a good option for patients with hypoPT who are taking proton pump inhibitors.

A randomised double-blind crossover trial compared calcium citrate and calcium carbonate in the management of chronic hypoPT. No difference in the calcium oxalate index or serum calcium and phosphate concentrations was observed between the two study groups. Calcium citrate was associated with a significant reduction in oxalate/creatinine ratio and with less constipation. No difference was found in quality of life (QoL) scores. Although calcium citrate did not modify the calcium oxalate index, it showed a reduction in urinary oxalate excretion that could have a beneficial effect on the risk of nephrolithiasis.

Research has shown little difference in serum calcium levels between patients given calcitriol and those given recombinant PTH.

Complications
Co-morbidities associated with chronic hypoPT are, in part, dependent on the disease, biochemical parameters and treatment. A systematic review investigated the most prevalent complications and symptoms:[2]

- cataract (17%)
- nephrocalcinosis/nephrolithiasis (15%)
- renal insufficiency (12%)
- infection (11%)
- seizures (11%)
- depression (9%)
- arrhythmia (7%)
- ischaemic heart disease (7%)
- all-cause mortality (6%).

High phosphate levels are associated with increased mortality and risk of infection, including infections in the upper airways, and reduced QoL. Increased calcium–phosphate product increases mortality, risk of renal disease, neurological dysfunction and symptoms that reduce QoL. Low calcium levels are associated with increased risk of cardiovascular disease and neurological dysfunction. Phases of hypercalcaemia are associated with increased mortality and risk of infection and cardiovascular and renal

disease, though activated vitamin D may have a protective effect.

Factors influencing QoL
Patient-reported outcome measures are used to assess patient health status at a particular point in time. They can be used to compare different treatments for hypoPT. Currently three tools are available to evaluate QoL:

- HypoPT symptom diary[3]
- HypoPT patient experience scales (HPES-symptom and HPES-impact)[4]
- Hypoparathyroid patient questionnaire (HPQ), the first questionnaire for patients with hypoPT.[5]

A trial of the HPQ 28 questionnaire in patients receiving conventional therapy showed a dose-dependent increase in depression and anxiety, pain and cramps, and numbness and tingling in relation to calcitriol.[6] The effect was independent of age, gender, underlying disease, type of surgery and values for activated vitamin D, calcium and phosphate.

The use of magnesium and calcium supplements also influenced parameters of quality of life. This shows that not all symptoms are dependent on the disease.

The Path Trial investigated QoL in hypoPT patients treated with TransConPTH. Patients treated with TransConPTH showed a clear improvement in QoL, whereas those taking a placebo did not.

A two-centre study compared conventional therapy with PTH(1–84) in relation to QoL, in patients with post-surgical hypoPT. After a year, conventional therapy led to no change in QoL measures. Recombinant PTH was associated with significant improvements in most measures.

HPQ 28 has now been investigated in 2500 healthy subjects and 100 patients with hypoPT. It is available in several languages and also as an online tool (the latter is currently only in German).

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Emerging treatments for hypoparathyroidism

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There are many exciting developments in the treatment of hypoparathyroidism (hypoPT). Unlike conventional treatment, these novel approaches focus on replacing the missing hormone with parathyroid hormone (PTH) therapy.

PTH(1–34) and PTH(1–84) are two forms of PTH replacement therapy. Both have half-lives that are too short to cover 24 hours. It has been announced that PTH(1–84) production will cease at around the end of 2024.

An alternative approach to daily subcutaneous injections is continuous administration of PTH through an insulin pump. This has not yet been approved by the US Food and Drug Administration, but has been shown to be very effective in clinical trials, resulting in normalised urinary calcium excretion and bone turnover markers.[1] However, it is a complex treatment and therefore not practical for most patients.

Current therapies do not fully replace the function of endogenous PTH. Possible solutions include:

- long-acting PTH analogues
- oral PTH or PTH receptor agonists
- stem cell therapy
- negative allosteric modulator of calcium-sensing receptor (CaSR) for autosomal dominant hypocalcaemia type 1.

Long-acting PTH analogues
Two approaches are being developed to sustain PTH action for >24 hours after a single subcutaneous injection. The first is extending the protein's plasma half-life; the second is the use of biased agonists to the PTH1 receptor, a G protein-coupled receptor (GPCR).

Extending the plasma half-life
This first approach aims to create a PTH analogue with prolonged pharmacokinetics, so it remains in the bloodstream for over 24 hours.

One method of achieving this is to increase the protein's molecular size by fusion to another molecule, such as polyethylene glycol or albumin. Alternatively, one can use prolongation technology to create a prodrug that releases the unmodified protein in a controlled way, such as TransCon technology. This binds PTH(1–34) to an inactive prodrug using a modifiable linker that enables predictable, sustained release of PTH.

Phase 2 and phase 3 trials have shown this drug to be safe and effective, achieving independence from conventional treatment. Serum calcium and phosphate levels normalised, as did mean 24-hour urinary calcium levels, and quality of life scores improved.[2]

Biased agonists to GPCRs
This approach makes use of the fact that ligands to GPCRs can exhibit a bias towards a particular signalling pathway. This bias can be exploited so that different ligands work longer on the receptor.[3] In temporal bias, distinct ligands bind selectively to distinct receptor conformations to induce signalling responses of different durations.

For the PTH receptor, the relevant conformations are R^0 and R^G. The R^0 selective ligand leads to prolonged signalling which could be an excellent treatment for hypoPT. The R^G selective ligand leads to more transient signalling, which may be a good option for the treatment of osteoporosis.

AZP-3601 is a long-acting PTH/PTH-related protein hybrid ligand that preferentially binds to and activates the R^0 conformation of the PTH receptor. A phase 2a trial in patients with hypoPT who took it daily showed a reduction in 24-hour urinary calcium over 3 months.

Oral PTH(1–34)
Oral treatments may be preferable to injections for many patients. Enterobio Pharma has developed an oral PTH, where an excipient protects the PTH(1–34) peptide from proteolysis. Results in a small, uncontrolled trial led to a 42% reduction in calcium supplements in patients with hypoPT.[4]

An alternative approach uses oral small molecule agonists to the PTH receptor. PCO371 is such a molecule, which increased calcium in animals. However, the programme was terminated due to adverse events observed in a phase 1b trial. The possibility of a small molecule agonist for the PTH receptor remains open.

Stem cell therapy
The field of PTH is also embracing the hot topic of stem cell therapy. In the future, it may be possible to generate healthy parathyroid cells from patients' fibroblasts or blood cells, reprogramme them and differentiate them to parathyroid cells *in vitro*, which can be transplanted back into the patient. Several labs are attempting this using a stepwise protocol to replicate organogenesis, as shown in the Figure below. This is likely to be a long term project.

Negative allosteric modulator of CaSR
In autosomal dominant hypocalcaemia type 1, low calcium levels are caused by activating mutations of the CaSR. They lead to hypocalcaemia because PTH is suppressed even at low serum calcium levels.

A negative allosteric modulator is a small-molecule drug that blocks the activation of the CaSR, thus enabling appropriate production of endogenous PTH. A phase 2 study has shown that the drug encaleret is quite effective. Patients with lifelong hypocalcaemia took oral encaleret twice per day. Serum calcium increased into the normal range and urinary calcium decreased.

Point-of-care measurements
Blood calcium measurements are a weak link in the current management of hypoPT, because it often takes too long to obtain results from clinical laboratories. There is great interest from patients with hypoPT in having home calcium measurement capabilities, similar to the glucose fingerstick for patients with diabetes.

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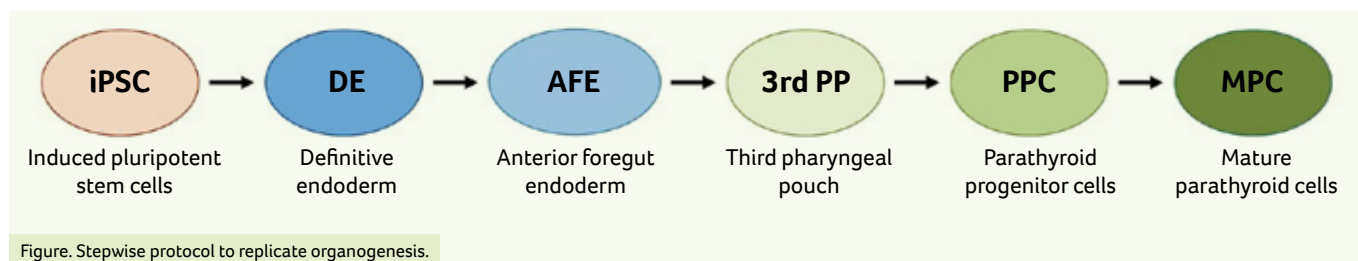4. Ish-Shalom *et al.* 2022 *Journal of Bone & Mineral Research* **36** 1060–1068.

iPSC	DE	AFE	3rd PP	PPC	MPC
Induced pluripotent stem cells	Definitive endoderm	Anterior foregut endoderm	Third pharyngeal pouch	Parathyroid progenitor cells	Mature parathyroid cells

Figure. Stepwise protocol to replicate organogenesis.

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