IN DEPTH REVIEW

La "triade malefica" di perdita di massa muscolare, osteoporosi e calcificazione vascolare richiede l'urgente sviluppo di nuove strategie terapeutiche

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Abstract

La malattia renale cronica (chronic kidney disease CKD) si distingue per le molteplici complicanze metaboliche, nutrizionali e cardiovascolari che la caratterizzano, comprese perdita di massa muscolare, osteoporosi e calcificazioni vascolari, e che contribuiscono alla elevata mortalità che colpisce questi pazienti. I processi patologici coinvolti in queste complicanze comprendono: il fattore della crescita 1 insulino-simile (IGF-1) o somatomedine, l'omeostasi del calcio e dei fosfati, la via del fattore di crescita fibrosi-23/ormone paratiroideo (PTH), il sistema dell'attivatore del recettore del fattore nucleare-kB (nuclear factor kB o NF-kB) e la via dell'osteoprotegerina (OPG). Questi sistemi interagiscono strettamente fra loro e possono anche risultare sinergici. La regolazione della lisi osteo-proteica e quello delle calcificazioni vascolari condividono alcuni mediatori e meccanismi, quindi sarà necessario focalizzare gli studi di ricerca su queste vie comuni. Lo scopo di questo breve riassunto è di sintetizzare le nostre conoscenze riguardo allo sviluppo di perdita di massa muscolare, osteoporosi e calcificazioni vascolari in pazienti affetti da CKD.

Parole chiave: ckd, osteoporosi

Introduzione

La malattia renale cronica (CKD) si distingue per le molteplici complicanze metaboliche e nutrizionali che la caratterizzano, compresa la perdita di massa muscolare, l'osteoporosi e le calcificazioni vascolari. Questi fattori, sia a livello di ogni singolo fattore, sia in modo sinergico come verrà illustrato, contribuiscono all'invecchiamento vascolare precoce e all'elevato tasso di mortalità tra i pazienti affetti da CKD [1] (full text), [2]. Mentre sono stati identificati alcuni dei meccanismi che mediano i processi patologici che producono ciascuna della complicanze già menzionate, diventa sempre più evidente che alcune vie fungono da 'collegamenti' che peggiorano gli outcome clinici e quindi anche la prognosi di perdita di massa muscolare, osteoporosi e calcificazioni vascolari. Esempi di tali collegamenti comprendono: il sistema dell'IGF-1, l'omeostasi del calcio e dei fosfati, la via del fattore della crescita fibrosi 23 (FGF-23) / ormone paratiroideo (PTH), il sistema dell'attivatore del recettore del fattore nucleare k-B (sistema RANK) e la via dell'osteoprotegerina (OPG). Lo scopo di questo breve riassunto è di sintetizzare gli ultimi sviluppi della nostra conoscenza dei meccanismi condivisi, talvolta sinergici, che producono 'la triade malefica' della perdita

di massa muscolare, osteoporosi e calcificazioni vascolari in pazienti affetti da CKD. Nuove strategie terapeutiche sono necessarie per migliorare tale situazione.

La perdita di massa muscolare nella ckd

Pazienti affetti da CKD possiedono scorte insufficienti di proteine, sia a livello viscerale (cioè bassi livelli sierici) sia a livello somatico (perdita di massa muscolare). Tale situazione è causata o da una riduzione nella sintesi di proteine o da un aumento nella degradazione di proteine, oppure da entrambe le cose. Studi osservazionali evidenziano come la riduzione della massa muscolare scheletrica, una componente importante nel depauperamento del bilancio proteico (protein-energy wasting o PEW) [3] frequente nella CKD e nei pazienti sottoposti a dialisi [4] (full text), sia associata ad un'elevata mortalità e morbilità in pazienti affetti da CKD [5] (full text), [6] (full text). In condizioni di catabolismo, la massa corporea magra, in maggior parte costituita dai muscoli scheletrici, viene rapidamente distrutta producendo una condizione di sarcopenia (atrofia muscolare) e di dinapenia (perdita di forza muscolare). I pazienti uremici hanno un'inefficiente utilizzo delle proteine, causato sia da un apporto inadeguato di proteine con la dieta [7] sia dall'aumentata distruzione o dalla ridotta sintesi delle proteine, tutti fattori che provocano l'atrofia muscolare [8] (full text). Studi del metabolismo muscolare e delle proteine corporee in pazienti stabili con CKD, dimostrano costantemente come la malattia produca una riduzione equilibrata sia della sintesi che della distruzione delle proteine. Tuttavia, nella malattia renale terminale (end stage renal disease o ESRD), e soprattutto in pazienti sottoposti a dialisi, il catabolismo proteico diventa preponderante [9], [10], risultando in una netta perdita di massa muscolare [11]. Il trattamento dialitico è associato a fattori catabolici come la perdita cronica di sangue e di nutrienti, compresi glucosio, aminoacidi, peptidi, proteine e vitamine solubili: tutto ciò contribuisce alla perdita delle scorte proteiche nell'ESRD. Tuttavia, la concentrazione intracellulare di aminoacidi può rimanere stabile durante l'emodialisi (HD) mediante un rilascio aumentato di aminoacidi dal catabolismo delle proteine muscolari [12] (full text).

Il sistema dell'ubiquitina-proteasoma nella perdita della massa muscolare

Esiste evidenza che la perdita di massa corporea magra sia mediata dal sistema dell'ubiquitina-proteasoma (UPS) dipendente dall' adenosina trifosfato (ATP), presente nel muscolo [13]. Durante il digiuno, e nei pazienti affetti da diabete mellito scompensato, cachessia oncologica, sepsi o lo stato uremico, il catabolismo proteico aumenta e il bilancio proteico muscolare si negativizza [14]. Tre componenti enzimatici sono necessari per legare le proteine dell'ubiquitina (Ub) destinate alla distruzione. Oltre all'enzima E1 (Ub-attivante) e l'enzima E2 (Ub-portante), l'E3 è l'enzima chiave necessario per identificare quali proteine devono essere degradate [15]. La maggior parte delle proteine muscolari è costituita da proteine miofibrillari che rappresentano la scorta di aminoacidi più importante per la sintesi di nuove proteine e per la gluconeogenesi. L'UPS degrada le proteine miofibrillari mediante l'enzima proteolitico caspasi-3 [16].

In questo modo, un importante meccanismo dell'atrofia muscolare in CKD coinvolge l'attivazione dell'UPS indotta dall'acidosi [17] (full text). Livelli maggiori di mRNA di certi componenti del sistema UPS e un pattern similare dell'espressione di geni legati all'atrofia (che vengono chiamati atrogeni), sono stati identificati in pazienti affetti da CKD [18] (full text). In vitro, è stato dimostrato come gli inibitori della proteasoma possano bloccare un aumentato catabolismo proteico nei ratti affetti da uremia cronica. L'insulino-resistenza (vedi sotto) è un'altra condizione che produce perdita di proteine muscolari ed atrofia muscolare mediante l'attivazione del sistema UPS [19] (full text).

La via GH/IGF-1 nella perdita di massa muscolare

Pazienti affetti da CKD dimostrano una varietà di anomalie metaboliche e nutrizionali, comprese anomalie dell'insulinoresistenza e dell'asse dell'ormone della crescita (GH)- insulino-simile (GH/IGF 1). L'insulina è un ormone fortemente anabolico ed inibisce il catabolismo proteico [20]. E' stato dimostrato che l'insulino-resistenza è fortemente associata al catabolismo muscolare in pazienti non-diabetici sottoposti ad HD cronica [21]. Molti pazienti affetti da CKD presentano segni di PEW, comprese la sarcopenia e la dinapenia, che potrebbero essere l'espressione di anomalie dell'asse GH/IGF-1. L'insulinoresistenza o l'utilizzo ridotto dell'insulina, [22], uniti ad anomalie dell'interazione tra insulina e IGF-1 [23], contribuiscono alla perdita di massa corporea magra in pazienti con ESRD. Intanto, la via dell'IGF-1/phosfoinositide 3-chinasi (PI3K)/Akt inibisce l'aumento (up-regulation) dei geni dell'atrofia muscolare che codificano per le ligasi dell'ubiquitina [24]. L'IGF-1 è in grado di stabilizzare gli effetti della miostatina, un'altro fattore importante nell'atrofia muscolare [25] (full text). Un'altro meccanismo strettamente legato a ridotta segnalazione dell'IGF-1 in CKD è rappresentato dalla disfunzione delle cellule satellite, ovvero cellule staminali residenti del muscolo scheletrico che fungono da precursori del muscolo scheletrico [26] (full text). Ridotti livelli di testosterone, frequentemente presente nei pazienti CKD, potrebbe provocare il catabolismo muscolare mediante un'alterata segnalazione dell'IGF-1 e l'aumentata espressione della miostatina, entrambi fattori che inibiscono la crescita muscolare [27].

Una riduzione o alterazione dell'azione del GH, che in parte rappresenta difetti della segnalazione del GH/IGF-1, è un'altra importante anomalia endocrina associata alla CKD che stimola il catabolismo muscolare e rallenta la crescita dei muscoli [28]. La somministrazione di GH aumenta la crescita dei bambini affetti da CKD e potrebbe anche migliorare il bilancio proteico nei pazienti sottoposti ad HD mediante l'aumento della massa magra corporea [29], con una riduzione inoltre, del rischio cardiovascolare [30] (full text). In più, l'esercizio fisico e l'uso degli agonisti dei recettori della grelina possono migliorare l'appetito ed aumentare il consumo di cibo in questi pazienti riducendo ulteriormente la perdita muscolare mediante i loro effetti sul sistema GH/IGF-1 [31], [32] (full text).

Altre vie regolatorie della perdita muscolare nella CKD

Alcune anomalie metaboliche frequenti nella CKD, come per esempio l'acidosi metabolica, l'insulinoresistenza e l'anoressia promuovono il catabolismo muscolare e la perdita della massa muscolare [33]. Uno dei modi in cui l'acidosi può stimolare la perdita di massa muscolare è mediante l'ossidazione irreversibile degli aminoacidi ramificati essenziali e l'attivazione della via ATP-dipendente [34]. Nell'acidosi, gli aminoacidi derivati dal muscolo vengono convertiti in glutamina, utilizzata dai reni per facilitare l'eliminazione degli acidi. L'infiammazione stimola la distruzione delle proteine e, negli stadi avanzati della CKD, sono elevati i livelli circolatori di citochine cataboliche, come IL-6 [35]. La CKD è anche caratterizzata dall'attivazione del sistema renina-angiotensina; è stato dimostrato che l'infusione di angiotensina II induce l'atrofia del muscolo scheletrico mediante l'aumento dello stress ossidativo e l'up-regulation mediata dalla miostatina e dalle E3 ligasi muscolo-specifiche, Atrogin-1 e la proteina muscle ring finger (MuRF1), risultando nella proteolisi mediata dall'ubiquitina-proteosoma e, conseguentemente, in ridotti livelli di IGF-1 in circolo e nel muscolo scheletrico [36], [37]. Il complesso proteico fattore nucleare-κB (NF-κB) inibisce la miogenesi mediante la promozione della crescita dei mioblasti e l'induzione della perdita di MyoD, uno stimolatore della differenziazione e della riparazione del muscolo [38]. La miostatina viene up-regolata nel muscolo scheletrico di pazienti affetti da CKD e questa up-regulation insieme con una accelerata perdita di miociti mediante l'apoptosi e con le variazioni della sintesi e della degradazione proteica indotte da una segnalazione alterata all'interno della via PI3-chinasi/Akt, rappresenta un meccanismo chiave che stimola la com-

parsa e la progressione della perdita di massa muscolare nei pazienti con CKD [39]. Recentemente, microRNAs (miRNAs), cioè segmenti di RNA non codificanti relativamente corti (21 a 24 nucleotidi), che agiscono come regolatori negativi sull'espressione dei geni, sono stati identificati come importanti mediatori nei processi che causano l'atrofia muscolare. Wang et al. [40] (full text) hanno riportato che la down-regulation di microRNAs, (miR-29a e miR-29b), e il successivo aumento del fattore di trascrizione factor Ying Yang-1 interferiscono con la normale differenziazione delle cellule progenitrici del muscolo, un processo necessario per la crescita muscolare, il mantenimento della sintesi proteica e la riparazione dei danni muscolari. Studi sperimentali effettuati da Xu et al. [41] hanno dimostrato che miR-486 mimetico migliorava l'atrofia muscolare nei topi affetti da CKD, indicando che l'uso dei mimetici di miR potrebbe rappresentare una nuova frontiera terapeutica per la cura dell'atrofia muscolare nella CKD. In conclusione, sono state scoperte recentemente diverse nuove vie strettamente associate all'induzione dei processi che causano l'atrofia muscolare in pazienti affetti da CKD.

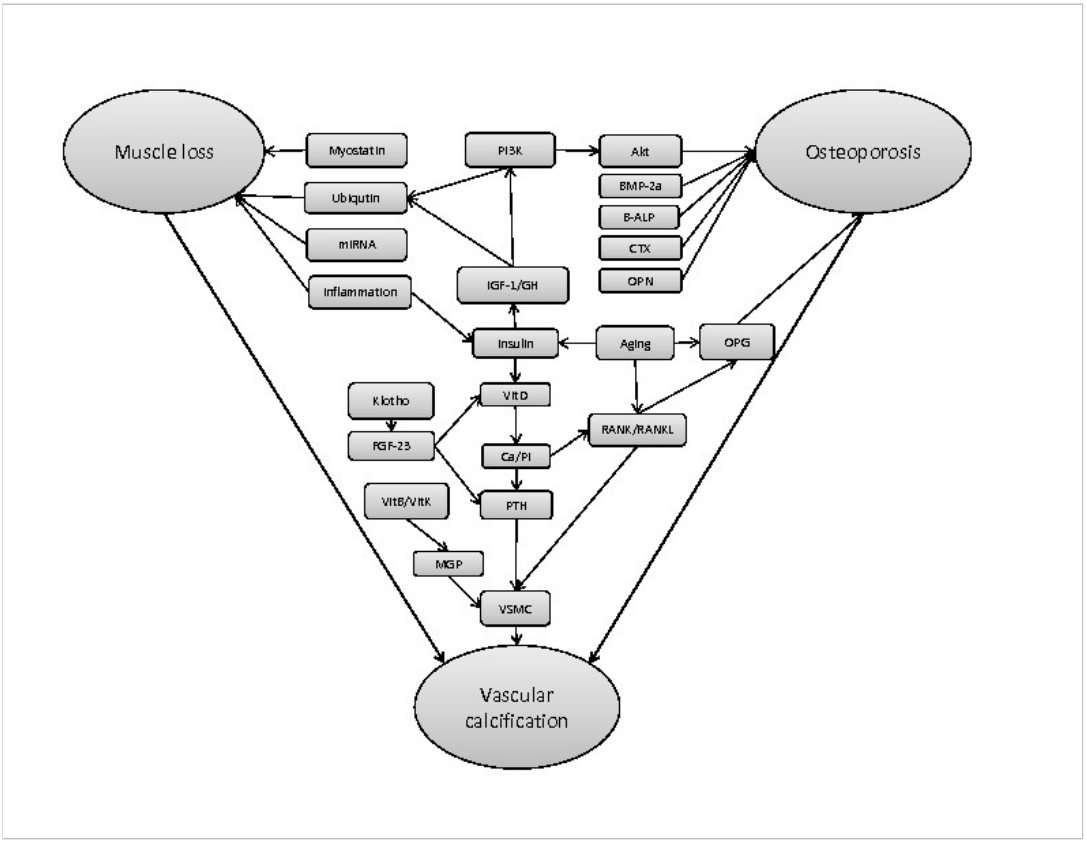

Figura 1.
Nello stato uremico, la perdita di massa muscolare, l'osteoporosi e la calcificazione vascolare sono frequentemente associate, riflettendo, in senso patologico, i meccanismi e le vie comuni che collegano i diversi sistemi, come per esempio, l'IGF/GH, il metabolismo del calcio e dei fosfati, la proteina Klotho/FGF23/PTH e le vie RANK/RANKL/OPG. Queste vie agiscono insieme,risultando nell'atrofia muscolare, la perdita di massa ossea e la calcificazione vascolare tipicamente osservate in pazienti affetti da CKD.

Abbreviazioni: BMP, bone morphogenetic protein; CTX, C-telopeptide cross-laps; FGF-23, fibrosis growth factor-23; GH, growth hormone; MGP, matrix gla protein; IGF-1,insulin-like growth hormone-1; OPG, Osteoprotegerin; Pi, phosphate; PTH, parathyroid hormone; RANK, receptor activator of nuclear-factor ?B, RANKL, receptor activator of nuclear-factor kB ligand; VitD, vitamin D; VitB, vitamin B; VitK, Vitamin K; Ca, calcium; VSMC, vascular smooth muscle cell; PI3K, phosphoinositide 3-kinase

L'osteoporosi nella ckd

Alterazioni del metabolismo minerale compaiono precocemente nella CKD, provocando variazioni della massa ossea, del ricambio osseo, dello stato di mineralizzazione e della resistenza ossea. L'osteoporosi viene definita come disturbo scheletrico caratterizzato dalla perdita di massa ossea e dal deterioramento della micro-architettura dell'osso, risultando nell'aumento del rischio di fratture, la manifestazione clinica più importante dell'osteoporosi [42] (full text). Pazienti affetti da CKD, a qualsiasi età, presentano una prevalenza maggiore di fratture vertebrali e del collo femorale rispetto alla popolazione generale, con conseguente netto aumento della mortalità e della morbilità, riflettendo probabili legami con un peggioramento dello stato nutrizionale e un aumento delle calcificazioni vascolari [43]. L'età costituisce un fattore di rischio per l'osteoporosi e per il rischio di fratture, sia nella popolazione generale che nei pazienti con CKD [44] (full text). L'alta incidenza di frattura del collo femorale in pazienti affetti da CKD stadio 5, 17 volte l'incidenza nella popolazione generale negli Stati Uniti [45], con tutta probabilità è legata all'alta prevalenza di osteopenia ed osteoporosi in pazienti affetti da ESRD [46] (full text), [47].

La diagnosi di osteoporosi viene generalmente basata sulla riduzione del contenuto minerale osseo (bone mineral density o BMD), come evidenziata dal metodo densitometrico DEXA (dual energy X-ray absorptiometry). Tuttavia, è importante ricordare come la fragilità ossea sia provocata anche da molti altri fattori, come per esempio il ricambio osseo anormale e il rimodellamento che portano a loro volta a deficit di micro-architettura ossea, e che non sono determinati dalla BMD. L'uso della BMD, quindi, rimane controverso come mezzo diagnostico per l'osteoporosi e per il rischio di fratture nei pazienti con CKD. Jamal et al. [48] non hanno trovato una correlazione tra BMD, misurata con la DEXA, e la comparsa di fratture in 104 pazienti anziani sottoposti ad emodialisi, mentre Atsumi et al. [49] hanno dimostrato invece un valore predittivo affidabile per la presenza di BMD nella colonna lombare di pazienti in emodialisi e il verificarsi di fratture vertebrali. Recentemente, alcuni studi hanno sottolineato come potrebbe non essere appropriata la definizione OMS dell'osteoporosi e dell'osteopenia per la classificazione dello status osseo in pazienti CKD stadio 4 o 5, in quanto la determinazione della BMD mediante DEXA produce dei risultati scarsamente attendibili e troppo elevati, a causa della sclerosi dei somi vertebrali e della calcificazione delle arterie di grande calibro [50] (full text). Tuttavia, se la DEXA non risulta l'esame ideale per la valutazione della qualità e della resistenza ossea [51] (full text), la biopsia ossea seguita dall'analisi istomorfometrica fornisce informazioni sia quantitative che qualitative sul rimodellamento e sullo status dell'osso, in particolare, riguardo alle variazioni micro-architettoniche nel tessuto osseo, ma è un metodo troppo invasivo per essere adoperato su larga scala nella valutazione dell'osteoporosi nei pazienti CKD [52]. Invece, nuove tecniche di imaging meno invasive, come ad esempio la tomografia computerizzata quantitativa (QCT), la risonanza magnetica ad alta risoluzione (HR-MRI) e la tomografia quantitativa periferica ad alta risoluzione (HR-pQCT) sono sempre più utilizzate negli studi clinici per la valutazione dello stato di salute osseo [53].

La via RANK/RANKL/OPG nell'osteoporosi

Il sistema RANK/RANKL/OPG gioca un'importante ruolo nella regolazione della formazione, dell'attività e della sopravvivenza degli osteoclasti nel rimodellamento osseo, sia normale che patologico. Markers biochimici, come per esempio i crosslap C-telopeptide (CTX) e la fosfatasi alcalina osteo-specifica (B-ALP), vengono utilizzati oggi nella ricerca clinica come marcatori del riassorbimento e della formazione ossea e per stimare il rischio di fratture, indipendentemente da altri metodi usati per la valutazione dell'osteoporosi, come la BMD

[54]. Gli studi *in vitro* hanno dimostrato come la citochina OPG giochi un ruolo importante nella regolazione negativa del riassorbimento osseo mediato dagli osteoclasti, risultando in un aumento della BMD e del volume osseo dovuto ad una riduzione del numero degli osteoclasti attivi [55]. Il livello di OPG aumenta con l'età, e potrebbe rivelarsi un meccanismo controregolatorio per contenere la perdità ossea nei soggetti anziani. L'OPG, ed anche la FGF-23, sono associate in modo indipendente al danno miocardico e alla aortic pulse wave velocity in pazienti affetti da CKD, fornendo un legame tra CKD-BMD e le patologie cardio-vascolari [56]. L'OPG e la leptina sono entrambe legate al controllo dell'osteoporosi, fornendo un legame tra perdità di massa ossea e massa grassa [57]. Il riscontro di un legame tra OPG e BMD del collo femorale in pazienti sottoposti ad emodialisi indica che l'OPG potrebbe essere utilizzata nello screening per la perdità di massa ossea e per la presenza di CKD-MBD in pazienti affetti da ESRD [58], [59]. L'OPG è stata anche proposta come biomarcatore nella valutazione della severità delle calcificazioni coronariche in pazienti con CKD non sottoposti ancora ad emodialisi [60] (full text). In modo dissimile all' OPG, il RANKL libero e il RANKL totale diminuiscono con l'età, grazie probabilmente ad una diminuzione dell'attività cellulare in generale con l'avanzarsi dell'età [61]. L'OPG sierica è aumentata in pazienti uremici indipendentemente dai livelli sierici di PTH [62]. In pazienti pre-dialisi affetti da CKD in fase 1-5, il RANKL sierico era inversamente correlato alla BMD del collo femorale, mentre il livello sierico dell'OPG era direttamente correlato alla BMD del collo femorale [63].

Il legame tra perdita di massa muscolare ed osteoporosi

La letteratura non ha fornito una chiara spiegazione del legame tra perdita di massa muscolare e perdita ossea. Alcuni studi hanno dimostrato una chiara associazione tra la massa ossea e il tessuto magro [64] [65] [66], ma altri non lo hanno confermato [67], [68]. L'osteoporosi è associata alla sarcopenia nei soggetti anziani [69] (full text), probabilmente a causa dell'impatto sul rimodellamento osseo della minore forza di carico del tessuto magro [70] (full text). L'IGF-1 è anche un regolatore importante della crescita ossea ed è stato indicato come potenziale marker per identificare una ridotta massa ossea nelle donne in pre e in post menopausa [71]. Uno studio recente ha dimostrato che la via IGF-1/Akt è coinvolta nell'atrofia muscolare legata all'osteoporosi, indicando che la BMD potrebbe essere utilizzata come marker nutrizionale dell'atrofia muscolare nei pazienti osteoporotici [72]. Nella CKD e nella ESRD, soltanto una manciata di studi hanno indicato un rapporto positivo tra la concentrazione di IGF-1 e la BMD [73] (full text). Una regolare e mirata attività fisica, soprattutto l'esercizio sotto carico, è un altro fattore che può essere promosso per il mantenimento di una buona qualità dell'osso con, probabilmente, una conseguente riduzione del rischio di fratture [31].

Si è visto che un aumento dei fosfati nella dieta dei topi aumenta le strutture trabecolari e corticali dell'osso, causando un aumento del rischio di fratture [74], e ciò potrebbe essere di importanza clinica per i pazienti affetti da CKD. Il trattamento anti-osteoporosi con bifosfonati consigliato per pazienti con CKD per la prevenzione di fratture è stato anche consigliato per tentare di ridurre la progressione di calcificazione extra-ossea e per inibire lo sviluppo di aterosclerosi; tuttavia, nella CKD avanzata, I bifosfonati dovrebbero essere impiegati con cautela in pazienti selezionati [75] (full text). Mentre la vitamina D non sembra ridurre l'incidenza di fratture in pazienti con ESRD, gli studi effettuati sugli animali dimostrano che la vitamina D potrebbe ridurre i livelli sierici di PTH con conseguente miglioramento della forza ossea [76] (full text), indicando che la vitamina D potrebbe migliorare la BD in pazienti affetti da CKD.

Le calcificazioni vascolari

La calcificazioni vascolari, chiamate anche "ossificazioni", in quanto le modifiche vascolari sono simili a tessuto osseo, sono conseguenza di uno squilibrio della mineralizzazione che accade molto precocemente nei pazienti con CKD se confrontati con soggetti con la funzione renale normale [77] (full text). Nella CKD di qualsiasi stadio, le calcificazioni vascolari sono associate ad un aumentato rischio di mortalità [78] (full text). Le calcificazioni si verificano a livello dell'intima o della media, (sclerosi di Monckeberg) o in entrambi questi strati. Lo strato mediale è composto da cellule del muscolo liscio vascolare (vascular smooth muscle cells o VSMC) e una matrice ricca di elastina. Le calcificazioni arteriose provocano un irrigidamento della parete arteriosa con conseguente aumento della pulse wave velocity (PWV) carotide-femorale ed aumento della pressione arteriosa. Nei pazienti con CKD, sono associate ad un aumentato rischio di perdita di massa ossea e di mortalità [79] (full text), [80] (full text). Le calcificazioni dei tessuti molli e, in particolare modo la calcificazione vascolare, sono manifestazioni importanti di un insieme di disturbi della mineralizzazione e dell'osso nella CKD, conosciuta come la sindrome CKD-MBD [81].

Il ruolo del GF-23/PTH e dell'omeostasi Ca/Pi nella calcificazione vascolare

Le vie del fosfato-FGF-23-PTH giocano un ruolo importante nel regolamento del metabolismo dei fosfati e nello sviluppo della calcificazione vascolare nei pazienti con CKD. Negli studi basati sulle popolazioni, il FGF-23 è associato in modo indipendente e positivo alla calcificazione aortica e coronaria nei pazienti con ESRD [82] (full text), ma in modo inverso con la progressione della calcificazione aortica [83] (full text), indicando un ruolo protettivo del FGF-23 contro lo sviluppo della iperfosfatemia. La resistenza all'azione del FGF-23 durante la progressione della malattia, provoca livelli sierici di FGF-23 estremamente elevati nei pazienti con CKD, i quali, ciononostante, non riescono più a normalizzare l'iperfosfatemia [84]. E' interessante osservare come mancano degli studi che documentino un valore predittivo chiaro per il FGF-23 sulla PWV, [85] sebbene un'associazione debole tra il FGF-23 e la PWV fosse stata osservata in un unico studio: tuttavia, il suo significato statistico svanì dopo un'accurata analisi multivariata [56]. In un altro studio, il FGF-23 non era associato alla calcificazione vascolare e non la iniziava [86]. Sia i fosfati che il FGF-23 sembrano essere legati alle patologie cardiovascolari mediante meccanismi distinti. E' stato osservato come la combinazione di un legante di fosfati e una restrizione dietetica dei fosfati sia in grado di ridurre i livelli sierici di FGF-23 in modo sinergico, indicando un possibile intervento per controllare gli alti livelli di FGF-23 nei pazienti con CKD [87].

L'espressione di Klotho, una proteina trans-membrana e secreta che agisce come co-fattore del recettore FGF ed viene espressa soprattutto nel rene umano, diminuisce con la progressione della CKD [88]. Ciò potrebbe spiegare, almeno in parte, la resistenza al FGF-23, e le elevate concentrazioni di FGF-23 e di fosfati nei pazienti con CKD. Klotho sopprime l'uptake sodio-dipendente dei fosfati e anche la differenziazione cellulare nelle VSMC [89] (full text): una riduzione dell'espressione genetica della klotho potrebbe contribuire quindi agli alti livelli di fosfati e alla calcificazione arteriosa nella CKD. Il FGF-23 induce l'ipertrofia ventricolare sinistra (LVH) in modo indipendente dalla klotho, indicando che alti livelli di FGF-23 potrebbero contribuire alla LVH con conseguente aumento della mortalità nei pazienti con CKD [90]. E' interessante che la klotho interferisca anche con la via insulina/IGF-1 mediante la regolazione del recettore dell'IGF [91] (full text); questo rappresenta un altro, nuovo legame che unisce lo status osseo alla calcificazione vascolare e la perdita di massa muscolare nei pazienti con CKD.

La deposizione di calcio-fosfati, prevalentemente come apatite, è presente nel miocardio, nelle valvole cardiache e nell'aorta di pazienti con ESRD [92]. Mentre si conosce il ruolo di PTH come regolatore chiave dei disturbi della mineralizzazione e dell'osso e anche della deposizione calcio-fosfatica nei pazienti con CKD, gli studi non concordano sugli effetti che il PTH potrebbe avere sulla calcificazione vascolare. Il PTH-(1-34) sembra inibire la calcificazione delle VSMC *in vivo* e anche la calcificazione vascolare in topi con assenza di recettori per le lipoproteine a bassa densità (topi LDLR-deficient) [93] (full text), [94] (full text). Un altro studi con modelli animali ha dimostrato che trattamento con PTH stimolava una intensa calcificazione della media aortica nei ratti [95]. La prevenzione della calcificazione vascolare mediante stretto controllo dei fosfati e del prodotto calcio-fosfatico è un obiettivo importante per i nuovo agenti terapeutici. Sevelamer sembra ridurre i livelli sierici di fosfati, prodotto calcio-fosfatico e PTH, e pare essere attivo anche nel prevenire la calcificazione dei tessuti molli e dell'aorta nei modelli animali di insufficienza renale [96]. Nuovi studi sono necessari per chiarire ulteriormente la complessa relazione tra il controllo del calcio-fosfati, il PTH e la calcificazione vascolare.

Studi epidemiologici hanno posto in evidenza come i livelli di vitamina D siano associati in modo inverso alla funzione endoteliale e la calcificazione aortica nei pazienti con ESRD [97]. Si è osservato come il metabolita attivo della vitamina D, 25-diidrossivitamina D3 (calcitriolo), induce la calcificazione delle cellule VSMC sia mediante la secrezione del peptide PTH-related (PTHrP), sia mediante la regolazione negativa del sistema renino-angiotensina (RAS) [98] (full text), [99]. Hirate et al [100] (full text) hanno riportato sull'efficacia del 22-oxacalcitriolo (OCT), un analogo 1, 25(OH)2D3 che possiede meno attività calcemica rispetto all' 1, 25(OH)2D3, nella soppressione dell'iperparatiroidismo secondario e della calcificazione dei tessuti molli in 5/6 ratti nefrectomizzati; i ratti trattati con OCT avevano un livello minore di calcificazione aortica e della funzionalità renale. Nel frattempo, in un grande studio basato sulla comunità, (community based) l'analogo di vitamina D, paricalcitolo, sembra essere associato a un vantaggio per la sopravvivenza se paragonato con il calcitriolo [101] (full text). Intanto, è anche vero che l'uso del calcitriolo, un down-regulator endocrino del sistema RAS, risulta in livelli sierici di calcio e fosfati più bassi, un down-regulation del sistema renina-angiotensina e l'inibizione della proliferazione del muscolo liscio [99].

Altri regolatori delle calcificazioni vascolari nella CKD

Oltre ai fattori di rischio classici per le calcificazioni vascolari, come l'iperfosfatemia, il PTH e la presenza di diabete mellito, esistono altri fattori di recente scoperta. La leptina adipocitichina sembra promuovere la differenziazione osteogenica e le calcificazioni vascolari, provocando un conseguente aumento del rischio cardiovascolare nei pazienti con CKD [102] (full text). Fetuin-A (Ahsg) è un inibitore importante della calcificazione ectopica: alti livelli di fetuin-A prevengono le calcificazioni vascolari precoci osservate nei pazienti con CKD [103] (full text). L' osteopontina (OPN) inibisce la calcificazione delle cellule VSMC [104] (full text), mentre Jono et al. [105] (full text) hanno dimostrato che la fosforilazione dell' OPN è un passo obbligatorio nell'inibizione della calcificazione delle VSMC. Contrariamente, alti livelli di osteocalcina, ALP, osteonectina e la proteina osso-morfogenetica-2a (bone morphogenetic protein o BMP)-2a sono stati associati alla trasformazione in senso osteogenetico di miofibroblasti e cellule VSMC con conseguente induzione delle calcificazioni [106] (full text), [107], [108] (full text). Ulteriori studi sono necessari per chiarire l'impatto dell'alterata espressione e funzione dei geni che codificano per queste proteine sulle calcificazioni vascolari nella ESRD. Una carenza di vitamina K, frequente in tutti gli stadi della CKD, è associata a una forma inattiva della matrice proteica Gla (MGP), a sua volta

associata ad un aumentato rischio di calcificazione vascolare [109] (full text). La vitamina K agisce come co-fattore durante la reazione enzimatica che consente a componenti di glutammato specifico (glu) nella MGP di essere trasformati in componenti di gamma-carbossiglutammato (gla), che inibiscono la calcificazione della parete arteriosa [110] (full text). Supplementi dietetici di vitamina K inibiscono la calcificazione vascolare nei topi con CKD [111] e in pazienti affetti da ESRD [112], indicando un potenziale ruolo nella terapia medica dei pazienti uremici. L'iperomocisteinemia probabilmente costituisce un legame tra l'osso e la calcificazione vascolare [113]; tuttavia, la terapia con l'acido folico e vitamine del gruppo B ad alto dosaggio non ha ridotto l'incidenza di patologie vascolari né ha migliorato la sopravvivenza dei pazienti con ESRD [114] e il ruolo dell'iperomocisteinemia in relazione alla comparsa di calcificazioni vascolari nella CKD rimane da definire. Recentemente, uno studio ha evidenziato che FT3 (triiodotirossina libera) è strettamente associata all'irrigidamento della arterie, alle calcificazione delle coronarie e alla mortalità, indicando un legame tra l'ormone tiroideo e le calcificazioni vascolari (Meuwese et al in press, AJKD 2013).

Legami tra la via -RANK/RANKL/OPG ossea e vascolare e la via FGF-23

Negli ultimi dieci anni, l'evidenza clinica suggerisce che i deficit del metabolismo osseo e minerale legati alle calcificazioni vascolari nei pazienti con CKD siano legati a certe proteine osteo-correlate, come per esempio B-ALP, osteocalcina, OPN, e Runx2. La presenza di tali proteine è stata osservata all'interno di lesioni vascolari calcificate. Pare sempre più probabile che la via RANK/RANKL/OPG, una regolatrice chiave nella formazione dell'osso, sia anche coinvolta nelle calcificazioni vascolari. Osteoblasti e cellule T attive sintetizzano RANKL, e RANK viene espresso dagli osteoclasti, dalle cellule endoteliali e dalle cellule VSMC. In alcuni studi, si è osservato come il sistema RANKL sia legato agli eventi avversi cardiovascolari e anche alla calcificazione delle arterie coronarie [115] (full text), [116] (full text). Il sistema RANK/RANKL promuove la calcificazione delle VSMC anche direttamente [117] (full text) mediante l'attivazione della via NF-κB e il rilascio di TNF e IL-6, mentre l'OPG inibisce la calcificazione vascolare [118] (full text). Nei pazienti in fase avanzata di CKD, cellule osteoclasta-simili esprimono fosfatasi acida tartrato-resistente (TRAP) nelle lesioni calcificate e il sistema RANK/RANKL stimola direttamente la formazione di cellule osteoclasta-simili positive per TRAP, indicando che siano gli osteoclasti a stimolare la calcificazione vascolare [119] (full text). Alcuni trattamenti medici per l'osteoporosi, come i bifosfonati (pirofosfati-analoghi), il denosumab (un inibitore monoclonale del RANKL) ed una proteina di fusione ricombinante dell'OPG, sembrano inibire le calcificazioni vascolari [120], [121] (full text). La CKD, e soprattutto l'ESRD, sono associate ad alti livelli sierici di FGF-23 e ad una ridotta attività della proteina klotho. Il FGF-23 (e anche l' 1, 25-diidrossivitamina D) aumentano il rischio di fratture [122], la demineralizzazione ossea post-trapianto [123] (full text) mentre la carenza della proteina klotho contribuisce ad un'invecchiamento precoce con calcificazioni arteriose ed osteoporosi [124]. I legami fisiopatologici e biologici tra le anormalità ossee e vascolari sembrano indicare l'esistenza di molteplici fattori regolatori comuni per entrambi i sistemi.

Conclusioni

La perdita di massa muscolare, l'osteoporosi e le calcificazioni vascolari sono tre problemi molto comuni nei pazienti affetti da CKD. Singolarmente, ma soprattutto insieme, rappresentano fattori prognostici negativi per questi pazienti e sono collegati fra loro mediante molteplici vie biochimiche condivise. Pertanto, dato il legame tra i meccanismi che governano la regolazione sia della distruzione delle proteine, che la crescita ossea e le calci-

ficazioni vascolari, sembra probabile che presto vedremo nuovi approcci diagnostici, preventivi e terapeutici per curare questa 'triade malefica', anche contemporaneamente.

Ringraziamenti

Vorremo ringraziare il Centro Diabetico del Karolinska Institutet, il Consiglio Svedese per le Ricerche, le Fondazioni Loo e Hans Osterman e Stig e Gunborg Westman. Jia Ting riceve una borsa di studio dal Consiglio Cinese per l'Istruzione (2009601096). Bengt Lindholm lavora per il Baxter Healthcare Corporation. Baxter Novum è stato fondato presso il Karolinska Institutet grazie a una donazione dal Healthcare Corporation.

Bibliografia

[1] Sarnak MJ, Levey AS, Schoolwerth AC et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 2003 Oct 28;108(17):2154-69 (full text)

[2] Stenvinkel P, Larsson TE Chronic Kidney Disease: A Clinical Model of Premature Aging. American journal of kidney diseases : the official journal of the National Kidney Foundation 2013 Jan 25;

[3] Fouque D, Kalantar-Zadeh K, Kopple J et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney international 2008 Feb;73(4):391-8

[4] Qureshi AR, Alvestrand A, Danielsson A et al. Factors predicting malnutrition in hemodialysis patients: a cross-sectional study. Kidney international 1998 Mar;53(3):773-82 (full text)

[5] Wolfe RR The underappreciated role of muscle in health and disease. The American journal of clinical nutrition 2006 Sep;84(3):475-82 (full text)

[6] Stenvinkel P, Heimbürger O, Paultre F et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney international 1999 May;55(5):1899-911 (full text)

[7] Dwyer JT, Cunniff PJ, Maroni BJ et al. The hemodialysis pilot study: nutrition program and participant characteristics at baseline. The HEMO Study Group. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 1998 Jan;8(1):11-20

[8] Meireles CL, Price SR, Pereira AM et al. Nutrition and chronic renal failure in rats: what is an optimal dietary protein? Journal of the American Society of Nephrology : JASN 1999 Nov;10(11):2367-73 (full text)

[9] Garibotto G, Bonanni A, Verzola D et al. Effect of kidney failure and hemodialysis on protein and amino acid metabolism. Current opinion in clinical nutrition and metabolic care 2012 Jan;15(1):78-84

[10] Lim VS, Bier DM, Flanigan MJ et al. The effect of hemodialysis on protein metabolism. A leucine kinetic study. The Journal of clinical investigation 1993 Jun;91(6):2429-36

[11] Carrero JJ, Stenvinkel P, Cuppari L et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2013 Mar;23(2):77-90

[12] Raj DS, Welbourne T, Dominic EA et al. Glutamine kinetics and protein turnover in end-stage renal disease. American journal of physiology. Endocrinology and metabolism 2005 Jan;288(1):E37-46 (full text)

[13] Mitch WE, Goldberg AL Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. The New England journal of medicine 1996 Dec 19;335(25):1897-905

[14] Pupim LB, Flakoll PJ, Majchrzak KM et al. Increased muscle protein breakdown in chronic hemodialysis patients with type 2 diabetes mellitus. Kidney international 2005 Oct;68(4):1857-65

[15] Ardley HC, Robinson PA E3 ubiquitin ligases. Essays in biochemistry 2005;41:15-30

[16] Du J, Wang X, Miereles C et al. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. The Journal of clinical investigation 2004 Jan;113(1):115-23

[17] Pickering WP, Price SR, Bircher G et al. Nutrition in CAPD: serum bicarbonate and the ubiquitin-proteasome system in muscle. Kidney international 2002 Apr;61(4):1286-92 (full text)

[18] Lecker SH, Jagoe RT, Gilbert A et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2004 Jan;18(1):39-51 (full text)

[19] Wang X, Hu Z, Hu J et al. Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling. Endocrinology 2006 Sep;147(9):4160-8 (full text)

[20] May RC, Kelly RA, Mitch WE et al. Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. The Journal of clinical investigation 1987 Apr;79(4):1099-103

[21] Siew ED, Pupim LB, Majchrzak KM et al. Insulin resistance is associated with skeletal muscle protein breakdown in non-diabetic chronic hemodialysis patients. Kidney international 2007 Jan;71(2):146-52

[22] Pupim LB, Heimbürger O, Qureshi AR et al. Accelerated lean body mass loss in incident chronic dialysis patients with diabetes mellitus. Kidney international 2005 Nov;68(5):2368-74

[23] Glass DJ Molecular mechanisms modulating muscle mass. Trends in molecular medicine 2003 Aug;9(8):344-50

[24] Stitt TN, Drujan D, Clarke BA et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Molecular cell 2004 May 7;14(3):395-403

[25] Frost RA, Lang CH Protein kinase B/Akt: a nexus of growth factor and cytokine signaling in determining muscle mass. Journal of applied physiology (Bethesda, Md. : 1985) 2007 Jul;103(1):378-87 (full text)

[26] Zhang L, Wang XH, Wang H et al. Satellite cell dysfunction and impaired IGF-1 signaling cause CKD-induced muscle atrophy. Journal of the American Society of Nephrology : JASN 2010 Mar;21(3):419-27 (full text)

[27] Carrero JJ, Stenvinkel P The vulnerable man: impact of testosterone deficiency on the uraemic phenotype. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2012 Nov;27(11):4030-41

[28] Mak RH, Cheung WW, Roberts CT Jr et al. The growth hormone-insulin-like growth factor-I axis in chronic kidney disease. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society 2008 Feb;18(1):17-25

[29] Garibotto G, Barreca A, Russo R et al. Effects of recombinant human growth hormone on muscle protein turnover in malnourished hemodialysis patients. The Journal of clinical investigation 1997 Jan 1;99(1):97-105

[30] Feldt-Rasmussen B, Lange M, Sulowicz W et al. Growth hormone treatment during hemodialysis in a randomized trial improves nutrition, quality of life, and cardiovascular risk. Journal of the American Society of Nephrology : JASN 2007 Jul;18(7):2161-71 (full text)

[31] Andreoli A, Celi M, Volpe SL et al. Long-term effect of exercise on bone mineral density and body composition in post-menopausal ex-elite athletes: a retrospective study. European journal of clinical nutrition 2012 Jan;66(1):69-74

[32] Deboer MD, Zhu X, Levasseur PR et al. Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. Endocrinology 2008 Feb;149(2):827-35 (full text)

[33] Guarnieri G, Barazzoni R Fighting protein-energy wasting in chronic kidney disease: a challenge of complexity. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2011 Jan;21(1):2-6

[34] Mitch WE, Medina R, Grieber S et al. Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. The Journal of clinical investigation 1994 May;93(5):2127-33

[35] Stenvinkel P, Ketteler M, Johnson RJ et al. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. Kidney international 2005 Apr;67(4):1216-33

[36] Song YH, Li Y, Du J et al. Muscle-specific expression of IGF-1 blocks angiotensin II-induced skeletal muscle wasting. The Journal of clinical investigation 2005 Feb;115(2):451-8

[37] Grune T, Merker K, Sandig G et al. Selective degradation of oxidatively modified protein substrates by the proteasome. Biochemical and biophysical research communications 2003 Jun 6;305(3):709-18

[38] McFarlane C, Plummer E, Thomas M et al. Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism. Journal of cellular physiology 2006 Nov;209(2):501-14

[39] Verzola D, Procopio V, Sofia A et al. Apoptosis and myostatin mRNA are upregulated in the skeletal muscle of patients with chronic kidney disease. Kidney international 2011 Apr;79(7):773-82

[40] Wang XH, Hu Z, Klein JD et al. Decreased miR-29 suppresses myogenesis in CKD. Journal of the American Society of Nephrology : JASN 2011 Nov;22(11):2068-76 (full text)

[41] Xu J, Li R, Workeneh B et al. Transcription factor FoxO1, the dominant mediator of muscle wasting in chronic kidney disease, is inhibited by microRNA-486. Kidney international 2012 Aug;82(4):401-11

[42] Tarantino U, Capone A, Planta M et al. The incidence of hip, forearm, humeral, ankle, and vertebral fragility fractures in Italy: results from a 3-year multicenter study. Arthritis research & therapy 2010;12(6):R226 (full text)

[43] Klawansky S, Komaroff E, Cavanaugh PF Jr et al. Relationship between age, renal function and bone mineral density in the US population. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2003 Jul;14(7):570-6

[44] Stehman-Breen CO, Sherrard DJ, Alem AM et al. Risk factors for hip fracture among patients with end-stage renal disease. Kidney international 2000 Nov;58(5):2200-5 (full text)

[45] Brauer CA, Coca-Perraillon M, Cutler DM et al. Incidence and mortality of hip fractures in the United States. JAMA : the journal of the American Medical Association 2009 Oct 14;302(14):1573-9

[46] Alem AM, Sherrard DJ, Gillen DL et al. Increased risk of hip fracture among patients with end-stage renal disease. Kidney international 2000 Jul;58(1):396-9 (full text)

[47] Stehman-Breen CO, Sherrard D, Walker A et al. Racial differences in bone mineral density and bone loss among end-stage renal disease patients. American journal of kidney diseases : the official journal of the National Kidney Foundation 1999 May;33(5):941-6

[48] Jamal SA, Chase C, Goh YI et al. Bone density and heel ultrasound testing do not identify patients with dialysis-dependent renal failure who have had fractures. American journal of kidney diseases : the official journal of the National Kidney Foundation 2002 Apr;39(4):843-9

[49] Atsumi K, Kushida K, Yamazaki K et al. Risk factors for vertebral fractures in renal osteodystrophy. American journal of kidney diseases : the official journal of the National Kidney Foundation 1999 Feb;33(2):287-93

[50] Miller PD Fragility fractures in chronic kidney disease: an opinion-based approach. Cleveland Clinic journal of medicine 2009 Dec;76(12):715-23 (full text)

[51] Nickolas TL, Stein E, Cohen A et al. Bone mass and microarchitecture in CKD patients with fracture. Journal of the American Society of Nephrology : JASN 2010 Aug;21(8):1371-80 (full text)

[52] Barreto FC, Barreto DV, Moyses RM et al. Osteoporosis in hemodialysis patients revisited by bone histomorphometry: a new insight into an old problem. Kidney international 2006 May;69(10):1852-7

[53] Pelletier S, Chapurlat R Optimizing bone health in chronic kidney disease. Maturitas 2010 Apr;65(4):325-33

[54] Vasikaran S, Eastell R, Bruyère O et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2011 Feb;22(2):391-420

[55] Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997 Apr 18;89(2):309-19

[56] Ford ML, Smith ER, Tomlinson LA et al. FGF-23 and osteoprotegerin are independently associated with myocardial damage in chronic kidney disease stages 3 and 4. Another link between chronic kidney disease-mineral bone disorder and the heart. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2012 Feb;27(2):727-33

[57] Pobeha P, Ukropec J, Skyba P et al. Relationship between osteoporosis and adipose tissue leptin and osteoprotegerin in patients with chronic obstructive pulmonary disease. Bone 2011 May 1;48(5):1008-14

[58] Doumouchtsis KK, Kostakis AI, Doumouchtsis SK et al. Associations between osteoprotegerin and femoral neck BMD in hemodialysis patients. Journal of bone and mineral metabolism 2008;26(1):66-72

[59] Jiang JQ, Lin S, Xu PC et al. Serum osteoprotegerin measurement for early diagnosis of chronic kidney disease-mineral and bone disorder. Nephrology (Carlton, Vic.) 2011 Aug;16(6):588-94

[60] Morena M, Jaussent I, Halkovich A et al. Bone biomarkers help grading severity of coronary calcifications in non dialysis chronic kidney disease patients. PloS one 2012;7(5):e36175 (full text)

[61] Kerschan-Schindl K, Wendlova J, Kudlacek S et al. Serum levels of receptor activator of nuclear factor kappaB ligand (RANKL) in healthy women and men. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2008 Aug;116(8):491-5

[62] Kazama JJ, Shigematsu T, Yano K et al. Increased circulating levels of osteoclastogenesis inhibitory factor (osteoprotegerin) in patients with chronic renal failure. American journal of kidney diseases : the official journal of the National Kidney Foundation 2002 Mar;39(3):525-32

[63] Fahrleitner-Pammer A, Dobnig H, Dimai HP et al. The effect of RANKL and OPG on bone mineral density in pre-dialysis chronic renal failure. Clinical nephrology 2009 Jun;71(6):652-9

[64] Mussolino ME, Gillum RF Low bone mineral density and mortality in men and women: the Third National Health and Nutrition Examination Survey linked mortality file. Annals of epidemiology 2008 Nov;18(11):847-50

[65] Takada H, Washino K, Iwata H et al. Risk factors for low bone mineral density among females: the effect of lean body mass. Preventive medicine 1997 Sep-Oct;26(5 Pt 1):633-8

[66] Andreoli A, Bazzocchi A, Celi M et al. Relationship between body composition, body mass index and bone mineral density in a large population of normal, osteopenic and osteoporotic women. La Radiologia medica 2011 Oct;116(7):1115-23

[67] Reid IR, Evans MC, Ames RW et al. Volumetric bone density of the lumbar spine is related to fat mass but not lean mass in normal postmenopausal women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1994 Nov;4(6):362-7

[68] Lindsay R, Cosman F, Herrington BS et al. Bone mass and body composition in normal women. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 1992 Jan;7(1):55-63

[69] Cruz-Jentoft AJ, Baeyens JP, Bauer JM et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing 2010 Jul;39(4):412-23 (full text)

[70] Wardlaw GM Putting body weight and osteoporosis into perspective. The American journal of clinical nutrition 1996 Mar;63(3 Suppl):433S-436S (full text)

[71] Liu JM, Zhao HY, Ning G et al. IGF-1 as an early marker for low bone mass or osteoporosis in premenopausal and postmenopausal women. Journal of bone and mineral metabolism 2008;26(2):159-64

[72] Terracciano C, Celi M, Lecce D et al. Differential features of muscle fiber atrophy in osteoporosis and osteoarthritis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2013 Mar;24(3):1095-100

[73] Park SH, Jia T, Qureshi AR et al. Determinants and survival implications of low bone mineral density in end-stage renal disease patients. Journal of nephrology 2013 May 16;26(3):485-94 (full text)

[74] Lau WL, Linnes M, Chu EY et al. High phosphate feeding promotes mineral and bone abnormalities in mice with chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2013 Jan;28(1):62-9

[75] Toussaint ND, Elder GJ, Kerr PG et al. Bisphosphonates in chronic kidney disease; balancing potential benefits and adverse effects on bone and soft tissue. Clinical journal of the American Society of Nephrology : CJASN 2009 Jan;4(1):221-33 (full text)

[76] Rix M, Eskildsen P, Olgaard K et al. Effect of 18 months of treatment with alfacalcidol on bone in patients with mild to moderate chronic renal failure. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2004 Apr;19(4):870-6 (full text)

[77] Goodman WG, Goldin J, Kuizon BD et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. The New England journal of medicine 2000 May 18;342(20):1478-83 (full text)

[78] London GM, Guérin AP, Marchais SJ et al. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2003 Sep;18(9):1731-40 (full text)

[79] Blacher J, Guerin AP, Pannier B et al. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999 May 11;99(18):2434-9 (full text)

[80] Blacher J, Pannier B, Guerin AP et al. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. Hypertension 1998 Sep;32(3):570-4 (full text)

[81] Moe S, Drüeke T, Cunningham J et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international 2006 Jun;69(11):1945-53

[82] Nasrallah MM, El-Shehaby AR, Salem MM et al. Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2010 Aug;25(8):2679-85 (full text)

[83] Tamei N, Ogawa T, Ishida H et al. Serum fibroblast growth factor-23 levels and progression of aortic arch calcification in non-diabetic patients on chronic hemodialysis. Journal of atherosclerosis and thrombosis 2011;18(3):217-23 (full text)

[84] Larsson T, Nisbeth U, Ljunggren O et al. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. Kidney international 2003 Dec;64(6):2272-9

[85] Desjardins L, Liabeuf S, Renard C et al. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2012 Jul;23(7):2017-25

[86] Scialla JJ, Lau WL, Reilly MP et al. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney international 2013 Feb 6;

[87] Isakova T, Barchi-Chung A, Enfield G et al. Effects of Dietary Phosphate Restriction and Phosphate Binders on FGF23 Levels in CKD. Clinical journal of the American Society of Nephrology : CJASN 2013 Mar 7;

[88] Karsli Ceppio?lu S, Yurdun T, Canbakan M et al. Assessment of matrix Gla protein, Klotho gene polymorphisms, and oxidative stress in chronic kidney disease. Renal failure 2011;33(9):866-74

[89] Hu MC, Shi M, Zhang J et al. Klotho deficiency causes vascular calcification in chronic kidney disease. Journal of the American Society of Nephrology : JASN 2011 Jan;22(1):124-36 (full text)

[90] Faul C, Amaral AP, Oskouei B et al. FGF23 induces left ventricular hypertrophy. The Journal of clinical investigation 2011 Nov;121(11):4393-408

[91] Château MT, Araiz C, Descamps S et al. Klotho interferes with a novel FGF-signalling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in Caenorhabditis elegans. Aging 2010 Sep;2(9):567-81 (full text)

[92] Giachelli CM The emerging role of phosphate in vascular calcification. Kidney international 2009 May;75(9):890-7

[93] Jono S, Nishizawa Y, Shioi A et al. Parathyroid hormone-related peptide as a local regulator of vascular calcification. Its inhibitory action on in vitro calcification by bovine vascular smooth muscle cells. Arteriosclerosis, thrombosis, and vascular biology 1997 Jun;17(6):1135-42 (full text)

[94] Shao JS, Cheng SL, Charlton-Kachigian N et al. Teriparatide (human parathyroid hormone (1-34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. The Journal of biological chemistry 2003 Dec 12;278(50):50195-202 (full text)

[95] Neves KR, Graciolli FG, dos Reis LM et al. Vascular calcification: contribution of parathyroid hormone in renal failure. Kidney international 2007 Jun;71(12):1262-70

[96] Katsumata K, Kusano K, Hirata M et al. Sevelamer hydrochloride prevents ectopic calcification and renal osteodystrophy in chronic renal failure rats. Kidney international 2003 Aug;64(2):441-50

[97] Mizobuchi M, Ogata H, Koiwa F et al. Vitamin D and vascular calcification in chronic kidney disease. Bone 2009 Jul;45 Suppl 1:S26-9

[98] Jono S, Nishizawa Y, Shioi A et al. 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. Circulation 1998 Sep 29;98(13):1302-6 (full text)

[99] Li YC, Kong J, Wei M et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. The Journal of clinical investigation 2002 Jul;110(2):229-38

[100] Hirata M, Makibayashi K, Katsumata K et al. 22-Oxacalcitriol prevents progressive glomerulosclerosis without adversely affecting calcium and phosphorus metabolism in subtotally nephrectomized rats. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2002 Dec;17(12):2132-7 (full text)

[101] Teng M, Wolf M, Lowrie E et al. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. The New England journal of medicine 2003 Jul 31;349(5):446-56 (full text)

[102] Zeadin M, Butcher M, Werstuck G et al. Effect of leptin on vascular calcification in apolipoprotein E-deficient mice. Arteriosclerosis, thrombosis, and vascular biology 2009 Dec;29(12):2069-75 (full text)

[103] Heiss A, DuChesne A, Denecke B et al. Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. The Journal of biological chemistry 2003 Apr 11;278(15):13333-41 (full text)

[104] Speer MY, McKee MD, Guldberg RE et al. Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. The Journal of experimental medicine 2002 Oct 21;196(8):1047-55 (full text)

[105] Jono S, Peinado C, Giachelli CM et al. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. The Journal of biological chemistry 2000 Jun 30;275(26):20197-203 (full text)

[106] Cheng SL, Shao JS, Charlton-Kachigian N et al. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. The Journal of biological chemistry 2003 Nov 14;278(46):45969-77 (full text)

[107] Giachelli CM Ectopic calcification: new concepts in cellular regulation. Zeitschrift fur Kardiologie 2001;90 Suppl 3:31-7

[108] Jono S, McKee MD, Murry CE et al. Phosphate regulation of vascular smooth muscle cell calcification. Circulation research 2000 Sep 29;87(7):E10-7 (full text)

[109] Holden RM, Morton AR, Garland JS et al. Vitamins K and D status in stages 3-5 chronic kidney disease. Clinical journal of the American Society of Nephrology : CJASN 2010 Apr;5(4):590-7 (full text)

[110] Price PA, Faus SA, Williamson MK et al. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arteriosclerosis, thrombosis, and vascular biology 1998 Sep;18(9):1400-7 (full text)

[111] McCabe KM, Booth SL, Fu X et al. Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. Kidney international 2013 May;83(5):835-44

[112] Westenfeld R, Krueger T, Schlieper G et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. American journal of kidney diseases : the official journal of the National Kidney Foundation 2012 Feb;59(2):186-95

[113] Herrmann M, Peter Schmidt J, Umanskaya N et al. The role of hyperhomocysteinemia as well as folate, vitamin B(6) and B(12) deficiencies in osteoporosis: a systematic review. Clinical chemistry and laboratory medicine : CCLM / FESCC 2007;45(12):1621-32

[114] Jamison RL, Hartigan P, Kaufman JS et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. JAMA : the journal of the American Medical Association 2007 Sep 12;298(10):1163-70

[115] Schoppet M, Schaefer JR, Hofbauer LC et al. Low serum levels of soluble RANK ligand are associated with the presence of coronary artery disease in men. Circulation 2003 Mar 25;107(11):e76; author reply e76 (full text)

[116] Crisafulli A, Micari A, Altavilla D et al. Serum levels of osteoprotegerin and RANKL in patients with ST elevation acute myocardial infarction. Clinical science (London, England : 1979) 2005 Oct;109(4):389-95 (full text)

[117] Panizo S, Cardus A, Encinas M et al. RANKL increases vascular smooth muscle cell calcification through a RANK-BMP4-dependent pathway. Circulation research 2009 May 8;104(9):1041-8 (full text)

[118] Deuell KA, Callegari A, Giachelli CM et al. RANKL enhances macrophage paracrine pro-calcific activity in high phosphate-treated smooth muscle cells: dependence on IL-6 and TNF-?. Journal of vascular research 2012;49(6):510-21 (full text)

[119] Byon CH, Sun Y, Chen J et al. Runx2-upregulated receptor activator of nuclear factor ?B ligand in calcifying smooth muscle cells promotes migration and osteoclastic differentiation of macrophages. Arteriosclerosis, thrombosis, and vascular biology 2011 Jun;31(6):1387-96 (full text)

[120] Nitta K, Akiba T, Suzuki K et al. Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. American journal of kidney diseases : the official journal of the National Kidney Foundation 2004 Oct;44(4):680-8

[121] Morony S, Tintut Y, Zhang Z et al. Osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in ldlr(-/-) mice. Circulation 2008 Jan 22;117(3):411-20 (full text)

[122] Mirza MA, Karlsson MK, Mellström D et al. Serum fibroblast growth factor-23 (FGF-23) and fracture risk in elderly men. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2011 Apr;26(4):857-64

[123] Kanaan N, Claes K, Devogelaer JP et al. Fibroblast growth factor-23 and parathyroid hormone are associated with post-transplant bone mineral density loss. Clinical journal of the American Society of Nephrology : CJASN 2010 Oct;5(10):1887-92 (full text)

[124] Kuro-o M Klotho as a regulator of oxidative stress and senescence. Biological chemistry 2008 Mar;389(3):233-41

IN DEPTH REVIEW

The triple whammy of muscle loss, osteoporosis and vascular calcification in chronic kidney disease patients calls out the need for novel treatment strategies

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Abstract

Chronic kidney disease (CKD) is characterized by numerous metabolic, nutritional and cardiovascular complications including loss of muscle mass, osteoporosis and vascular calcification contributing to the high mortality among these patients. Factors involved in the underlying pathology processes resulting in these complications such as insulin-like growth factor-1 system, calcium and phosphate homeostasis, fibrosis growth factor-23/parathyroid hormone pathway, receptor activator of nuclear-factor κB system and osteoprotegerin pathway are closely interrelated and may act synergistically. Thus, the regulation of protein degradation, bone loss and vascular calcification share several common mediators and mechanisms, underlining the need for research approaches focusing on these shared mechanisms and pathways. This brief review aims at summarizing advances in our current understanding of the basis for development of muscle loss, osteoporosis and vascular calcification and also the possible connections among them in the CKD patients.

Key words: chronic kidney disease, osteoporosis

Introduction

Chronic kidney disease (CKD) is characterized by numerous metabolic and nutritional complications including muscle loss, osteoporosis and vascular calcification, which separately – and in synergy as will be discussed in the following – contribute to premature vascular aging and high mortality among CKD patients [1] (full text), [2]. Whereas several specific mechanisms mediating pathology processes leading to each of these complications have been identified, it has become apparent that some pathways also may act as bridges amplifying the clinical and prognostic consequences of muscle loss, osteoporosis and vascular calcification. Examples of such links include: insulin-like growth factor-1 (IGF-1) system, calcium and phosphate homeostasis, fibrosis growth factor-23 (FGF-23) / parathyroid hormone (PTH) pathway and receptor activator of nuclear-factor κB (RANK) system and osteoprotegerin (OPG) pathway. This brief review summarizes current advances in our understanding of the often shared and sometimes synergistic mechanisms leading to the triple whammy of muscle loss, osteoporosis and vascular calcification in CKD patients which we believe calls out the need for novel treatment strategies.

Muscle loss in chronic kidney disease

Patients with CKD have low visceral (i.e. low serum protein concentration) and low somatic (i.e., loss of muscle mass) protein stores, caused by a decrease in protein synthesis or an increase in protein degradation, or both. Observational reports suggest that a decrease of skeletal muscle mass, an important component of protein-energy wasting (PEW) [3] frequently found in CKD and dialysis patients [4] (full text), is associated with high morbidity and mortality in CKD patients [5] (full text), [6] (full text). In catabolic conditions, lean body mass, i.e., mainly skeletal muscle, degrades rapidly, resulting in skeletal muscle atrophy (sarcopenia) and loss of muscle strength (dynapenia). In uremic patients, both inadequate dietary protein intake [7] and increased protein degradation or reduced synthesis leading to low efficiency of protein utilization are the major factors contributing to muscle atrophy [8] (full text). Muscle and whole body protein turnover studies in stable CKD have consistently shown that there is a balanced reduction in protein synthesis and degradation; however, in end-stage renal disease (ESRD), and especially in patients on dialysis treatment, a condition of net protein catabolism is induced [9], [10], resulting in accelerated net muscle protein loss [11]. Dialysis treatment is associated with catabolic factors such as chronic losses of blood and nutrients, such as glucose, amino acids, peptides, proteins and soluble vitamins, during dialysis contributing to loss of protein stores in ESRD. However, the intracellular amino acid concentration can be maintained during hemodialysis (HD) by augmented release of amino acids from muscle protein catabolism [12] (full text).

Ubiquitin-proteasome pathway in muscle loss

There is evidence that loss of lean body mass is mediated by the action of the adenosine triphosphate (ATP)-dependent ubiquitin-proteasome system (UPS) in muscle. [13] In fasting state, and in patients with uncontrolled diabetes mellitus, cancerous cachexia, sepsis or uremic, the breakdown of protein increases and muscle protein balance becomes negative [14]. Three enzymatic components are required to link ubiquitin (Ub) proteins that are destined for degradation. Besides E1 (Ub-activating) enzyme and E2 (Ub-carrier) enzyme, E3 is the key enzyme accounting for the specificity of proteins to be degraded [15]. Myofibrilla proteins comprise most of muscle protein, representing the major store of amino acids for new protein synthesis and gluconeogenesis. UPS readily degrades the myofibrilla proteins through the protein-cleaving action of caspase-3 [16].

Thus, a principal mechanism involved in muscle atrophy in CKD involves activation of UPS which is induced by acidosis [17] (full text). Higher levels of mRNAs of certain components of the UPS system and a similar pattern of expression of atrophy-related genes (named atrogenes) have been found in CKD patients [18] (full text). In vitro, it has been shown that proteasome inhibitors block the increased protein degradation in rats with chronic uremia. Insulin resistance (see below) also causes loss of muscle protein and muscle atrophy through activation of the UPS system [19] (full text).

GH/IGF-1 pathway in muscle loss

Patients with CKD display a variety of metabolic and nutritional abnormalities, including abnormalities in insulin resistance and growth hormone (GH)/insulin-like growth factor (IGF) -1 axis. Insulin is an anabolic hormone and exerts a powerful inhibitory effect on protein catabolism [20]. Insulin resistance has been shown to strongly associate with muscle breakdown in non-diabetic chronic HD patients [21]. Many CKD patients show signs of PEW reflected by sarcopenia and dynapenia which at least in part could be due to abnormalities in the GH/IGF-1 axis. Impaired insulin signaling or insulin resistance [22], together with abnormalities in the interplay between insulin and IGF-1 [23], contribute to the loss

of lean body mass loss in ESRD patients. Meanwhile, the IGF-1/phosphoinositide 3-kinases (PI3K)/Akt pathway inhibits the up-regulation of muscle atrophy genes which encode ubiquitin ligases [24]. IGF-1 is able to stabilize the effects of myostatin, a major factor involved in muscle atrophy [25] (full text). Another mechanism closely linked to impaired IGF-1 signaling in CKD is dysfunction of satellite cells, resident stem cells of skeletal muscle serving as skeletal muscle precursors [26] (full text). Testosterone deficiency, which commonly appears in CKD patients, might cause muscle catabolism by altering IGF-1 signaling and increasing myostatin expression leading to suppressed muscle growth [27].

Decreased or impaired action of GH, in part representing defects in GH/IGF-1 signaling, is another uremic endocrine disturbance associated with CKD that is a key driver of muscle protein catabolism and muscle growth retardation [28]. Administration of GH increases the growth in children with CKD and may also improve protein balance in HD patients increasing lean body mass [29], and decreasing the cardiovascular risk [30] (full text). In addition, exercise and use of ghrelin receptor agonists, apart from their other effects such as improved appetite and therefore increased dietary intakes, also may reduce muscle loss by influencing the GH/IGF-1 system in CKD patients [31], [32] (full text) further underlining the potential value of strategies aiming at improving the GH/IGF-1 system.

Other regulatory pathways of muscle loss in chronic kidney disease

Several common metabolic abnormalities in CKD, such as metabolic acidosis, insulin resistance and anorexia promote muscle protein degradation and loss of muscle mass [33]. One way by which acidosis stimulates muscle mass loss is by irreversible oxidation of essential branched-chain amino acids and activation of the ATP-dependent pathway [34]. In acidosis, amino acids from muscle protein are converted to glutamine, which is used by the kidneys to facilitate acid excretion. Inflammation triggers protein degradation and advanced stages of CKD are associated with increased levels of circulating catabolic cytokines, such as IL-6 [35]. CKD is characterized also by activation of the renin-angiotensin system, and angiotensin-?infusion has been shown to induce skeletal muscle atrophy through increases of oxidative stress and myostatin mediated up-regulation of the muscle-specific E3 ligases, Atrogin-1 and muscle ring finger protein 1 (MuRF1), leading to ubiquitin-proteasome mediated proteolysis, and decreased levels of circulating and skeletal muscle IGF-1 [36], [37]. The nuclear factor-κB (NF-κB) protein complex inhibits myogenesis by promoting myoblast growth and inducing loss of MyoD, which stimulates skeletal muscle differentiation and repair [38]. Myostatin is up-regulated in the skeletal muscle of patients with CKD and this together with an accelerated loss of myonuclei by apoptosis and changes in protein synthesis and degradation induced by altered signaling through the PI3-kinase/Akt pathway represent key mechanisms driving the onset and progression of muscle loss in CKD patients [39]. Recently, microRNAs, i.e., relatively short (21 to 24 nucleotides), non-coding RNAs that function as negative regulators of gene expression, have been found to be important mediators in processes leading to muscle wasting. Wang et al. [40] (full text) reported that down-regulation of miRNAs (miR-29a and miR-29b) and subsequent increase of the transcription factor Ying Yang-1 interferes with the normal differentiation of muscle progenitor cells, a process that is necessary for muscle growth, the maintenance of protein synthesis, and the repair of injuries in muscle. Studies by Xu et al. [41] showed that a miR-486 mimetic ameliorated muscle wasting in mice with CKD, suggesting that use of miR mimetics could represent a new therapeutic frontier for treatment of muscle wasting in CKD. Thus, multiple novel pathways have recently been discovered and are thought to be of key importance for the induction of processes leading to muscle atrophy in patients with CKD.

Osteoporosis in CKD

Disruption in mineral metabolism occurs already at an early stage of CKD, leading to alterations in bone mass, bone turnover, mineralization and bone strength. Osteoporosis is defined as a skeletal disorder characterized by loss of bone strength and micro-architectural deterioration of bone tissue, leading to increased risk of fractures - the main clinical manifestation of osteoporosis [42] (full text). There is an increased prevalence of vertebral and hip fractures among CKD patients compared with the general population in all age groups, with profound effects on morbidity and mortality, possibly reflecting also links with nutritional status and vascular calcification [43]. Age is a risk factor for osteoporosis and fracture risk, both in the general population and in CKD patients [44] (full text). The high incidence of hip fractures in CKD stage 5 patients, 17 times higher incidence than in the general population in the United States [45], is most likely linked to the high prevalence of osteopenia and osteoporosis in ESRD patients [46] (full text), [47].

The diagnosis of osteoporosis is usually based on reduction in bone mineral content reflected by reduced bone mineral density (BMD) as assessed by dual energy X-ray absorptiometry (DXA). However, it should be noted that bone fragility is due also to many other factors that cannot fully be described by BMD such as abnormal bone turnover and remodeling, leading to impairments of bone micro-architecture. Thus, the use of BMD as a diagnostic tool for osteoporosis and fracture risk in CKD patients is controversial. Jamal et al. [48] failed to find correlation between BMD measured by DXA and fractures in 104 elderly HD patients, whereas Atsumi et al. [49] showed a good predictive value of lumbar-spine BMD for vertebral fractures in HD patients. Recently, some studies underlined that the standard WHO classification of osteoporosis or osteopenia to classify bone status in CKD stage 4 or 5 patients may not be appropriate, since BMD determined by DXA may be falsely elevated in these patients due to sclerosis of posterior elements and calcification of large arteries [50] (full text). Thus, whereas DXA is not an ideal tool for assessing bone quality and bone strength [51] (full text), bone biopsy, followed by histomorphometric analysis, provides qualitative and quantative information about bone remodeling and bone status, in particular regarding the micro-architectural changes in bone tissue, but this invasive approach is not widely used in the evaluation of osteoporosis in CKD patients [52]. Instead, new noninvasive imaging techniques for assessment of bone health, such as quantitative computed tomography (QCT), high resolution magnetic resonance imaging (HR-MRI) and high resolution peripheral quantitative computed tomography (HR-pQCT) are increasingly used in clinical studies [53].

RANK/RANKL/OPG pathway in osteoporosis

The RANK/RANKL/OPG system plays an important role in the regulation of osteoclast formation, activity, and survival in normal and pathological states of bone remodeling. Biochemical markers, such as C-telopeptide crosslaps (CTX) and bone-specific alkaline phosphatase (B-ALP), are currently used in clinical research as markers of bone resorption and bone formation and for prediction of fracture risks, independent of other methods for monitoring osteoporosis, such as BMD [54]. OPG is a cytokine that plays an important role in the negative regulation of osteoclastic bone resorption thereby increasing BMD and bone volume by decreasing the active osteoclasts as demonstrated by *in vitro* studies [55]. The OPG level increases with increasing age, and this age-dependent increase in OPG might be a counter-regulatory mechanism preventing further bone loss in elderly subjects. OPG, and also FGF-23, are independently associated with myocardial damage and aortic pulse wave velocity in CKD patients, thus linking CKD-BMD with cardiovascular disease [56]. OPG and

leptin are both related with osteoporosis, linking losses of bone mass and muscle mass as well as with fat mass [57]. The finding that there is a positive relation between OPG and femoral neck BMD in HD patients, indicates that OPG perhaps could be used as an early screening tool of bone loss and presence of CKD-MBD in ESRD patients [58], [59]. OPG has been proposed as a practical bone biomarker to grade the severity of coronary calcification in non-dialysis CKD patients [60] (full text). Unlike OPG, free RANKL and total RANKL decrease with age, possibly due to a general age-related decrease of cell activity [61]. Circulating OPG is increased in uremic patients independent of serum PTH [62]. In pre-dialysis CKD stage 1-5 patients, serum RANKL was negatively while OPG was positively related with femoral neck BMD [63].

Linkage between muscle loss and osteoporosis

The literature describing the relationship of skeletal muscle and bone loss is equivocal. Some studies demonstrated a clear association between bone mass and lean tissue [64] [65] [66], but others could not confirm this [67], [68]. Osteoporosis is associated with sarcopenia in elderly people [69] (full text), putatively due to an impact on bone remodeling through increased mechanical load forces of lean tissue [70] (full text). IGF-1 is also an important regulator of bone growth and has been suggested as an early marker for low bone

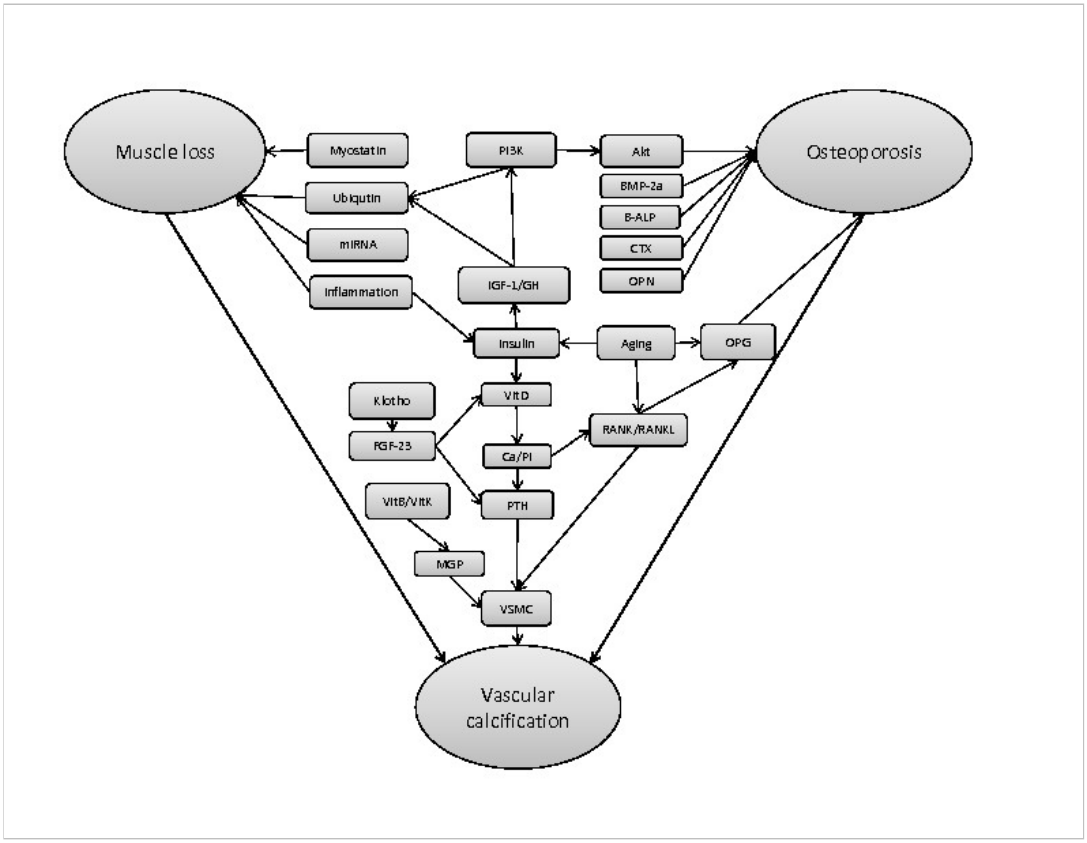

Figura 1.
In the uremic milieu, the clinical correlates of muscle loss, osteoporosis and vascular calcification often appear together reflecting close interrelations via multiple common mechanisms and perturbations in pathways such as IGF/GH, calcium and phosphate metabolism, Klotho/FGF23/PTH and RANK/RANKL/OPG pathways which act together to induce muscle atrophy, bone loss and vascular calcification in patients with CKD.

Abbreviation: BMP, bone morphogenetic protein; CTX, C-telopeptide cross-laps; FGF-23, fibrosis growth factor-23; GH, growth hormone; MGP, matrix gla protein; IGF-1,insulin-like growth hormone-1; OPG, Osteoprotegerin; Pi, phosphate; PTH, parathyroid hormone; RANK, receptor activator of nuclear-factor ?B, RANKL, receptor activator of nuclear-factor kB ligand; VitD, vitamin D; VitB, vitamin B; VitK, Vitamin K; Ca, calcium; VSMC, vascular smooth muscle cell; PI3K, phosphoinositide 3-kinases.

mass in pre- and post-menopausal women [71]. A recent study showed that the IGF-1/Akt pathway is involved in osteoporosis-related muscle atrophy, suggesting that BMD could be used as a nutrition marker of muscle atrophy in osteoporotic patients [72]. In CKD and ESRD, only a few studies have so far demonstrated a positive relationship between IGF-1 concentration and BMD ([73] (full text). Appropriate regular physical activity and especially weight-bearing exercise could be recommended as measures aiming at maintaining muscle mass and muscle strength and thereby improving bone quality and possibly reducing fracture risk [31].

As dietary phosphate overloading in CKD mice increases the trabecular and cortical bone structures, leading to high fracture risk [74], it is possible that this could be of relevance also for CKD patients. Anti-osteoporosis treatment with bisphosphonates which have been used for preventing fractures in patients with CKD have been suggested to reduce progression of extra-osseous calcification and inhibit the development of atherosclerosis; however, in severe CKD, bisphosphonates should only be used with caution in carefully selected patients [75] (full text). Whereas vitamin D has not been demonstrated to reduce fracture incidence in patients with ESRD, animal studies show that vitamin D may reduce serum PTH levels and improve bone strength [76] (full text), suggesting that vitamin D might improve BMD in patients with CKD.

Vascular calcification

Vascular calcification, also called “ossification” as alterations in the vasculature are bone-like, is the consequence of mineralization imbalance occurring at a much earlier age in CKD patients as compared with subjects with similar age but with normal renal function [77] (full text). In all stages of CKD, vascular calcification associates with a marked increase of mortality risk [78] (full text). Arterial vessel calcification takes place in the intima or in the media (Monckeberg sclerosis), or most commonly, in both these locations. The medial layer of the vessel wall is composed of vascular smooth muscle cells (VSMC) and elastin-rich matrix. Because medial calcification alters the elasticity of peritoneal arteries, leading to stiffening of the arterial wall, thereby increasing the carotid-femoral pulse wave velocity (PWV), and raising blood pressure in CKD patients, it is strongly associated with increasing risks of bone loss and mortality [79] (full text), [80] (full text). Extraskeletal soft-tissue calcification, and in particular vascular calcification, are important manifestations of the clinical syndrome of systemic mineral and bone disorders in CKD, collectively named as the CKD-MBD syndrome [81].

Role of FGF-23/PTH and Ca/Pi homeostasis in vascular calcification

The phosphate-FGF-23-PTH pathways play an important role in the regulation of phosphate metabolism and vascular calcification in CKD patients. In population-based studies, FGF-23 is independently and positively associated with aortic and coronary calcification in ESRD patients [82] (full text), but negatively associated with the progression of aortic calcification [83] (full text), indicating a protective role of FGF-23 against hyperphosphatemia. However, resistance to the action of FGF-23 effect during the course of CKD progression, leads to extremely high levels of FGF-23 in CKD patients, who nevertheless still are unable to normalize the hyperphosphatemia [84]. Interestingly, studies documenting a clear predictive value of FGF-23 on PWV are lacking [85] although a weak crude association between FGF-23 and PWV was seen in one study; however, its statistical significance disappeared after adjustment in a multivariate analysis [56]. Whereas according to another study, FGF-23 was not associated with and did not induce arterial calcification [86]. both phosphate and FGF-23

appear to be linked with cardiovascular disease through distinct mechanisms. A combination of phosphate binder and restricted phosphate intake was reported to reduce the FGF-23 levels in a synergetic way, suggesting that this could be possible intervention for regulation of the high FGF-23 levels in CKD patients [87].

The expression of Klotho, a trans-membrane and secreted protein acting as a co-factor of the FGF receptor and expressed mostly in the kidney in humans, decreases with CKD progression [88]. This could explain at least in part the resistance to FGF-23, and also the elevated concentrations of FGF-23 and plasma phosphate in CKD patients. Klotho suppresses the sodium-dependent phosphate uptake and cell differentiation in VSMC [89] (full text). Thus, a decrease of klotho gene expression may contribute to increased phosphate levels and arterial calcification in CKD. FGF-23 induces left ventral hypertrophy (LVH) independent of klotho, suggesting that high FGF-23 levels may contribute to LVH and increased mortality in CKD patients [90]. Interestingly, klotho also interferes with the insulin/IGF-1 pathway through regulating the IGF-receptor [91] (full text); this represents another novel bridge linking bone health, vascular calcification, and muscle loss in CKD patients.

Calcium-phosphate deposition, mostly in the form of apatite, is present in the myocardium, cardiac valves and aortae in patients with ESRD [92]. Whereas PTH is a key regulator of mineral and bone disorders and calcium-phosphate deposition in CKD patients, the results of studies regarding the effects of PTH on vascular calcification is conflicting. PTH-(1-34) was shown to inhibit the VSMC calcification *in vivo* and vascular calcification in low-density lipoprotein receptor (LDLR)-deficient mice [93] (full text), [94] (full text). Another animal study showed that PTH therapy stimulated intense aortic median calcification in rats [95]. Prevention of vascular calcification through a strict control of phosphate and the calcium-phosphate product is a major goal for new therapeutic agents. Sevelamer has been reported to reduce the serum phosphate, calcium-phosphate product and PTH levels, as well as preventing ectopic calcification of soft tissues and aortic calcification in animal models of renal failure [96]. The complicated relationships between calcium-phosphate control and PTH in relation to vascular calcification need further studies.

Epidemiological studies found vitamin D levels to be inversely associated with endothelial function and aortic calcification in ESRD patients [97]. The active form of vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol), was found to induce VSMC calcification by effecting PTH-related peptide (PTHrP) secretion, but also by negative regulation of the renin-angiotensin (RAS) system [98] (full text), [99]. Hirate et al [100] (full text) reported the efficacy of 22-oxacalcitriol (OCT), a 1, 25(OH)2 D3 analogue with lesser calcemic activity than 1, 25(OH)2 D3, in suppressing secondary hyperparathyroidism and soft tissue calcification in 5/6 nephrectomized rats; the rats treated with OCT had less aortic calcification and less decline of renal function. Meanwhile, the vitamin D analogue paricalcitol was reported to be associated with a survival advantage, compared with calcitriol, in a large community-based study [101] (full text). On the other hand, the use of calcitriol, a negative endocrine regulator of theRAS, results in milder elevation of serum phosphate and calcium, down-regulation of renin-angiotensin system, and inhibition of smooth muscle proliferation [99].

Other regulators of vascular calcification in CKD

Besides the classical risk factors such as phosphate, PTH, and presence of diabetes mellitus, there are several novel risk factors for vascular calcification, besides the classic risk factors. The adipocytokine leptin has been proposed to promote osteogenic differentiation and vascular calcification, leading to high cardiovascular risk in CKD patients [102] (full text). Fetuin-A (Ahsg) is an important inhibitor of ectopic calcification; a high level of fetuin-A prevents the accelerated extra-skeletal calcification observed in CKD patients [103] (full

text). Osteopontin (OPN) acts as an inhibitor of calcification of VSMC [104] (full text), while Jono et al. [105] (full text) demonstrated that the phosphorylation of OPN was a mandatory step to inhibit VSMC calcification. In contrast, high levels of osteocalcin, ALP, osteonectin and bone morphogenetic protein (BMP)-2a were associated with myofibroblasts and VSMCs being diverted to osteogenic lineage, resulting in induction of calcification [106] (full text), [107], [108] (full text). More studies are needed to clarify the impact of altered expression and function of these gene products in the vascular calcification in ESRD. Vitamin K deficiency, which is common in all stages of CKD, is associated with an inactive form of matrix Gla protein (MGP), which has been linked to increased risk for vascular calcification [109] (full text). This is because vitamin K serves as a co-factor in the enzymatic reaction that allows specific glutamate (glu) residues in MGP to be changed into gamma-carboxyglutamate (gla) residues, which inhibit the calcification of the arterial wall [110] (full text). Vitamin K supplementation inhibits the vascular calcification in a CKD mice model [111] and in ESRD patients [112], suggesting a potential therapeutic role of vitamin K in the management of uremia patients. Hyperhomocysteinemia is thought to represent a link between bone status and vascular calcification [113]; however, treatment with high doses of folic acid and B vitamins did not reduce the incidence of vascular disease, or improve survival, in patients with ESRD [114], but the role of hyperhomocysteinemia in relation to vascular calcification in CKD is still largely unexplored. Recently, one study showed that free triiodothyronine associated strongly with arterial stiffness, coronary artery calcification and mortality, which could suggest a link between thyroid hormone and vascular calcification (Meuwese et al in press, AJKD 2013).

Links between bone and vascular -RANK/RANKL/OPG pathway and FGF-23 pathway

In the last decade, evidence suggests that the disturbances in mineral and bone metabolism in patients with CKD that associate with vascular calcification are linked to bone-related proteins such as B-ALP, osteocalcin, OPN, and Runx2; these proteins have been observed in calcified vascular lesions. Increasing evidences suggest that the RANK/RANKL/OPG pathway, a key regulator of bone formation, may be involved in vascular calcification. Osteoblasts and active T cells synthesize RANKL, and RANK is expressed by osteoclasts, endothelial cells and VSMC. The RANKL system has been reported to be related to cardiovascular events and coronary artery calcification in several studies [115] (full text), [116] (full text). The RANK/RANKL directly promotes VSMC calcification [117] (full text) through activating NF-κB pathway and release of TNF and IL-6, while OPG inhibits the vascular calcification [118] (full text). In patients with advanced stages of CKD, osteoclast-like cells express tartrate-resistance acid phosphatase (TRAP) in calcified lesions, and the RANK/RANKL directly stimulates TRAP positive osteoclast-like cell formation, suggesting that osteoclasts might actually promote vascular calcification [119] (full text). Some osteoporosis therapies, such as bisphosphonates (pyrophosphate analogs), denosumab (a monoclonal inhibitor of RANKL) and a recombinant fusion protein of OPG have been shown to inhibit vascular calcification [120], [121] (full text). CKD and especially ESRD are associated with elevated circulating FGF-23 and reduced klotho activity; FGF-23 (and 1, 25-dihydroxyvitamin D) influence fracture risk [122] and post-transplant bone mineral loss [123] (full text) while klotho protein deficiency contributes to accelerated aging with arterial calcifications and osteoporosis [124]. The pathophysiology and biological links between bone and vascular abnormalities suggest the existence of multiple common regulatory factors shared by vascular and bone systems.

Conclusions

Muscle loss, osteoporosis and vascular calcification, three common complications in CKD patients that one by one and even more so together herald a poor prognosis, are closely linked to each other via multiple shared pathways. Thus, as the regulation of protein degradation, bone growth and vascular calcification share several common mechanisms, this suggests that novel diagnostic, preventive and therapeutic approaches could emerge that could treat this triple whammy of severe complications simultaneously.

Acknowledgment

We are grateful to the Karolinska Institutet Diabetic Theme Center, the Swedish Research Council, the Loo and Hans Osterman and the Stig and Gunborg Westman Foundations. Jia Ting has a grant from the China Scholarship Council (2009601096). Baxter Healthcare Corporation employs Bengt Lindholm. Baxter Novum is the result of a grant from Baxter Healthcare Corporation to Karolinska Institutet.

Bibliografia

[1] Sarnak MJ, Levey AS, Schoolwerth AC et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 2003 Oct 28;108(17):2154-69 (full text)

[2] Stenvinkel P, Larsson TE Chronic Kidney Disease: A Clinical Model of Premature Aging. American journal of kidney diseases : the official journal of the National Kidney Foundation 2013 Jan 25;

[3] Fouque D, Kalantar-Zadeh K, Kopple J et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney international 2008 Feb;73(4):391-8

[4] Qureshi AR, Alvestrand A, Danielsson A et al. Factors predicting malnutrition in hemodialysis patients: a cross-sectional study. Kidney international 1998 Mar;53(3):773-82 (full text)

[5] Wolfe RR The underappreciated role of muscle in health and disease. The American journal of clinical nutrition 2006 Sep;84(3):475-82 (full text)

[6] Stenvinkel P, Heimbürger O, Paultre F et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney international 1999 May;55(5):1899-911 (full text)

[7] Dwyer JT, Cunniff PJ, Maroni BJ et al. The hemodialysis pilot study: nutrition program and participant characteristics at baseline. The HEMO Study Group. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 1998 Jan;8(1):11-20

[8] Meireles CL, Price SR, Pereira AM et al. Nutrition and chronic renal failure in rats: what is an optimal dietary protein? Journal of the American Society of Nephrology : JASN 1999 Nov;10(11):2367-73 (full text)

[9] Garibotto G, Bonanni A, Verzola D et al. Effect of kidney failure and hemodialysis on protein and amino acid metabolism. Current opinion in clinical nutrition and metabolic care 2012 Jan;15(1):78-84

[10] Lim VS, Bier DM, Flanigan MJ et al. The effect of hemodialysis on protein metabolism. A leucine kinetic study. The Journal of clinical investigation 1993 Jun;91(6):2429-36

[11] Carrero JJ, Stenvinkel P, Cuppari L et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2013 Mar;23(2):77-90

[12] Raj DS, Welbourne T, Dominic EA et al. Glutamine kinetics and protein turnover in end-stage renal disease. American journal of physiology. Endocrinology and metabolism 2005 Jan;288(1):E37-46 (full text)

[13] Mitch WE, Goldberg AL Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. The New England journal of medicine 1996 Dec 19;335(25):1897-905

[14] Pupim LB, Flakoll PJ, Majchrzak KM et al. Increased muscle protein breakdown in chronic hemodialysis patients with type 2 diabetes mellitus. Kidney international 2005 Oct;68(4):1857-65

[15] Ardley HC, Robinson PA E3 ubiquitin ligases. Essays in biochemistry 2005;41:15-30

[16] Du J, Wang X, Miereles C et al. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. The Journal of clinical investigation 2004 Jan;113(1):115-23

[17] Pickering WP, Price SR, Bircher G et al. Nutrition in CAPD: serum bicarbonate and the ubiquitin-proteasome system in muscle. Kidney international 2002 Apr;61(4):1286-92 (full text)

[18] Lecker SH, Jagoe RT, Gilbert A et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2004 Jan;18(1):39-51 (full text)

[19] Wang X, Hu Z, Hu J et al. Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell

signaling. Endocrinology 2006 Sep;147(9):4160-8 (full text)

[20] May RC, Kelly RA, Mitch WE et al. Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. The Journal of clinical investigation 1987 Apr;79(4):1099-103

[21] Siew ED, Pupim LB, Majchrzak KM et al. Insulin resistance is associated with skeletal muscle protein breakdown in non-diabetic chronic hemodialysis patients. Kidney international 2007 Jan;71(2):146-52

[22] Pupim LB, Heimbürger O, Qureshi AR et al. Accelerated lean body mass loss in incident chronic dialysis patients with diabetes mellitus. Kidney international 2005 Nov;68(5):2368-74

[23] Glass DJ Molecular mechanisms modulating muscle mass. Trends in molecular medicine 2003 Aug;9(8):344-50

[24] Stitt TN, Drujan D, Clarke BA et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Molecular cell 2004 May 7;14(3):395-403

[25] Frost RA, Lang CH Protein kinase B/Akt: a nexus of growth factor and cytokine signaling in determining muscle mass. Journal of applied physiology (Bethesda, Md. : 1985) 2007 Jul;103(1):378-87 (full text)

[26] Zhang L, Wang XH, Wang H et al. Satellite cell dysfunction and impaired IGF-1 signaling cause CKD-induced muscle atrophy. Journal of the American Society of Nephrology : JASN 2010 Mar;21(3):419-27 (full text)

[27] Carrero JJ, Stenvinkel P The vulnerable man: impact of testosterone deficiency on the uraemic phenotype. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2012 Nov;27(11):4030-41

[28] Mak RH, Cheung WW, Roberts CT Jr et al. The growth hormone-insulin-like growth factor-I axis in chronic kidney disease. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society 2008 Feb;18(1):17-25

[29] Garibotto G, Barreca A, Russo R et al. Effects of recombinant human growth hormone on muscle protein turnover in malnourished hemodialysis patients. The Journal of clinical investigation 1997 Jan 1;99(1):97-105

[30] Feldt-Rasmussen B, Lange M, Sulowicz W et al. Growth hormone treatment during hemodialysis in a randomized trial improves nutrition, quality of life, and cardiovascular risk. Journal of the American Society of Nephrology : JASN 2007 Jul;18(7):2161-71 (full text)

[31] Andreoli A, Celi M, Volpe SL et al. Long-term effect of exercise on bone mineral density and body composition in post-menopausal ex-elite athletes: a retrospective study. European journal of clinical nutrition 2012 Jan;66(1):69-74

[32] Deboer MD, Zhu X, Levasseur PR et al. Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. Endocrinology 2008 Feb;149(2):827-35 (full text)

[33] Guarnieri G, Barazzoni R Fighting protein-energy wasting in chronic kidney disease: a challenge of complexity. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2011 Jan;21(1):2-6

[34] Mitch WE, Medina R, Grieber S et al. Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. The Journal of clinical investigation 1994 May;93(5):2127-33

[35] Stenvinkel P, Ketteler M, Johnson RJ et al. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. Kidney international 2005 Apr;67(4):1216-33

[36] Song YH, Li Y, Du J et al. Muscle-specific expression of IGF-1 blocks angiotensin II-induced skeletal muscle wasting. The Journal of clinical investigation 2005 Feb;115(2):451-8

[37] Grune T, Merker K, Sandig G et al. Selective degradation of oxidatively modified protein substrates by the proteasome. Biochemical and biophysical research communications 2003 Jun 6;305(3):709-18

[38] McFarlane C, Plummer E, Thomas M et al. Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism. Journal of cellular physiology 2006 Nov;209(2):501-14

[39] Verzola D, Procopio V, Sofia A et al. Apoptosis and myostatin mRNA are upregulated in the skeletal muscle of patients with chronic kidney disease. Kidney international 2011 Apr;79(7):773-82

[40] Wang XH, Hu Z, Klein JD et al. Decreased miR-29 suppresses myogenesis in CKD. Journal of the American Society of Nephrology : JASN 2011 Nov;22(11):2068-76 (full text)

[41] Xu J, Li R, Workeneh B et al. Transcription factor FoxO1, the dominant mediator of muscle wasting in chronic kidney disease, is inhibited by microRNA-486. Kidney international 2012 Aug;82(4):401-11

[42] Tarantino U, Capone A, Planta M et al. The incidence of hip, forearm, humeral, ankle, and vertebral fragility fractures in Italy: results from a 3-year multicenter study. Arthritis research & therapy 2010;12(6):R226 (full text)

[43] Klawansky S, Komaroff E, Cavanaugh PF Jr et al. Relationship between age, renal function and bone mineral density in the US population. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2003 Jul;14(7):570-6

[44] Stehman-Breen CO, Sherrard DJ, Alem AM et al. Risk factors for hip fracture among patients with end-stage renal disease. Kidney international 2000 Nov;58(5):2200-5 (full text)

[45] Brauer CA, Coca-Perraillon M, Cutler DM et al. Incidence and mortality of hip fractures in the United States. JAMA : the journal of the American Medical Association 2009 Oct 14;302(14):1573-9

[46] Alem AM, Sherrard DJ, Gillen DL et al. Increased risk of hip fracture among patients with end-stage renal disease. Kidney international 2000 Jul;58(1):396-9 (full text)

[47] Stehman-Breen CO, Sherrard D, Walker A et al. Racial differences in bone mineral density and bone loss among end-stage renal disease patients. American journal of kidney diseases : the official journal of the National Kidney Foundation 1999 May;33(5):941-6

[48] Jamal SA, Chase C, Goh YI et al. Bone density and heel ultrasound testing do not identify patients with dialysis-dependent renal failure who have had fractures. American journal of kidney diseases : the official journal of the National Kidney Foundation 2002 Apr;39(4):843-9

[49] Atsumi K, Kushida K, Yamazaki K et al. Risk factors for vertebral fractures in renal osteodystrophy. American journal of kidney diseases : the official journal of the National Kidney Foundation 1999 Feb;33(2):287-93

[50] Miller PD Fragility fractures in chronic kidney disease: an opinion-based approach. Cleveland Clinic journal of medicine 2009 Dec;76(12):715-23 (full text)

[51] Nickolas TL, Stein E, Cohen A et al. Bone mass and microarchitecture in CKD patients with fracture. Journal of the American Society of Nephrology : JASN 2010 Aug;21(8):1371-80 (full text)

[52] Barreto FC, Barreto DV, Moyses RM et al. Osteoporosis in hemodialysis patients revisited by bone histomorphometry: a new insight into an old problem. Kidney international 2006 May;69(10):1852-7

[53] Pelletier S, Chapurlat R Optimizing bone health in chronic kidney disease. Maturitas 2010 Apr;65(4):325-33

[54] Vasikaran S, Eastell R, Bruyère O et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2011 Feb;22(2):391-420

[55] Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997 Apr 18;89(2):309-19

[56] Ford ML, Smith ER, Tomlinson LA et al. FGF-23 and osteoprotegerin are independently associated with myocardial damage in chronic kidney disease stages 3 and 4. Another link between chronic kidney disease-mineral bone disorder and the heart. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2012 Feb;27(2):727-33

[57] Pobeha P, Ukropec J, Skyba P et al. Relationship between osteoporosis and adipose tissue leptin and osteoprotegerin in patients with chronic obstructive pulmonary disease. Bone 2011 May 1;48(5):1008-14

[58] Doumouchtsis KK, Kostakis AI, Doumouchtsis SK et al. Associations between osteoprotegerin and femoral neck BMD in hemodialysis patients. Journal of bone and mineral metabolism 2008;26(1):66-72

[59] Jiang JQ, Lin S, Xu PC et al. Serum osteoprotegerin measurement for early diagnosis of chronic kidney disease-mineral and bone disorder. Nephrology (Carlton, Vic.) 2011 Aug;16(6):588-94

[60] Morena M, Jaussent I, Halkovich A et al. Bone biomarkers help grading severity of coronary calcifications in non dialysis chronic kidney disease patients. PloS one 2012;7(5):e36175 (full text)

[61] Kerschan-Schindl K, Wendlova J, Kudlacek S et al. Serum levels of receptor activator of nuclear factor kappaB ligand (RANKL) in healthy women and men. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2008 Aug;116(8):491-5

[62] Kazama JJ, Shigematsu T, Yano K et al. Increased circulating levels of osteoclastogenesis inhibitory factor (osteoprotegerin) in patients with chronic renal failure. American journal of kidney diseases : the official journal of the National Kidney Foundation 2002 Mar;39(3):525-32

[63] Fahrleitner-Pammer A, Dobnig H, Dimai HP et al. The effect of RANKL and OPG on bone mineral density in pre-dialysis chronic renal failure. Clinical nephrology 2009 Jun;71(6):652-9

[64] Mussolino ME, Gillum RF Low bone mineral density and mortality in men and women: the Third National Health and Nutrition Examination Survey linked mortality file. Annals of epidemiology 2008 Nov;18(11):847-50

[65] Takada H, Washino K, Iwata H et al. Risk factors for low bone mineral density among females: the effect of lean body mass. Preventive medicine 1997 Sep-Oct;26(5 Pt 1):633-8

[66] Andreoli A, Bazzocchi A, Celi M et al. Relationship between body composition, body mass index and bone mineral density in a large population of normal, osteopenic and osteoporotic women. La Radiologia medica 2011 Oct;116(7):1115-23

[67] Reid IR, Evans MC, Ames RW et al. Volumetric bone density of the lumbar spine is related to fat mass but not lean mass in normal postmenopausal women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1994 Nov;4(6):362-7

[68] Lindsay R, Cosman F, Herrington BS et al. Bone mass and body composition in normal women. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 1992 Jan;7(1):55-63

[69] Cruz-Jentoft AJ, Baeyens JP, Bauer JM et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing 2010 Jul;39(4):412-23 (full text)

[70] Wardlaw GM Putting body weight and osteoporosis into perspective. The American journal of clinical nutrition 1996 Mar;63(3 Suppl):433S-436S (full text)

[71] Liu JM, Zhao HY, Ning G et al. IGF-1 as an early marker for low bone mass or osteoporosis in premenopausal and postmenopausal women. Journal of bone and mineral metabolism 2008;26(2):159-64

[72] Terracciano C, Celi M, Lecce D et al. Differential features of muscle fiber atrophy in osteoporosis and osteoarthritis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2013 Mar;24(3):1095-100

[73] Park SH, Jia T, Qureshi AR et al. Determinants and survival implications of low bone mineral density in end-stage renal disease patients. Journal of nephrology 2013 May 16;26(3):485-94 (full text)

[74] Lau WL, Linnes M, Chu EY et al. High phosphate feeding promotes mineral and bone abnormalities in mice with chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2013 Jan;28(1):62-9

[75] Toussaint ND, Elder GJ, Kerr PG et al. Bisphosphonates in chronic kidney disease; balancing potential benefits and adverse effects on bone and soft tissue. Clinical journal of the American Society of Nephrology : CJASN 2009 Jan;4(1):221-33 (full text)

[76] Rix M, Eskildsen P, Olgaard K et al. Effect of 18 months of treatment with alfacalcidol on bone in patients with mild to moderate chronic renal failure. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2004 Apr;19(4):870-6 (full text)

[77] Goodman WG, Goldin J, Kuizon BD et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. The New England journal of medicine 2000 May 18;342(20):1478-83 (full text)

[78] London GM, Guérin AP, Marchais SJ et al. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrology, dialysis, transplantation : official publication of the Eu-

ropean Dialysis and Transplant Association - European Renal Association 2003 Sep;18(9):1731-40 (full text)

[79] Blacher J, Guerin AP, Pannier B et al. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999 May 11;99(18):2434-9 (full text)

[80] Blacher J, Pannier B, Guerin AP et al. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. Hypertension 1998 Sep;32(3):570-4 (full text)

[81] Moe S, Drüeke T, Cunningham J et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international 2006 Jun;69(11):1945-53

[82] Nasrallah MM, El-Shehaby AR, Salem MM et al. Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2010 Aug;25(8):2679-85 (full text)

[83] Tamei N, Ogawa T, Ishida H et al. Serum fibroblast growth factor-23 levels and progression of aortic arch calcification in non-diabetic patients on chronic hemodialysis. Journal of atherosclerosis and thrombosis 2011;18(3):217-23 (full text)

[84] Larsson T, Nisbeth U, Ljunggren O et al. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. Kidney international 2003 Dec;64(6):2272-9

[85] Desjardins L, Liabeuf S, Renard C et al. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2012 Jul;23(7):2017-25

[86] Scialla JJ, Lau WL, Reilly MP et al. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney international 2013 Feb 6;

[87] Isakova T, Barchi-Chung A, Enfield G et al. Effects of Dietary Phosphate Restriction and Phosphate Binders on FGF23 Levels in CKD. Clinical journal of the American Society of Nephrology : CJASN 2013 Mar 7;

[88] Karsli Ceppio?lu S, Yurdun T, Canbakan M et al. Assessment of matrix Gla protein, Klotho gene polymorphisms, and oxidative stress in chronic kidney disease. Renal failure 2011;33(9):866-74

[89] Hu MC, Shi M, Zhang J et al. Klotho deficiency causes vascular calcification in chronic kidney disease. Journal of the American Society of Nephrology : JASN 2011 Jan;22(1):124-36 (full text)

[90] Faul C, Amaral AP, Oskouei B et al. FGF23 induces left ventricular hypertrophy. The Journal of clinical investigation 2011 Nov;121(11):4393-408

[91] Château MT, Araiz C, Descamps S et al. Klotho interferes with a novel FGF-signalling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in Caenorhabditis elegans. Aging 2010 Sep;2(9):567-81 (full text)

[92] Giachelli CM The emerging role of phosphate in vascular calcification. Kidney international 2009 May;75(9):890-7

[93] Jono S, Nishizawa Y, Shioi A et al. Parathyroid hormone-related peptide as a local regulator of vascular calcification. Its inhibitory action on in vitro calcification by bovine vascular smooth muscle cells. Arteriosclerosis, thrombosis, and vascular biology 1997 Jun;17(6):1135-42 (full text)

[94] Shao JS, Cheng SL, Charlton-Kachigian N et al. Teriparatide (human parathyroid hormone (1-34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. The Journal of biological chemistry 2003 Dec 12;278(50):50195-202 (full text)

[95] Neves KR, Graciolli FG, dos Reis LM et al. Vascular calcification: contribution of parathyroid hormone in renal failure. Kidney international 2007 Jun;71(12):1262-70

[96] Katsumata K, Kusano K, Hirata M et al. Sevelamer hydrochloride prevents ectopic calcification and renal osteodystrophy in chronic renal failure rats. Kidney international 2003 Aug;64(2):441-50

[97] Mizobuchi M, Ogata H, Koiwa F et al. Vitamin D and vascular calcification in chronic kidney disease. Bone 2009 Jul;45 Suppl 1:S26-9

[98] Jono S, Nishizawa Y, Shioi A et al. 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. Circulation 1998 Sep 29;98(13):1302-6 (full text)

[99] Li YC, Kong J, Wei M et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. The Journal of clinical investigation 2002 Jul;110(2):229-38

[100] Hirata M, Makibayashi K, Katsumata K et al. 22-Oxacalcitriol prevents progressive glomerulosclerosis without adversely affecting calcium and phosphorus metabolism in subtotally nephrectomized rats. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2002 Dec;17(12):2132-7 (full text)

[101] Teng M, Wolf M, Lowrie E et al. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. The New England journal of medicine 2003 Jul 31;349(5):446-56 (full text)

[102] Zeadin M, Butcher M, Werstuck G et al. Effect of leptin on vascular calcification in apolipoprotein E-deficient mice. Arteriosclerosis, thrombosis, and vascular biology 2009 Dec;29(12):2069-75 (full text)

[103] Heiss A, DuChesne A, Denecke B et al. Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. The Journal of biological chemistry 2003 Apr 11;278(15):13333-41 (full text)

[104] Speer MY, McKee MD, Guldberg RE et al. Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. The Journal of experimental medicine 2002 Oct 21;196(8):1047-55 (full text)

[105] Jono S, Peinado C, Giachelli CM et al. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. The Journal of biological chemistry 2000 Jun 30;275(26):20197-203 (full text)

[106] Cheng SL, Shao JS, Charlton-Kachigian N et al. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. The Journal of biological chemistry 2003 Nov 14;278(46):45969-77 (full text)

[107] Giachelli CM Ectopic calcification: new concepts in cellular regulation. Zeitschrift fur Kardiologie 2001;90 Suppl 3:31-7

[108] Jono S, McKee MD, Murry CE et al. Phosphate regulation of vascular smooth muscle cell calcification. Circulation research 2000 Sep 29;87(7):E10-7 (full text)

[109] Holden RM, Morton AR, Garland JS et al. Vitamins K and D status in stages 3-5 chronic kidney disease. Clinical journal of the American Society of Nephrology : CJASN 2010 Apr;5(4):590-7 (full text)

[110] Price PA, Faus SA, Williamson MK et al. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arteriosclerosis, thrombosis, and vascular biology 1998 Sep;18(9):1400-7 (full text)

[111] McCabe KM, Booth SL, Fu X et al. Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. Kidney international 2013 May;83(5):835-44

[112] Westenfeld R, Krueger T, Schlieper G et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. American journal of kidney diseases : the official journal of the National Kidney Foundation 2012 Feb;59(2):186-95

[113] Herrmann M, Peter Schmidt J, Umanskaya N et al. The role of hyperhomocysteinemia as well as folate, vitamin B(6) and B(12) deficiencies in osteoporosis: a systematic review. Clinical chemistry and laboratory medicine : CCLM / FESCC 2007;45(12):1621-32

[114] Jamison RL, Hartigan P, Kaufman JS et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. JAMA : the journal of the American Medical Association 2007 Sep 12;298(10):1163-70

[115] Schoppet M, Schaefer JR, Hofbauer LC et al. Low serum levels of soluble RANK ligand are associated with the presence of coronary artery disease in men. Circulation 2003 Mar 25;107(11):e76; author reply e76 (full text)

[116] Crisafulli A, Micari A, Altavilla D et al. Serum levels of osteoprotegerin and RANKL in patients with ST elevation acute myocardial infarction. Clinical science (London, England : 1979) 2005 Oct;109(4):389-95 (full text)

[117] Panizo S, Cardus A, Encinas M et al. RANKL increases vascular smooth muscle cell calcification through a RANK-BMP4-dependent pathway. Circulation research 2009 May 8;104(9):1041-8 (full text)

[118] Deuell KA, Callegari A, Giachelli CM et al. RANKL enhances macrophage paracrine pro-calcific activity in high phosphate-treated smooth muscle cells: dependence on IL-6 and TNF-?. Journal of vascular research 2012;49(6):510-21 (full text)

[119] Byon CH, Sun Y, Chen J et al. Runx2-upregulated receptor activator of nuclear factor ?B ligand in calcifying smooth muscle cells promotes migration and osteoclastic differentiation of macrophages. Arteriosclerosis, thrombosis, and vascular biology 2011 Jun;31(6):1387-96 (full text)

[120] Nitta K, Akiba T, Suzuki K et al. Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. American journal of kidney diseases : the official journal of the National Kidney Foundation 2004 Oct;44(4):680-8

[121] Morony S, Tintut Y, Zhang Z et al. Osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in ldlr(-/-) mice. Circulation 2008 Jan 22;117(3):411-20 (full text)

[122] Mirza MA, Karlsson MK, Mellström D et al. Serum fibroblast growth factor-23 (FGF-23) and fracture risk in elderly men. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2011 Apr;26(4):857-64

[123] Kanaan N, Claes K, Devogelaer JP et al. Fibroblast growth factor-23 and parathyroid hormone are associated with post-transplant bone mineral density loss. Clinical journal of the American Society of Nephrology : CJASN 2010 Oct;5(10):1887-92 (full text)

[124] Kuro-o M Klotho as a regulator of oxidative stress and senescence. Biological chemistry 2008 Mar;389(3):233-41