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In Focus

Combining exercise and growth hormone therapy: how can we translate from animal models to chronic kidney disease children?

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Renal osteodystrophy (ROD) and growth retardation are frequent complications of paediatric chronic kidney disease (CKD) secondary to an association of abnormalities including impaired growth hormone (GH) metabolism, hypo- or hyperparathyroidism, vitamin D deficiency, malnutrition, hypogonadism and drug toxicity [1]. The impact of CKD-associated bone and mineral disorders (CKD-MBDs) can be a short-term issue (abnormal mineral metabolism), but also a long-term one (ROD, fractures, impaired growth, vascular calcifications and mortality) [1]. These complications affect the quality of life because of their consequences on mental and physical well-being, but they also strongly impair mineral metabolism and bone quality and ultimately cause cardiovascular disease, which is the major cause of death in paediatric CKD [1].

Linear growth is specific to paediatric patients: it is a highly regulated process occurring through the modelling of new bone by skeletal accretion and longitudinal growth at the growth plate. Chondrocytes are a cornerstone of this process, along with GH [2]. One-third of the total growth occurs during infancy (i.e. the first 2 years of life), depending mainly on nutritional parameters [2]. Later childhood is marked by a constant growth velocity (but of lesser intensity than in the early stages); this phenomenon is driven by GH and thyroid hormone. When puberty occurs, testosterone and oestrogen further increase growth velocity. The epiphyseal cartilage goes through a process of progressive maturation during infancy, childhood and adolescence. When no additional epiphyseal cartilage remains to allow further growth of long bones, fusion occurs between the shaft and the epiphysis, ending the linear growth process.

In children, bone formation occurs by to two different mechanisms: the first mechanism is endochondral ossification (i.e. modelling of new bone by longitudinal growth and skeletal accretion from the growth plate by chondrocytes), while the second one is similar to that observed in adults, corresponding to the skeletal remodelling of existing mineralized bone tissue by osteoblasts and osteoclasts [3].

The growth plate is an avascular tissue located between the metaphyses and epiphyses in long bones; endochondral bone formation corresponds to its progressive replacement by bone. Its regulation corresponds to a complex cascade of nutritional, cellular, paracrine and endocrine factors [4]. The growth plate consists of three different zones: resting, proliferative and hypertrophic, with each zone containing chondrocytes at different stages of differentiation, i.e. small chondrocytes with low replication rates, flat chondrocytes with high replication rates and chondrocytes at the later stage of differentiation, respectively [5]. The cellular factors involved in endochondral ossification include apoptosis, autophagy, hypoxia and trans-differentiation [5]. In terms of paracrine factors involved in the proliferation and differentiation of chondrocytes, the PTH/PTH-related protein-receptor axis, the Indian Hedgehog pathway and the Runx2 transcription factors are key components of the cascade, along with fibroblast growth factor receptor and bone morphogenetic protein signalling [5]. GH and insulin-like growth factor-1 (IGF-1) are obviously the most important endocrine factors involved, but oestrogens and androgens also play a role [5]. GH exerts at least four main actions on the growth plate: (i) increased local synthesis of IGF-1 for a paracrine effect (i.e. clonal expansion of proliferating chondrocytes), (ii) a stimulating effect on cellular differentiation to convert chondrocytes into osteogenic cells, (iii) increased cellular proliferation rate and (iv) increased synthesis and deposition of proteins by chondrocytic and osteogenic

cells, thus inducing bone growth [4]. Many other signalling molecules and transcription factors have been described in this process, as recently thoroughly reviewed by Kozhemyakina *et al.* [6]. The role of another important regulator, active vitamin D sterol, especially in the context of kidney disease, is important to stress out. Indeed, 1,25-dihydroxyvitamin D [1,25(OH_2)D] has an intracrine role in endochondral ossification and chondrocyte development *in vivo*: in young rats with normal renal function, concomitant with altered chondrocyte maturation, 1,25(OH_2)D increased chondrocyte proliferation and apoptosis in terminal hypertrophic cells. Similarly, *in vitro* treatment of the chondrocytic cell line ATDC5 with 1,25(OH_2)D lowered differentiation and increased proliferation on a dose- and time-dependent manner [7].

Longitudinal growth begins with the proliferation of early chondrocytes, followed by their alignment in columns and their maturation into hypertrophic chondrocytes. The size of the columns depends on the dynamic equilibrium between cartilage production and the bone apposition rate [8]. Eventually hypertrophic cells die, inducing a break in the cartilage matrix septa, thus allowing invading cells from the ossification front (vessels, precursors of osteoblasts, osteoclasts) to settle in the primary ossification centre. Blood vessel formation is also a crucial part of this ossification process, with a key role for vascular endothelial growth factor (VEGF), but also Runx2, IGF-1 and platelet-derived growth factor [5].

CKD modifies the morphology and dynamics of the growth plate, but the data have provided inconsistent results: some authors have described a greater thickness of the growth cartilage (due to an expansion of hypertrophic chondrocytes), whereas others have found decreased thickness in young uraemic rats in comparison with controls [8]. Anyway, the increased growth plate length does not appear to be due to metabolic acidosis or malnutrition, but rather to the degree of renal failure and its duration [8]. Interestingly, the underlying type of ROD may modify the morphology of the growth plate: in animals with mild secondary hyperparathyroidism, there were no changes at the growth plate in comparison with controls, whereas in animals with severe secondary hyperparathyroidism, the thickness of the growth plate was significantly decreased [9].

Another important point to stress is the profound interference of CKD with the process of chondrocyte maturation: chondrocytes of uraemic rats display a significantly smaller size. Moreover, there is a positive correlation between the volume of chondrocytes in the terminal hypertrophic zone and longitudinal growth rate [8]. Other abnormalities have also been described in young uraemic rats, such as a disorganization of the columns, a metaphyseal irregularity at the bone/cartilage interface, modification in the expression of collagen X and a decrease of IGF-1 and GH receptor expression [8]. Initially there was no evidence for disturbed angiogenesis during the endochondral ossification in the first reports of animal models with CKD [8]. However, a recent study highlighted impaired vascularization in CKD rats, namely reduced blood vessels and blood cells in the primary ossification centre, with a marked decrease of VEGF expression [10].

Discrepancies were also observed when using calcitriol in young uraemic rats; some authors reporting a correction of the increased thickness of the growth plate [11], while others demonstrated an inhibitory calcitriol effect [9, 12]. In rats, while a treatment with recombinant human GH (rhGH) increased growth plate thickness in rats with secondary hyperparathyroidism, in contrast, the administration of calcitriol attenuated these responses, thus demonstrating that calcitriol counteracts the trophic actions of rhGH on epiphyseal growth plate cartilage in rats, modifying chondrocyte differentiation *in vivo* [9]. Interestingly, rhGH appears to have a beneficial effect on chondrocyte maturation [8], with increased IGF-1 expression and an increased number of proliferative chondrocytes [13].

In paediatric patients with CKD, growth failure develops early, affecting up to 35% of this population [14]. The causes of poor growth are multifactorial, including potentially modifiable factors (e.g. anaemia, salt-wasting, metabolic acidosis, malnutrition and hypothyroidism), but also less modifiable factors (e.g. ROD, abnormalities in the GH–IGF-1 axis, inflammation and therapies) [14].

Defects in skeletal mineralization are the first skeletal abnormality in early stages of CKD and the prevalence increases with CKD progression, being present in up to 80% of dialysis patients, but bone turnover abnormalities become apparent only at late CKD stages [15]. Furthermore, in paediatric dialysis patients, the mineralization defects persist despite therapy with active vitamin D analogues, thus it is not related to 25-hydroxyvitamin D deficiency [16]. In dialysis patients, high bone turnover (due to high serum PTH levels and 1,25(OH_2)D deficiency) is the primary skeletal lesion of paediatric ROD, present in virtually all untreated incident patients, but low turnover lesions (also known as adynamic bone disease, characterized by low PTH and alkaline phosphatase levels with increased calcium levels) may also be seen, especially when there has been vitamin D analogues and calcium salts in excess. Both low and high bone turnover have been associated with an increased risk of vascular calcifications and severe growth retardation. In humans, the impact of CKD on the growth plate has not been thoroughly studied, but marked chondroclastic erosions and abnormal vascularization have been described from autopsy studies in haemodialysis children with high bone turnover [17].

In children with CKD, diminished linear growth was observed in individuals undergoing chronic peritoneal dialysis during intermittent calcitriol therapy. In this study, evaluating 16 prepubertal patients with bone biopsy–proven secondary hyperparathyroidism who completed a 12-month prospective clinical trial of intermittent calcitriol therapy (after a 12-month period of daily calcitriol therapy), Kuizon *et al.* [18] showed that *Z*-scores for height did not change during 12 months of daily calcitriol therapy, whereas they significantly decreased from -1.8 ± 0.3 to -2.0 ± 0.3 during intermittent calcitriol therapy. The largest reductions were seen in patients who developed adynamic bone lesions. Thus, these data suggested that high-dose intermittent calcitriol therapy adversely affects linear growth, particularly in patients with adynamic bone disease. As such, the higher doses of calcitriol or the intermittent schedule of calcitriol administration may directly inhibit chondrocyte activity in the growth plate cartilage of children with end-stage renal disease. However, these data were described during an era when patients were also given large doses of

calcium-based binders, thus questioning their validity in 2016, with lower daily doses of vitamin D analogues and the availability of calcium-free phosphate binders. Indeed, the use of such binders with high doses of active vitamin D sterols in dialysis patients was associated with skeletal improvement of the lesions of secondary hyperparathyroidism without increments in serum calcium levels in dialysis patients [16].

In paediatric patients with mild to moderate renal insufficiency (CKD stages 2–4), once all metabolic abnormalities that can worsen growth (namely anaemia, hypocalcaemia, hyperphosphatemia, metabolic acidosis, vitamin D deficiency and malnutrition) are corrected, the use of supraphysiologic doses of rhGH should be discussed since this has been proved to be safe and effective in increasing growth and final adult height [19]. In addition to these effects on growth, rhGH therapy also increases bone formation rates in paediatric dialysis patients with low-turnover ROD; conversely, in patients with high-turnover ROD at baseline, calcitriol therapy attenuates the increased bone formation rate observed with rhGH therapy [20].

In this issue of *NDT*, Troib *et al.* provide new insights into the impact of therapy with GH in an experimental model of CKD [29], in addition to their 2013 study demonstrating significant inhibition of GH receptor signalling in the epiphyseal growth plate of nephrectomized rats [10]. Indeed, in this new report, they demonstrated that a combination of endurance exercise and GH therapy in a rat model of CKD improves both bone formation and linear growth. Briefly, nephrectomized young rats displayed an enlargement of the epiphyseal growth plate that was not fully corrected by treadmill exercise (except for tibial length). Exercise alone improved the expression of endochondral ossification factors, such as IGF-1, VEGF, receptor activator of nuclear factor κB ligand (RANKL) and osteocalcin. However, combining exercise with rhGH therapy increased trabecular bone volume, as well as the expression in the growth plate of runt-related transcription factor 2, RANKL and VEGF. Thus, the authors suggested that the potential benefit of combining exercise and rhGH in paediatric CKD may improve both growth and bone quality.

The potential beneficial effects of physical activity have been proposed in adults with CKD because exercise was shown to improve IGF-1 muscular expression in rat [21]. It is well known that adults with CKD are inactive compared with their healthy counterparts; in this setting, lower levels of physical activity are associated with higher mortality. In CKD, interventions targeting increased fitness and physical activity appear to be effective and may have multiple potential benefits [22]. Recommendations regarding physical activity have been incorporated into the kidney disease: improving global outcomes CKD guidelines, which suggest that patients perform 30 min of moderate-intensity exercise on most days of the week. Exercise as simple, popular and inexpensive as walking appears to be associated with significant health benefits. More vigorous exercise requires increased supervision but can be safely prescribed to patients with a broad range of comorbidities and may be associated with greater gains in health [23].

In healthy children, physical activity has been shown to be associated with better bone status and bone mass (as assessed by dual-energy X-ray absorptiometry), but not with improved growth [24, 25]. Data on physical activity in paediatric CKD are scarce [26]. Preliminary data have suggested that children and teenagers suffering from CKD display physical activity that is significantly less than the recommended levels; however, the effects of physical activity on outcomes have not been carefully evaluated in paediatric CKD patients [27]. Recent data have nevertheless shown that changes in physical activity can be associated with a significant increase in physical functioning in a 12-week intervention trial (pedometer-based) in a cohort of 44 children with CKD [28]. Even though still under investigation in terms of long-term outcomes, the incorporation of exercise programmes to increase physical activity in children with CKD appears to be a cheap clinical tool that can be used worldwide.

In conclusion, the physiology of enchondral ossification in the growth plate is a very complex, fascinating and highly regulated process. During CKD, linear growth retardation is one of the major features observed both in animal models and in patients. The complete pathophysiology of such impairment remains to be fully determined, but the results of the study by Troib *et al.* [10] highlight a possible promising therapeutic option in children. Exercise and rhGH appears to be a win/win combination for bone and growth …., and maybe the vasculature system later in life.

CONFLICT OF INTEREST STATEMENT

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