Body composition, arteriosclerosis and exercise training in patients with chronic kidney disease

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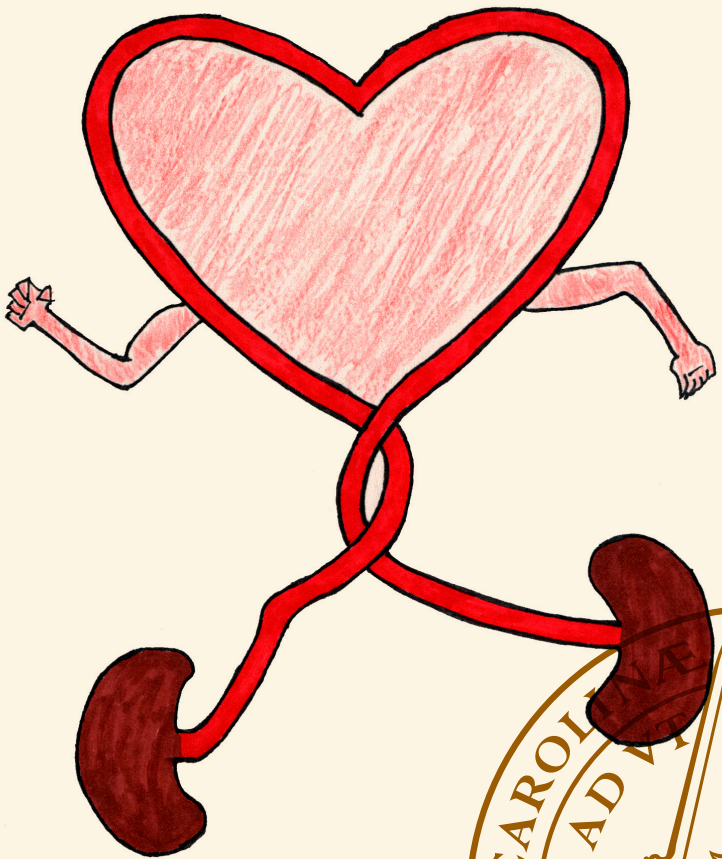

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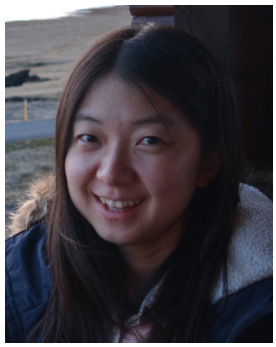

Chronic kidney disease (CKD) is a global health problem with a prevalence of ~10%. Cardiovascular disease is the leading cause of death in patients with CKD. This thesis explores how exercise training effects muscle wasting, inflammation and arteriosclerosis, which are three clinical entities associated with higher risk of cardiovascular events.

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Body composition, arteriosclerosis and exercise training in patients with chronic kidney disease

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Abstract

Background: Muscle wasting, inflammation and arteriosclerosis are common in patients with chronic kidney disease (CKD) and associated with increased cardiovascular morbidity and mortality.

Aims: Firstly, to investigate the relationships between muscle mass, physical function, plasma myostatin and GFR and the effects of exercise training in non-dialysis dependent patients with CKD stages 3-5. Secondly, to investigate the relationships between vascular calcification, plasma markers of arteriosclerosis and some cardiac indices and GFR and the effects of exercise training in these patients.

Methods: 151 non-dialysis dependent patients, average measured GFR 23±8 mL/min/1.73m^2, irrespective of age or comorbidity, were randomly assigned to 90 minutes per week of either strength or balance training both in combination with 60 minutes endurance training per week for an intervention period of 12 months. Handgrip strength, isometric quadriceps strength, functional reach, Berg's balance test and six minutes walking test were used to measure physical performance. Body composition was measured by dual-energy X-ray absorptiometry. Abdominal aortic calcification (AAC) was measured by X-ray. Plasma myostatin, fibroblast growth factor 23 (FGF23), interleukin 6 (IL6), fetuin-A were analyzed using ELISA kits.

Results: *In study 1*, 14% of the patients had sarcopenia. Muscle mass was positively related to measured GFR, and physical performance was positively related to muscle mass. *In study 2,* the prevalence of sarcopenia was unchanged after 12 months of exercise training, leg- and whole-body lean mass increased in the balance group, and was maintained in the strength group. Whole fat mass decreased in both groups. There were no significant between group differences in sarcopenia or body composition. Plasma myostatin levels increased in both groups, with a significant difference in favor of the strength group. Plasma myostatin was significantly positively related to muscle mass and physical performance at baseline, but these relationships were attenuated after 12 months. *In study 3*, 73% of the patients had AAC. AAC score was related to GFR, plasma albumin, plasma phosphate, pulse pressure, left ventricular mass, left atrial volume and left atrial volume index. *In study 4,* AAC score, parathyroid hormone and $1,25(OH)_2D_3$ increased significantly, plasma lipoprotein (a) decreased significantly after 12 months of exercise training. Plasma triglycerides, total cholesterol, high-density lipoprotein- and low-density lipoprotein cholesterol, FGF23, phosphate, calcium, fetuin-A, IL6, C-reactive protein, and albumin were unchanged. The increase in AAC score was positively related to baseline levels of triglycerides.

Conlusions: Among non-dialysis dependent patients with CKD stages 3–5, muscle mass was positively related to GFR and physical performance was positively related to muscle mass. Exercise training seemed to be effective in preventing sarcopenia, maintaining and even increasing muscle mass in these patients. Myostatin increased significantly after exercise training. However, further studies are needed to understand the role of plasma myostatin on muscle mass and physical performance in patients with CKD. AAC was negatively related to GFR and positively related to plasma phosphate, but no casual relationships were found. Although exercise training could not stop the progression of AAC, it might have contributed to the reduced levels of lipoprotein (a) and unchanged levels of calcific- and anti-inflammatory markers. Hypertriglyceridemia and aging emerged as longitudinal predictors of vascular calcification in these patients. Further studies on the progression of AAC during the natural course of CKD are required.

Key words: Chronic kidney disease, exercise training, muscle mass, sarcopenia, arteriosclerosis, abdominal aortic calcification, myostatin, lipids, lipoproteins, fibroblast growth factor 23, fetuin-A and interleukin 6**.**

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Study 1

Sarcopenia and relationships between muscle mass, measured GFR and physical function in patients with CKD 3-5

Yunan Zhou, Matthias Hellberg, Philippa Svensson, Peter Höglund and Naomi Clyne. Nephrology Dialysis Transplantation, Volume 33, Issue 2, Pages 342–348, February 2018.

Study 2

Muscle mass and plasma myostatin after exercise training - a sub-study of RENEXC– a randomized controlled trial

Yunan Zhou, Matthias Hellberg, Thomas Hellmark, Peter Höglund, Naomi Clyne.

Nephrology Dialysis Transplantation, in press, 2019.

Study 3

Relationships between abdominal aortic calcification, GFR and cardiovascular risk factors in patients with non-dialysis dependent CKD

Yunan Zhou, Matthias Hellberg, Evangelia Kouidi, Asterios Deligiannis, Peter Höglund, and Naomi Clyne. Clinical Nephrology, Volume 90, No.6/2018, Pages 380-389. October 2018.

Study 4

Abdominal aortic calcification, plasma markers of arteriosclerosis and exercise training in CKD – a sub-study of RENEXC

Yunan Zhou, Matthias Hellberg, Thomas Hellmark, Peter Höglund, Naomi Clyne.

Submitted to Nephron.

Abbreviations

AAC	abdominal aortic calcification
ADMA	asymmetric dimethylarginine
ASM	appendicular skeletal muscle
ASMI	appendicular skeletal muscle index
BCM	body composition monitor
BMI	body mass index
BSA	body surface area
CAC	coronary artery calcification
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRP	C-reactive protein
CT	computed tomography
CVC	cardiovascular comorbidities
DEXA	dual-energy X-ray absorptiometry
eNOS	endothelial nitric oxide synthase
FGF23	fibroblast growth factor 23
GDF-8	growth differentiation factor 8
GFR	glomerular filtration rate
eGFR	estimated glomerular filtration rate
mGFR	measured glomerular filtration rate
HDL-C	high-density lipoprotein cholesterol
IGF-1	insulin-like growth factor-1
IL6	interleukin 6

LAV	left atrial volume
LAVI	left atrial volume index
LDL-C	low-density lipoprotein cholesterol
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
MDRD	Modification of Diet in Renal Disease
MIA	Malnutrition - inflammation - atherosclerosis
mTOR	mammalian target of rapamycin
NO	nitric oxide
nPCR	normalized protein catabolic rate
RENEXC	Renal Exercise
PTH	parathyroid hormone
Stat3	signal transducer and activator of transcription-3
SGA	subjective global assessment
SWEDAC	Swedish Board for Accreditation and Conformity Assessment
TGF-β	transforming growth factor beta
TNF-α	tumour necrosis factor α
VSMC	vascular smooth muscle cells

Background

There are two kidneys in our body located in the retroperitoneal space. The kidneys participate in the regulation of body fluid, fluid osmolality, acid-base balance, electrolyte concentrations, and removal of toxins. They also have endocrine functions, like production of renin, erythropoietin and activation of vitamin D. The nephron is the structural and functional unit of the kidney. Each human adult kidney contains around one million nephrons. Glomerular filtration rate (GFR) is a measure used to evaluate renal function. GFR is the volume of fluid filtered from the renal glomerular capillaries into Bowman's capsule per minute. It is usually normalized to a body surface area of 1.73 m^2.

Chronic kidney disease (CKD)

CKD is defined as abnormalities of kidney structure or function, present for at least 3 months, with implications for health [1]. The main causes of CKD include hypertensive kidney disease, diabetes nephropathy, chronic glomerulonephritis, interstitial nephritis and polycystic kidney disease. According to the level of estimated GFR (eGFR), CKD is staged from 1 to 5 (Table 1) [1].

Table 1. GFR Categories in CKD [1]

GFR Category (CKD stage)	eGFR (mL/mim/1.73m^2)	Terms
G1	≥90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

Measured GFR (mGFR)

Measured GFR is determined by the clearance of an exogenous substance which is neither reabsorbed nor secreted by the kidneys after glomerular filtration. The exogenous substance is given as an intravenous injection. The rate of excretion is

directly proportional to the rate of filtration of water and solutes across the glomerular filter. The classic inulin clearance method is considered to be the golden standard method and a reference for GFR measurement [2]. But it is a cumbersome method and not practical in clinical routine. Other tracer substances, like iohexol, ^{51}Cr-EDTA and ^{131}I-iothalamate are more widely used [3]. In our hospital iohexol clearance is used in clinical routine. Iohexol is a non-ionic contrast medium, which is water soluble and non-protein bound, and is freely filtered through the glomeruli. Iohexol clearance has a good correlation with inulin clearance and is easily performed [4,5].

Estimated GFR (eGFR)

Although mGFR provides the most accurate evaluation of renal function, the methods of measuring mGFR are invasive and labour intensive. Therefore some endogenous substances, like creatinine and cystatin C are used to estimate GFR. Most well-founded, generally used and recommended creatinine-based GFR prediction equations are the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. Both equations use the same variables (age, sex, race, and serum creatinine level) but with different coefficients [6]. Creatinine is a metabolic end product of muscle activity and metabolism, so it is highly related to muscle mass. Therefore, the mean muscle mass of a specified age, sex and ethnic origin in the population is employed to derive the equations to compensate for the influence of muscle mass on the creatinine-level used for estimating GFR. However, the MDRD-equation was found to underestimate GFR [7,8].

Cystatin C is a protein produced by all nuclear cells. Cystatin C is removed from the bloodstream by glomerular filtration and neither reabsorbed nor secreted by the kidney. Cystatin C is not influenced by muscle mass, so GFR estimation based on cystatin C is more accurate than that based on creatinine [9,10].

In our hospital, the Lund model is used to estimate GFR. This comprises simultaneous use of a cystatin C- and a creatinine-based GFR prediction equation. If the GFRs predicted agree, the mean value is used and is regarded as a reliable GFR-estimate. If the GFRs predicted do not agree, the clinical situation must be considered e.g. concerning the presence of an abnormal muscle mass or use of high doses of glucocorticoids, and preferably one of the estimates should be used. If no reasons for the difference in predicted GFRs are found, GFR should be measured [11].

In our studies, we chose mGFR rather than eGFR as the measure of kidney function, as mGFR would not be affected by muscle mass and is more accurate than eGFR.

Cardiovascular disease and Malnutrition - inflammation - arteriosclerosis

Cardiovascular disease is the leading cause of mortality and morbidity in patients with CKD, especially in those with eGFR <60 mL/min/1.73 m^2 [12]. More than half of the deaths in patients on dialysis are related to cardiovascular disease [13]. A large study including 1,120,295 subjects showed that a reduced eGFR was independently associated with increased risks of cardiovascular events and death even in people with non-dialysis dependent CKD [13].

Many studies have reported that protein energy malnutrition and inflammation are two strong factors associated with cardiovascular events, decreased quality of life and increased hospitalization in patients with CKD [14-16]. One study has shown that malnutrition may aggravate heart failure by inducing morphologic and functional deterioration of the myocardium [17].

Some inflammatory markers, such as interleukin 6 (IL6) and C-reactive protein (CRP), have also been reported to be strong predictors of cardiovascular events [18,19]. These pro-inflammatory cytokines not only contribute to malnutrition by inducing proteolysis in muscle [20], but are also involved in vascular calcification [21]. Unlike the arterial plaques in non-CKD patients, autopsy studies have shown that the plaques in CKD not only involved the intimal, but also the medial layer of the artery [22]. The changes to the intimal layer were mainly caused by atherosclerosis and lipids, and the changes to the media were associated with the disorder of mineral metabolism in CKD [23].

In a cross-sectional study in 1999, Stenvinkel et al found a high prevalence of malnutrition, inflammation and carotid plaques in pre-dialysis patients that these three conditions frequently coincided and were strongly associated with each other [24]. Malnutrition - inflammation - atherosclerosis (MIA) syndrome was used to describe the status where the three clinical entities, malnutrition, inflammation and atherosclerosis, coexist and interact with each other in CKD [25].

Since atherosclerosis is a category of arteriosclerosis, and the vascular disease in CKD not only involves atherosclerosis, which affects the intimal layer, but also calcification in the medial layer, we use the term arteriosclerosis instead of the atherosclerosis in MIA to include both types of vascular lesions in CKD. This will be elucidated further in the “Arteriosclerosis in CKD” section below.

Malnutrition in CKD

Protein energy malnutrition and muscle wasting

Muscle plays a key role not only in physical performance but also in whole body protein metabolism. In states of starvation, stress, under nutrition or disease, muscle supplies abundant amino acids to meet the increased demands of protein [26]. Consequently, patients with limited reserves of muscle mass respond poorly to disease [27,28].

Protein energy malnutrition is a state of decreased body pools of protein with or without fat depletion or a state of diminished functional capacity [29]. Protein energy malnutrition with muscle wasting is present in a large proportion of patients with CKD. In CKD, both consequences of uraemia, such as inflammation, metabolic acidosis, insulin resistance, loss of appetite and its treatment, comprising restriction of protein intake and dialysis, contribute to loss of muscle mass [30,31]. Studies have shown that loss of muscle mass is associated with a decline in kidney function [32,33]. It is also an important predictor of muscle strength, physical performance and even survival in patients with CKD [33-35].

Sarcopenia

Sarcopenia is a word with Greek roots, sarx for flesh and penia for loss. It describes not only the degenerative loss of skeletal muscle mass, but also the loss of muscle quality and function associated with aging. Although sarcopenia was originally known as a condition related to aging, it is also prevalent and independent of age, in the CKD population because of protein energy malnutrition [36]. Decreased muscle mass and muscle function are two dominant conditions of the diagnosis of sarcopenia. In 2010 the European Working Group for Sarcopenia in Older People defined sarcopenia as low muscle mass plus either low muscle strength or low physical performance [37].

The prevalence of sarcopenia varies depending on the populations studied and the methods applied. In 60-70 years old it was reported to be 5 to 13% [37]; in people >80 years it was 11 to 50% [37]. In patients with non-dialysis dependent CKD, sarcopenia ranged from 6 to 16% [33,36] and could reach up to 37% in patients on dialysis [38]. Sarcopenia has been shown to be associated with multiple adverse clinical outcomes and has been reported be an independent predictor of mortality in patients with CKD [36,39].

As it is increasingly recognized that muscle strength is better than muscle mass in predicting adverse outcomes [40-42]. The new guidelines from 2018, issued by the

European Working Group for Sarcopenia in Older People, proposed that decreased muscle strength should come to the forefront. Probable sarcopenia is diagnosed by low muscle strength; sarcopenia is confirmed by additional low muscle quantity or quality; and sarcopenia is considered severe if low muscle strength, low muscle quantity or quality and low physical performance are all met [42].

Body composition measurement

Dual-energy X-ray absorptiometry (DEXA) and bioimpedance are two commonly used methods for body composition measurement in CKD. DEXA is regarded as the gold standard method in the general population [43]. However, it cannot measure body water, so may not be an optimal method in patients with CKD [44]. Bioimpedance comprises bioimpedance analysis and bioimpedance spectroscopy. It has more measurement compartments than DEXA and can also provide information on body water. In a previous study, we showed that changes in body composition might affect the measurement differences of DEXA and bioimpedance [45]. Most importantly, because of the limited agreement between DEXA and bioimpedance, the same measurement should be used for one patient over time.

DEXA

DEXA is a means of measuring body composition. Two X-ray beams, with different energy levels, are aimed at the subject's bones. DEXA is regarded as a gold standard measurement especially for bone and fat mass [43]. The differential attenuation of two energies by the subject is used to estimate the bone mineral content and soft tissue composition. Then fat mass and lean mass are separated by the ratio of attenuations of body mass [46]. Since the ratio of additional body fluid is similar to lean mass, DEXA usually measures additional body fluid as lean mass [44].

Body composition monitor (BCM)

In contrast to DEXA, bioimpedance can detect total body water, extracellular water, intracellular water, and overhydration. BCM is a portable bioimpedance monitor, which is cheaper and easier to perform than DEXA. In BCM, lean mass measurement is calculated by an equation after taking body water into account [47]. However, fat mass is calculated simply by subtracting fat free mass from total body weight, which means that the measurement of fat mass in BCM might be less accurate than in DEXA [47].

Subjective global assessment (SGA)

SGA is a nutritional assessment tool widely used in clinical practice. SGA was standardized in 1987 by Detsky et al. [48]. It is based on features of the patient's history and physical examination [48]. According to the subjective evaluation of the observer, the nutritional diagnosis is defined and the patient is classified as: A, well-nourished, B, moderately (or suspected of being) malnourished, or C, severely malnourished. SGA is widely used because it is simple, non-invasive, inexpensive, and is capable of identifying patients at higher nutritional risk [49]. However it is hard to detect low grade nutritional changes with SGA, and its subjectivity may also limit the accuracy in routine care [49].

Normalized protein catabolic rate (nPCR)

nPCR is expressed in grams of protein degraded daily divided by body weight. It has been proposed as a measure of dietary protein intake and ultimately nutrition. However nPCR only reflects the protein intake of the day of the analysis and does not represent the long term nutritional status.

Myostatin

Myostatin, also known as growth differentiation factor 8 (GDF-8), is a 25 kDa myokine produced and released by myocytes. Myostatin acts on muscle cells' autocrine function to inhibit muscle cell growth and differentiation [50]. The gene encoding myostatin was discovered in 1997 by Alexandra McPherron et al. They produced a knockout strain of mice that lacked the gene, and found that these mice had approximately twice as much muscle as normal mice [50].

Myostatin is predominantly expressed in skeletal muscle [50] and has been found to be overexpressed in uremic sarcopenia [31,51]. Consequently, inhibition of myostatin expression has been suggested as a strategy to treat muscle wasting in CKD [51,52]. However, the literature presents conflicting evidence on the relationship between myostatin and muscle mass both in patients on dialysis and in people in general [52-55]. Moreover, myostatin levels have been shown to decrease after exercise training in the general population and in patients on dialysis [56,57], but the results concerning the expression of myostatin in response to exercise are not consistent [58,59].

Myostatin has also been implicated to be involved in vascular damage by activating transforming growth factor beta (TGF-β) signalling and increasing expression of proatherogenic adhesion molecules [60]. An increased expression was found both in the medial and intimal layers of arteries with progressive atherosclerotic lesions [60]. Additionally, myostatin was also upregulated in an inflammation environment and its expression was directly associated with IL6 [61].

In consequence, the activation of myostatin has been proposed to be a link between malnutrition, inflammation and arteriosclerosis [60].

We decided that measuring body composition was the most straightforward way to examine protein energy malnutrition. As our patients were non-dialysis dependent and without severe overhydration (1.2±1.0 L) [45], we chose DEXA to measure body composition and sarcopenia in studies 1 and 2. Myostatin was chosen as a marker of muscle growth in study 2.

Inflammation in CKD

It is well established that low-grade systemic inflammation, characterized by hypoalbuminemia and elevated levels of circulating inflammatory markers like CRP and IL6, is frequently observed in patients with CKD [62,63]. This is due to reduced cytokine clearance, oxidative stress, malnutrition, muscle wasting, metabolic acidosis, disorders in bone mineral metabolism, accumulation of advanced glycation end-products and toxins absorbed in the gut [63].

Earlier studies have indicated that these inflammatory processes could induce vascular calcification by promoting proliferation and infiltration of inflammatory cells into both the intimal and medial layers of arteries [19,23,29]. Inflammation-induced oxidative stress is a strong inducer of vascular smooth muscle cell (VSMC) damage [21]. Inflammation is not only a stimulus but also a result of calcification. The calcium and phosphate deposits can stimulate the production of some pro-inflammatory cytokines by residing in macrophages [64].

Albumin

Albumin is a 65 kDa protein that is synthesized in the liver. In CKD, conditions such as chronic metabolic acidosis and inflammation profoundly interfere with albumin synthesis [65]. In the clinical setting, albumin is widely used as a marker of nutritional status [65,66]. However, it has been proposed that albumin predominately should be considered as a marker of inflammation and illness because of its poor correlation with other nutritional markers, like DEXA and SGA [65-67]. Moreover, low levels of serum albumin have been shown to be strongly associated with systemic inflammation in patients on hemodialysis [67]. It is of consequence that the relationship between hypoalbuminemia and inflammation is strongly associated with mortality in patients with CKD [68,69].

CRP

Of all the acute phase proteins and plasma inflammatory markers, CRP is the one most widely used. CRP rises rapidly following an inflammatory stimulus and mainly responds to an elevation in IL6. It is also associated with all-cause and cardiovascular mortality in patients with CKD [70-72].

Interleukin 6 (IL6)

IL6 (22 to 27 kDa) is produced by numerous types of immune cells, and can be stimulated by bacterial endotoxins, oxidative stress and physical exercise [73]. In CKD, the IL6 levels can be elevated before the initiation of dialysis due to multiple factors as mentioned above [73]. Its activation mainly depends on the tumour necrosis factor α (TNF-α), which is also elevated in CKD [74]. IL6 can promote osteogenic transition and calcification directly in VSMCs. Additionally, IL6 can also contribute to vascular calcification by decreasing fetuin-A (a calcification inhibitor, will be elucidated below) expression in the liver and klotho expression in the kidney [21]. The levels of both IL6 and TNF-α have been shown to be associated with vascular calcification and cardiovascular mortality [75,76].

Plasma IL6 has also been shown to increase in direct response to exercise albeit without an increase in TNF-α [77]. The production of IL6 is associated with contracting muscle and myocytes could be the origin of IL6 [77]. IL6 is the first detectable cytokine released into the blood, and its response is sensitive to the exercise duration and intensity [78]. The levels of IL6 reach a peak at the end of exercise or shortly after, which is followed by a rapid decrease to pre-exercise levels [79].

Although there are a number of inflammatory markers, CRP and IL6 are most commonly used to demonstrate low-grade inflammation in patients with CKD. Additionally, CRP and albumin are laboratory analyses in clinical routine. In our studies, we chose these three markers to represent inflammatory status in studies 3 and 4.

Arteriosclerosis in CKD

The current classification of arteriosclerosis comprises three lesions: atherosclerosis, Mönckeberg's medial calcific sclerosis, and arteriolosclerosis [80].

Atherosclerosis is a disease of elastic and large arteries in which the atheroma is the characteristic lesion. It is considered to be a result of hyperlipidemia, lipid

oxidation and impairment of endothelial function, and is characterized by vascular intimal plaques [81,82].

The so-called Mönckeberg's medial calcific sclerosis is not purely a medial lesion with mineral deposits, but also involves the intimal layer [82,83]. This type of sclerosis is associated with disorders of calcium-phosphate metabolism and abnormal levels of bone-related proteins, such as fetuin-A and matrix-Gla protein in chronic kidney disease [23,81]. Medial calcification also results in vascular stiffness [23,81].

Arteriolosclerosis is a hardening of the wall of very small arteries by intimal fibromuscular tissue or hyaline deposition, typically it is associated with hypertension and diabetes [82].

The first two categories, atherosclerosis and Mönckeberg's medial calcific sclerosis, are very common in patients with CKD, and both are regarded to be promoted by inflammation, uremic toxins and oxidative stress [23,81,84].

A causal relationship between arterial calcification and cardiovascular events is well known [85,86]. Therefore, the presence of calcified lesions in arteries has been widely used as a measurement to evaluate arteriosclerosis also in patients with CKD.

Abdominal aortic calcification (AAC) score

In order to examine the relationship between the degree of vascular calcification and patient outcomes, some quantification approaches have been developed. Kauppila's AAC score is a grading system to assess the location and progression of calcification in the abdominal aorta from a lumbar radiograph [87]. Another commonly used approach is Agatston coronary artery calcification (CAC) score, which is measured by computed tomography (CT) [88]. Compared to CAC, AAC is more feasible and less costly. Additionally, the AAC score has been shown to correlate well with the CAC score [89,90] and has been shown to be reliable predictor of cardiovascular risk in patients with CKD [91,92].

The AAC score was suggested as a useful tool for assessment of vascular calcification in patients with CKD in the 2009 KDIGO clinical practice guideline on CKD mineral and bone disorder [93].

Lipids and lipoproteins

Atherosclerosis is initiated by low-density lipoprotein (LDL) entering the arterial wall and followed by oxidative stress and inflammation [94]. Dyslipidemia is common in patients with CKD [95], which is characterized as a decreasing high-

density lipoprotein cholesterol (HDL-C), while triglycerides and low-density lipoprotein cholesterol (LDL-C) increase with the decline in GFR [95]. It has been well established that the levels blood cholesterol and triglycerides are strongly associated with coronary events [96,97]. The high levels of LDL-C and low levels of HDL-C are both associated with an increased risk of cardiovascular morbidity and mortality [96,98]. Lipoprotein (a) is a lipoprotein variant which consists of an LDL-like particle and the specific apolipoprotein (a). Genetic evidence has confirmed that lipoprotein (a) is a direct cause of cardiovascular disease [99].

Fibroblast growth factor 23 (FGF23) - klotho endocrine axis

FGF23 is a 32kDa protein secreted by osteocytes and osteoblasts from bone and plays a key role in the regulation of mineral metabolism [100]. The action of FGF23 is dependent on the presence of its co-receptor klotho. As klotho is mainly expressed in kidney, parathyroid gland and brain, the function of FGF23 is considered to be restricted to these organs [101].

In healthy individuals, FGF23 levels fall and rise in parallel with the amount of phosphate intake to maintain the plasma phosphate within a normal range. When the phosphate intake is high, FGF23 levels increase to induce greater phosphaturia and, by lowering $1,25(OH)_2D_3$ (calcitriol) levels to reduce the absorption of phosphate in the gut [102]. When acting on the parathyroid gland, FGF23 suppresses the secretion and synthesis of parathyroid hormone (PTH) [103].

In CKD, klotho levels start declining during stage 1 due to albuminuria. Throughout the course of CKD there is a continued decrease in klotho, which in turn results in an increase in FGF23, which is followed by a decrease in $1,25(OH)_2D_3$, an increase in PTH and finally an increase in phosphate during CKD stage 5 [104].

Although several studies have suggested that the dysregulation of the FGF23 - klotho axis is associated with higher risks of morbidity and mortality in CKD [105-107], the effects of FGF23 on vascular calcification still remain controversial [108].

Fetuin-A

Fetuin-A is a 64 kDa glycoprotein which is mainly expressed and secreted from the liver and adipose tissue [109]. Fetuin-A is associated with many factors, such as insulin sensitivity [110], lipid levels [111], and inflammatory markers [112]. It is also an important inhibitor of calcification by binding calcium phosphate and calcium carbonate, especially in CKD [23,113].

The low-grade systemic inflammation in CKD could cause a decrease in fetuin-A secretion [21]. Cross-sectional studies have suggested that fetuin-A is independently associated with eGFR [114,115]. A lower level of fetuin-A was associated with a higher risk of cardiovascular and all-cause mortality in patients with CKD [115,116], suggesting that fetuin-A may be a protective factor of vascular calcification in CKD. Although the majority studies have shown that low fetuin-A serum levels are related to vascular calcification and increased mortality in patients with CKD, its exact role on vascular calcification is still controversial.

In our studies, we chose the AAC score as the index of arteriosclerosis in studies 3 and 4, because it is feasible in routine clinical, practice and inexpensive. Moreover AAC shows the actual calcific plaque in the aorta instead of the degree of arterial stiffness such as measured by pulse wave velocity. We chose the following markers of arteriosclerosis in study 4: lipids and lipoproteins as they contribute to atherosclerosis on the intimal arterial layers, FGF23 as dysregulation of the FGF23 - klotho axis is one of the underlying mechanisms of vascular calcification and fetuin-A as it is considered to be a powerful calcification inhibitor.

Exercise training in CKD

Exercise training in patients with CKD is generally accepted to have positive effects on physical capacity and cardiovascular disease with the earliest studies performed over 30 years ago [117,118]. The KDIGO guidelines from 2012 recommend that people with CKD undertake physical activity compatible with cardiovascular health and tolerance, i.e. aiming for at least 30 minutes 5 times per week [1]. In a systematic review and meta-analysis of 41 randomized clinical trials (928 participants) of exercise training in adult patients with CKD, Heiwe et al showed that regular exercise for at least 30 minutes per session 3 times a week would improve aerobic capacity, blood pressure, survival, muscular strength, and health-related quality of life in patients with CKD with or without renal replacement therapy [119]. However, most of the exercise trials were in patients on dialysis.

In previous studies from the Renal Exercise (RENEXC) trial, we have shown that exercise training was effective in improving physical performance in patients with non-dialysis dependent CKD stages 3 - 5 [120,121].

Effects of exercise training on muscle wasting

Augmented muscle degradation is common in patients with CKD and can lead to muscle wasting and impaired physical activity [122]. The activation of the ubiquitin - proteasome system and caspase-3 have been demonstrated to be key events in

inducing muscle catabolism in CKD [123,124]. Ubiquitin - proteasome system degrades actin and myosin in all cells and caspase-3 breakdowns the structure of muscle.

Exercise training may slow down the wasting of muscle by reducing the levels of oxidative stress and by limiting the protein degradation activation [125]. Experimental studies have shown that exercise could also promote anabolism in CKD [126,127]. Exercise was reported to activate protein synthesis through increasing the expression of the mammalian target of rapamycin (mTOR) gene [126], which regulates cell metabolism and organism lifespan [128]. Protein turnover is increased due to an acceleration of synthesis and degradation after exercise [127]. A post exercise acceleration of amino acid transport may also contribute to the relatively greater stimulation of protein synthesis [127].

In healthy people it is well established that exercise not only increases muscle strength but also can increase muscle mass. However, in patients with CKD [129-133] the evidence is scant and the results are inconsistent [134]. There are some randomized controlled trials on the effects of exercise training on muscle mass, but they were performed in relatively small groups of patients (10 to 20 patients in each group) and with short intervention periods (12 weeks) [131-133,135].

Effects of exercise training on inflammation

The anti-inflammatory effect of exercise training has been suggested to be mainly mediated by IL6 [79]. During acute exercise, IL6 is the first detectable cytokine released from the contracting muscle cells into the blood [79]. The increase in IL6 levels stimulates the synthesis of anti-inflammatory cytokines through inducing the production of IL1 receptor antagonist and IL10, one of the most important anti-inflammatory cytokines [136,137]. IL10 can inhibit the synthesis of pro-inflammatory cytokines, like TNF-α [138].

In one study, the levels of IL6 were reported to be reduced after 6 months of regular exercise, whereas the levels of IL10 tended to be increased, indicating an anti-inflammatory effect of exercise adaptation [139]. Moreover, these investigators reported that the activation of T-lymphocytes and monocytes were downregulated after regular exercise [139].

Regular exercise training has also been suggested to prevent the accumulation of abdominal fat, which is associated with low-grade inflammation, partly mediated by the upregulation of myokine IL15, an anabolic cytokine [140]. IL15 not only induces protein accumulation but also reduces adipose tissue mass [141,142]. IL15 may also be involved in decreasing or even inhibiting TNF-α in a state of low-grade inflammation[143].

Large observational studies have shown that higher levels of physical activity were associated with lower levels of inflammatory markers in healthy people, of which CRP and IL6 were the most commonly used inflammatory markers [144-146].

However, data from interventional studies in patients with CKD are less consistent, this might be due to different exercise regimes, different baseline inflammatory status, or underpowered study designs [147-149]. The effects of exercise training on systemic inflammation in patients with CKD are far from clear.

Effects of exercise training on arteriosclerosis

Nitric oxide (NO) is an important endothelium-derived vasoactive factor that relaxes VSMCs [150]. NO also inhibits leukocyte adhesion and platelet aggregation in the vascular endothelium [150]. In CKD, NO bioavailability was decreased because of the increased expression of the endothelial NO synthase (eNOS) inhibitors, such as caveolin-1 and asymmetric dimethylarginine (ADMA), as well as the decrease in eNOS promoters, like protein kinase B [81]. The decreased NO bioavailability is one of the most important factors involved in endothelial dysfunction.

Exercise training has been suggested to increase NO bioavailability through both enhancing eNOS activity and preventing eNOS uncoupling [81]. Moreover, exercise can increase the number and functionality of endothelial progenitor cells, which contributes to the repair of endothelial cell layer [151].

Oxidative stress increases with declining renal function due to various uremic toxins and inflammation [152]. Oxidative stress is associated with both atherosclerosis and arterial calcification [153]. Exercise training can reduce oxidative stress by increasing various anti-oxidative enzymes [154]. Additionally, the anti-inflammatory effects of exercise could also be beneficial for vascular disease in patients with CKD.

In subjects without CKD, clinical studies have shown that exercise training has a protective effect on cardiovascular disease, with a reduction in coronary atherosclerotic lesions [155,156]. In patients with CKD on dialysis, an improved vascular endothelial function and arterial stiffness/compliance has been reported after exercise training [157,158]. However, this was not confirmed in non-dialysis dependent patients with CKD [159]. Moreover, to our knowledge and to date, no study has investigated the effects of exercise training on medial layer calcification.

Aims

Study 1

- To investigate the prevalence of sarcopenia, the relationship between muscle mass and GFR and physical performance in non-dialysis dependent patients with CKD stages 3 to 5.

Study 2

- To investigate the effects of 12 months of exercise training on sarcopenia, muscle mass and plasma myostatin in non-dialysis dependent patients with CKD stages 3 to 5;
- To investigate the relationships between plasma myostatin and physical performance and muscle mass, respectively.

Study 3

- To investigate the relationship between abdominal aortic calcification score (AAC) and GFR in non-dialysis dependent patients with CKD stages 3 to 5;
- To investigate the relationship between AAC and pulse pressure, some calcific- and inflammatory markers and cardiac structure, respectively.

Study 4

- To investigate the effects of 12 months of exercise training on AAC and some markers of arteriosclerosis, respectively, in non-dialysis dependent patients with CKD stages 3 to 5;
- To investigate the relationships between AAC and GFR and these markers.

Methods and patients

Study design

These four studies are all pre-specified sub-studies of the RENEXC trial (Table 2).

RENEXC (www.ClinicalTrials.gov NCT02041156) is a single centre, consecutive, prospective, randomized controlled and interventional exercise training study in patients with CKD not on renal replacement therapy. The study was approved by the Regional Ethical Review Board in Lund (registration number 2011/369) and adhered to the Helsinki Declaration.

The inclusion criteria were: prevalent and incident patients at the Department of Nephrology in Lund, Skåne University Hospital, with an eGFR <30 mL/min/1.73m^2, age ≥18 years, all renal diagnoses, any number of comorbidities. The exclusion criteria were: orthopaedic impediment, severe neurological dysfunction, inability to understand patient information, renal replacement therapy, clinical signs and symptoms of heart failure (NYHA class ≥III) and estimated start of dialysis within 12 months of study start. All participants gave informed consent prior to inclusion after having received written and oral information.

Table 2. Study type

Study	Type	Number of patients
1	Cross-sectional baseline analysis	151
2	Pre-specified sub-study, randomized controlled trial	112. Balance group, 59; Strength group, 53
3	Cross-sectional baseline analysis	151
4	Pre-specified sub-study, longitudinal analysis	112

Intervention

151 patients irrespective of age or comorbidity were randomly assigned to either strength or balance training both in combination with endurance training. Both groups were prescribed 150 minutes per week of self-administered exercise training for an intervention period of 12 months. 60 minutes per week of

endurance training was part of the prescription in both groups and was combined with 90 minutes per week of either strength training or balance training.

Randomization and blinding

Random allocation was generated by program SAS ProcPlan. Patients were included and allocated sequential treatment according to a list which only the research physiotherapist had access to. The random allocation sequence was generated by one investigator (Peter Höglund). Then the nephrologists enrolled the patients and the research physiotherapist assigned them intervention. All the recruitment staff except the research physiotherapist were blinded to the randomization.

Comorbidity

The comorbidity of each patient was evaluated by the same clinician (Matthias Hellberg) using the Davies Comorbidity Score with seven different domains: malignancy, ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, diabetes mellitus, systemic collagen vascular disease or other significant pathology (a condition severe enough to have an impact on survival in the general population) [160]. We defined the following comorbidities as cardiovascular comorbidities (CVC): ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, and diabetes mellitus. Patients with at least one of those comorbidities were defined as having CVC.

GFR

Measured GFR

Iohexol clearance was used to assess the mGFR [161]. It was performed at the Department of Clinical Chemistry, Laboratory Medicine Skåne, which is accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC) according to international standards of ISO 15189:2012. First an intravenous injection of 5ml iohexol is given. Then the sample is taken either after 7 hours (eGFR 20-50 mL/min/1.73m^2) or 24 hours (eGFR <20 mL/min/1.73m^2). The serum iohexol concentration is analyzed with high performance liquid

chromatography. Then GFR is calculated using age, sex, weight, height, iohexol dose, time from injection to sample taken and iohexol concentration [162].

Estimated GFR

The eGFR was estimated using cystatin C- and creatinine-based equations, Lund model, created by Grubb A [11].

Nutritional assessments

Each patient was prescribed continued normal protein intake or a restricted protein diet consisting of 0.8 or 0.6 g/kg body weight/day by their physician. All patients were referred to a specialized renal dietician for dietary counselling at the start of the trial and were followed according to the department's standard procedure throughout the study period. SGA [48] and nPCR were used to describe the patients' nutritional status. SGA is classified as A, well-nourished, B, moderately (or suspected of being) malnourished, or C, severely malnourished. nPCR was calculated using the equation: 6.25× [(0.028×urine urea×24-h urine volume) + (0.031×weight)]/body weight.

Physical performance

Handgrip strength

Handgrip strength was measured in kilograms by a Jamar dynamometer, in a sitting position without back support with the elbow at a 90 degrees angle and the arm close to the body. For each hand, the scores of 3 successive trials were recorded and the mean registered [163].

Isometric quadriceps strength

Isometric quadriceps strength was tested by knee extension against resistance and evaluated in kilograms multiplied by the distance from the knee to the ankle. For each leg the scores of 3 successive trials were recorded and the mean registered [163].

Functional reach

The patient stood positioned next to a wall but not touching, the arm was extended at a 90 degrees angle from the shoulder, fist closed, and then asked to reach forward as far as possible without losing balance. The distance between the starting point and end point of three successive trials was recorded and the mean registered [163].

Berg's balance test

The test comprises a set of 14 simple balance related tasks with a full score of 56, ranging from being able to sit on a chair without back support, to standing on one foot. The degree of success in achieving each task was given a score of zero (unable) to four (independent), and the final measure was the sum of all the scores [164].

Six-minute walking test

The patient walked as fast as possible along a marked indoor corridor. The walking distance during 6 minutes was recorded [165].

Laboratory measurements

Clinical laboratory analyses and plasma $1,25(OH)_2D_3$

Routine clinical laboratory analyses and plasma $1,25(OH)_2D_3$ were measured at the Department of Clinical Chemistry, Laboratory Medicine Skåne, which is accredited by SWEDAC according to international standards of ISO 15189:2012.

ELISA measurements

Plasma myostatin, fetuin-A, IL6 and FGF23 were all measured using ELISA kits (R&D systems, Inc, Minneapolis, USA) at the Nephrology Laboratory, Biomedicine Centre at Lund University. The plasma was collected fasting and stored at -80°C. Since there are no standard reference ranges for these variables, the values from healthy subjects presented in previous studies were used [102,115,166,167].

Definition of sarcopenia

During this project, the European Working Group on Sarcopenia in Older People revised the guideline for sarcopenia in 2018. In study 1, sarcopenia was defined as low muscle mass [168] or low muscle strength [169] or both [40]. Appendicular skeletal muscle index (ASMI) <7.3 kg/m^2 for men and <5.5 kg/m^2 for women were defined as low muscle mass [168]. Handgrip strength <30 kg for men and <20 kg for women were defined as low muscle strength [169]. In study 2, sarcopenia was defined as a combination of low muscle strength and low muscle mass according to the latest guideline 2018 with new cut-offs to increase harmonisation with sarcopenia studies [42]. Handgrip strength <27 kg for men and <16 kg for women were defined as low muscle strength [42]. ASMI <7 kg/m^2 for men and <6 kg/m^2 for women were defined as low muscle mass [42].

Dual energy X-ray absorptiometry (DEXA)

Body composition was measured by DEXA at baseline and after 12 months. DEXA scanning was conducted in a standardized manner according to the procedures recommended by the manufacturer, at the Department of Diagnostic Radiology, Skåne University Hospital, which is accredited by SWEDAC according to ISO 15189:2012. The hospital changed from Lunar Prodigy to Lunar iDEXA during the study period. Lunar iDEXA is an upgrade from Lunar Prodigy and has a superior camera and gives a more precise measurement. The analysis software is the same as Lunar Prodigy.

Assessment of abdominal aortic calcification (AAC) score

AAC was evaluated by lateral lumbar X-ray, at the Department of Diagnostic Radiology, Skåne University Hospital, which is accredited by SWEDAC according to ISO 15189:2012. The scoring system described by Kauppila was used to calculate the AAC score [87]. Calcific deposits were graded on a scale of 0-3 on both posterior and anterior sides of each segment: 0= no calcific deposits, 1= calcific deposits filling less than 1/3 of the aortic wall, 2= 1/3 to 2/3 of the aortic wall calcified, 3= more than 2/3 of the aortic wall calcified. The grades of four segments (Lumbar 1 - Lumbar 4) were added up, giving a range between 0-24. 0 score is normal, 1-6 score is moderate calcification, 7 and above is severe

calcification [87]. The grading was performed by one investigator who was blinded to the randomization of each patient (Yunan Zhou).

24-hour blood pressure measurement

24-hour-blood pressure was measured at the Department of Clinical Physiology and Nuclear Medicine, Skåne University Hospital, which is accredited by SWEDAC according to ISO 15189:2012. All patients underwent monitoring with SpaceLabs Medical ABP-monitor model 90207 or 90217. The size of the blood pressure cuff was selected according to each patient's arm circumference. The blood pressure was measured using a completely automatic oscillometric device, which measured blood pressure every 20 minutes during the day and every 30 minutes during the night. The collected information was transferred to SpaceLabs computerized reporting system 90121-1 for Windows. 24-hour pulse pressure was calculated using the average value of 24-hour systolic blood pressure minus the average value of 24-hour diastolic blood pressure.

Echocardiographic measurements

The ultrasound machine used was Philips iE33 or Epic from Philips Healthcare, Eindhoven, Netherlands. Echocardiographic examinations of the patients were performed by different experienced sonographers using the same echocardiographic protocol at the Echo Laboratory, Department of Clinical Physiology and Nuclear Medicine, Skåne University Hospital, which is accredited by SWEDAC according to ISO 15189:2012. All measurements and calculations are according to ASE guidelines from 2015 [170].

In routine clinical practice at our hospital left ventricular ejection fraction (LVEF) is reported as: normal (LVEF ≥55%) or reduced (LVEF <55%). Mitral E/A ratio was assessed as a marker of LV diastolic function. E/A is the ratio of E (peak early transmitral filling wave velocity) to A (peak late transmitral filling wave velocity). The measurements were then indexed to body surface area (BSA), when appropriate. Thus, left ventricular mass index (LVMI) was calculated as left ventricular mass (LVM)/BSA and left atrial volume index (LAVI) as left atrial volume (LAV)/BSA [171].

Statistical analysis

To detect 60% differences at 5% significance level and 80% of power, we calculated that we needed to include 75 patients in each group in order to achieve complete data for 50 patients at the end of the intervention. Continuous variables are presented as mean±SD or median (25th-75th percentile). Categorical variables are given as frequencies and percentages. T-test was used to compare parametric variables, Wilcoxon signed rank test was used to compare nonparametric variables and Fisher test was used to compare binary variables. Linear regression analysis was used to analyze the relationships between variables. In our previous study, in which we reported the primary outcomes of the RENEXC trial, mixed model analyses showed that there were no significant differences between groups for changes in any of the measures of physical performance after 12 months of exercise training for the 151 patients who were randomized [121]. This is why we pooled the patients from the two groups in the linear regression analyses in study 2 and in all the analyses in study 4 in order to increase the power of the linear regression. A p-value <0.05 was considered statistically significant. Data were analyzed using R software (R foundation for Statistical Computing, Vienna, Austria).

Results

Patients

After 217 patients had been screened, 151 patients (98 men and 53 women) were randomized. These 151 patients were included in studies 1 and 3. After 12 months of exercise training, 112 patients (76 men and 36 women) had completed RENEXC. These 112 patients were included in studies 2 and 4. The CONSORT Flow Diagram is presented in Figure 1. The clinical characteristics are presented in Table 3.

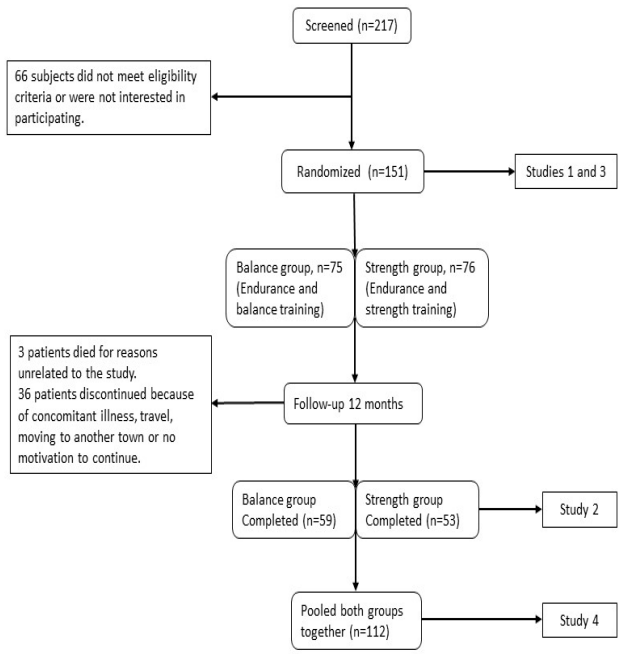

Figure 1. CONSORT Flow Diagram

Table 3. Clinical characteristics at baseline

Characteristics	Units	151 patients	112 patients
Age	year	66±14	67±13
Male/Female	n(%)	98(65)/53(35)	76(68)/36(32)
Weight	kg	82±18	81±17
Height	m	1.72±0.09	1.72±0.09
BMI	kg/m^2	28±5	27±5
SGA	score(A/B/C)	132A, 13B, 0C	102A, 10B, 0C
nPCR	g/kg body weight/day	0.97±0.44	0.99±0.47
mGFR	$mL/min/1.73m^2$	22.5±8.2	22.6±8.0
eGFR	$mL/min/1.73m^2$	19.7±7.4	20.4±7.3
P-creatinine	μmol/L	254±104	247±91
P-urea	mmol/L	16±5	15±5
P-PTH	pmol/L	12 (9-18)	11 (8-17)
P-Albumin	g/L	37±4	37±3
B-Hemoglobin	g/L	127±14	128±14
P-Triglyceride	mmol/L	1.8±1.0	1.8±1.0
P-Tatal cholesterol	mmol/L	4.8±1.3	4.8±1.2
P-HDL-C	mmol/L	1.3±0.4	1.3±0.4
P-LDL-C	mmol/L	3.0±1.1	2.9±1.0
P-Potassium	mmol/L	4.2±0.5	4.2±0.5
P-Calcium	mmol/L	2.3±0.1	2.3±0.1
P-Ca×P	$mmol^2/L^2$	2.7±0.7	2.6±0.6
P-Phosphate	mmol/L	1.2±0.3	1.1±0.2
Base excess	mmol /L	-1.2 (-3.2-0.1)	-1.2 (-2.8-0.1)
P-CRP	mg/L	3 (1.5-6.1)	3.1 (1.3-6.1)
Medication, n(%)			
Antihypertensive medication		143(95)	105(94)
Calcium channel blocker		86(57)	65(58)
Beta blocker		101(67)	73(65)
RAAS-blocker		91(61)	71(63)
Central antiadrenergic medication		16(11)	13(12)
Diuretic		108(72)	79(71)
Active vitamin D		95(63)	70(62)
Phosphate binder		67(44)	42(38)
Calcimimetic		2(1)	2(2)
Statin		79(52)	61(54)
Causes of CKD, n(%)			
Hypertensive kidney disease		62(41)	47(42)
Diabetes nephropathy		24(16)	21(19)
Interstitial nephritis		22(15)	18(17)
Chronic glomerulonephritis		23(15)	12(11)
Polycystic kidney disease		9(6)	6(5)
Others		11(7)	8(6)
Comorbidity, n(%)			
Malignancy		21(14)	16(14)
Ischemic heart disease		30(20)	22(20)
Peripheral vascular disease		32(21)	23(21)
Left ventricular dysfunction		17(11)	11(10)
Diabetes mellitus		49(32)	30(27)
Systemic collagen vascular disease		16(11)	10(9)
Others		116(77)	83(74)

Note: Data presented as mean±SD or median (25th-75th percentile) or n(%)

BMI= body mass index; SGA= subjective global assessment; nPCR= normalized protein catabolic rate; mGFR= measured glomerular filtration rate; eGFR= estimated glomerular filtration rate; P= plasma; B= blood; PTH= parathyroid hormone; HDL-C= high-density lipoprotein cholesterol; LDL-C= low-density lipoprotein cholesterol; Ca×P= calcium phosphate product; CRP= C-reactive protein; RAAS= renin–angiotensin–aldosterone system; CKD= chronic kidney disease.

Physical performance

Some measures of physical performance of the 151 included patients at baseline and the 112 completers at baseline and after 12 months of exercise training are presented in Table 4. There were no significant differences between groups for changes in any measures of physical performance. All measures increased with the exception of handgrip strength, which was unchanged.

Table 4. Physical performance

Physical performance	Baseline (n=151)	Baseline (n=112)	12 month (n=112)	P	95%CI
Handgrip strength right (kg)	32±11	32.4±10.8	32.8±11.3	0.3	-1.1 to 0.3
Handgrip strength left (kg)	29±11	29.9±11.0	30.1±11.0	0.6	-1.0 to 0.6
IQS right (kg×cm)	1144±409	1160±390	1269±433	**<0.001**	-134 to -53
IQS left (kg×cm)	1132±421	1143±423	1246±467	**<0.001**	-135 to -49
Functional reach (cm)	33±9	33.2±8.1	35.8±7.5	**<0.001**	-3.1 to -1.1
Six-minute walking test (m)	402±136	417±125	460±130	**<0.001**	-40 to -21
Berg's balance test (score)	54 (50-56)	55 (51-56)	56 (53-56)	**0.04**	-2.0 to -0.00004

Note: Data presented as mean±SD or median (25th-75th percentile)

IQS= isometric quadriceps strength, 95%CI= 95% confidence interval.

Study 1 - Body composition and mGFR in CKD

Prevalence of sarcopenia

When we used low muscle mass only, 54 (36%) patients were defined as having sarcopenia, 43 (44%) men and 11 (22%) women. When using low handgrip strength only, 43 (29%) patients were defined as having sarcopenia, 25 (26%) men and 18 (36%) women. If both low muscle mass and low muscle strength were used, 20 (14%) patients were defined as having sarcopenia, 16 (16%) men and 4 (8%) women.

Relationship between body composition and mGFR

After adjusting for sex, age and comorbidity, lean mass (p=0.04), fat mass (p=0.04), appendicular skeletal muscle (ASM) (p<0.0001), ASMI (p=0.002) and body mass index (BMI) (p=0.04) all showed significant positive relationships with mGFR, respectively.

Relationship between physical performance and muscle mass

After adjusting for sex, age and comorbidity, functional reach showed a significant positive relationship with lean mass in the legs (p=0.01). The Berg balance test score showed a significant positive relationship with lean mass in the trunk (p=0.04). Handgrip strength in the right and left hand, respectively, was significantly and positively related to arm lean mass (p<0.001). Isometric quadriceps strength in the right and left leg, respectively, was significantly positively related to leg lean mass (p<0.001).

Study 2 - Muscle mass and myostatin after exercise in CKD

mGFR and prevalence of sarcopenia after 12 months

There was a modest yet significant decrease in mGFR in both groups (1.0 mL/min/1.73m^2) after 12 months of exercise training. The prevalence of sarcopenia was unchanged after 12 months in both groups. There were no significant differences between groups for change in mGFR or in sarcopenia.

Body composition

DEXA measures of body composition in both groups at baseline and after 12 months are presented in Figure 2. In the balance group whole-body fat mass decreased by 1.3 kg (p=0.04), leg lean mass and whole-body lean mass increased by 0.3 kg and 0.9 kg, respectively (p=0.02, p=0.006). In the strength group, whole-body fat mass decreased by 1 kg (p=0.03) after 12 months of exercise training. In the whole group, leg lean mass and whole-body lean mass increased by 0.2 kg (p=0.01), and 0.6 kg (p=0.02), respectively. Whole-body fat mass decreased by 1.2 kg (p=0.003). There were no significant differences between groups for changes in any measures of body composition.

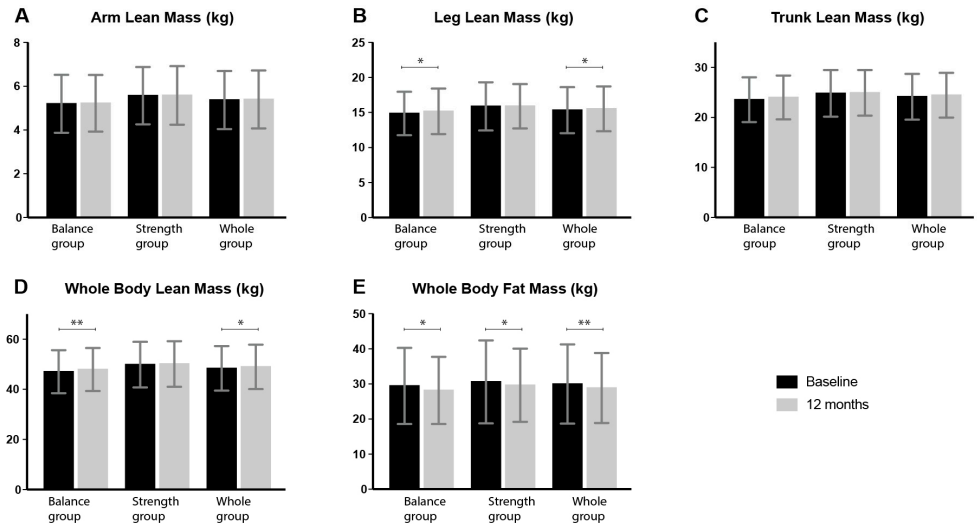

Figure 2. Body composition of strength group, balance group and whole group (112 patients) at baseline and after 12 months. * = p<0.05, ** = p<0.01.

Plasma myostatin

Plasma myostatin levels at baseline and after 12 months are presented in Figure 3. After 12 months of exercise training plasma myostatin increased by 1.42 ng/mL (p<0.0001) in the strength group, 0.81 ng/mL (p<0.0001) in the balance group and 1.1 ng/mL (p<0.0001) in the whole group. There was a significant between group difference in favour of the strength group (p<0.03).

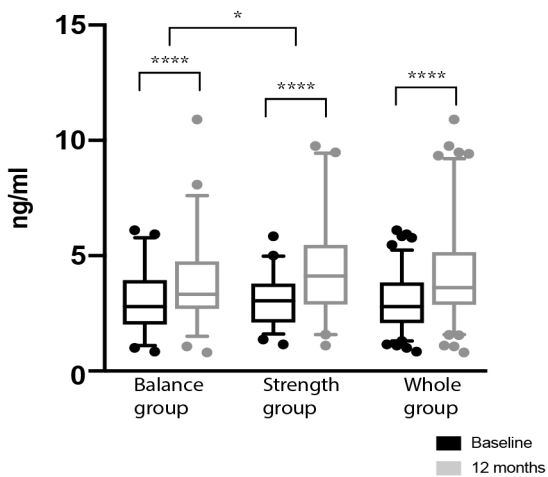

Figure 3. Plasma myostatin in the strength group, balance group and whole group (112 patients) at baseline and after 12 months. *=p<0.05, **** = p<0.0001.

Relationships between mGFR, physical performance, body composition and plasma myostatin

Plasma myostatin did not show any significant relationship with mGFR at baseline nor after 12 months. There were no significant relationships between delta myostatin and baseline mGFR.

Plasma myostatin showed a significant positive relationship with all measures of physical performance at baseline ($p<0.001$). After 12 months it only showed significant positive relationships with handgrip strength ($p=0.008$) and isometric quadriceps strength ($p<0.001$). There were no significant relationships between delta myostatin and any of the measures of physical performance at baseline.

At baseline, plasma myostatin showed a significant positive relationship with arm lean mass ($p<0.001$), leg lean mass ($p<0.001$) and trunk lean mass ($p=0.001$), respectively. After 12 months the only significant positive relationship was with arm lean mass ($p=0.01$). There were no significant relationships between delta myostatin and any of the measures of lean muscle mass at baseline.

Study 3 - Abdominal aortic calcification, GFR, and cardiovascular risk factors in CKD

AAC score, 24-hour blood pressure, pulse pressure and echocardiographic measures

Thirty-seven (27%) patients had an AAC score= 0 (normal), 36 (26%) patients had an AAC score 1-6 (moderate), and 66 (47%) patients had an AAC score ≥7 (severe). 69 (76%) men and 33 (69%) women had an AAC score >0.

Thirty (21%) patients had a LVEF <55%. Forty (28%) patients had a LAVI ≥34 mL/m^2. Of these patients 6 had an E/A ratio <0.8 (grade 1 diastolic dysfunction), 15 (14%) patients had an E/A ratio 0.8-1.5 (grade 2 diastolic dysfunction), and 5 patients had an E/A ratio ≥2 (grade 3 diastolic dysfunction) [171].

The results of AAC score, 24-hour blood pressure, pulse pressure and echocardiographic data are presented in Table 5.

Table 5. AAC score, 24-hour blood pressure, pulse pressure and echocardiographic measures

Measurements	Units	Completed number	Mean±SD or Median (25th-75th percentile) or n(%)	Reference range
AAC	score	139	6 (0~13)	0
Blood pressure	mmHg	133	130±15 / 75±10	≤130/80 [172]
Pulse pressure	mmHg	133	55±14	40
LVEF ≥55%	%	142	112(79%)	≥55% [173]
LAV	mL	92	Women: 53±21 Men: 73±32	Women: 22-52 Men: 18-58 [174]
LAVI	mL/m^2	70	35±15	16-28 [174]
E/A	ratio	110	Age 18-40: 1.70±0.54 Age 41-60: 0.99±0.26 Age >60: 0.97±0.50	Age 18-40: 1.53±0.40 Age 41-60: 1.28±0.25 Age >60: 0.96±0.18 [173]
LVM	g	107	Women: 158±55 Men: 213±58	Women: 67-162 Men: 88-224 [173]
LVMI	g/m^2	94	Women: 89±29 Men: 106±27	Women: 43-95 Men: 46-115 [173]

AAC= abdominal aortic calcification; LVEF= left ventricular ejection fraction; LAV= left atrial volume; LAVI= left atrial volume index; E/A: E/A ratio, it represents the ratio of peak velocity blood flow from gravity in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave); LVM= left ventricular mass; LVMI= left ventricular mass index. The reference ranges are given with appropriate references.

Relationship between AAC score and GFR

After adjustment for sex, age, CVC and hypertension, the AAC score showed a significant inverse relationship with both mGFR (p=0.03) and eGFR (p=0.006).

Relationship between AAC score and some calcific- and inflammatory markers and pulse pressure

After adjustment for sex, age, CVC and hypertension, the AAC score showed a significant inverse relationship with plasma albumin (p=0.006), a significant positive relationship with plasma phosphate (p=0.01) and a significant positive relationship with 24-hour pulse pressure (p=0.004).

Relationship between AAC score and some echocardiographic measures

After adjustment for sex, age and hypertension, the AAC score showed a significant positive relationship with LVM (p=0.02), LAV (p<0.001) and LAVI (p=0.001), respectively.

Study 4 - Abdominal aortic calcification, some markers of arteriosclerosis after exercise in CKD

AAC score, mGFR, and some markers of arteriosclerosis

AAC score, mGFR, and some markers of arteriosclerosis at baseline and after 12 months are presented in Table 6. After 12 months of exercise training, the AAC score increased by 2 points ($p<0.001$), mGFR decreased by 1.0 mL/min/1.73m^2 ($p<0.001$), plasma lipoprotein (a) decreased by 49 nmol/L ($p<0.001$), plasma PTH increased by 2 pmol/L ($p=0.01$), and plasma $1,25(OH)_2D_3$ increased by 6 nmol/L ($p=0.04$).

Table 6. AAC score and some markers of arteriosclerosis at baseline and after 12 months

Variables	Baseline	12 months	p	95%CI
AAC (score)	5 (0-12.5)	7 (2-14)	**<0.001**	-2 to -1
mGFR (mL/min/1.73m^2)	22.6±8.0	21.6±8.8	**<0.001**	1.0 to 3.0
Lipids and lipoproteins				
P-Total cholesterol (mmol/L)	4.8±1.2	4.7±1.2	0.5	-0.1 to 0.3
P-Triglyceride (mmol/L)	1.8±1.0	1.8±1.0	0.2	-0.05 to 0.2
P-HDL-C (mmol/L)	1.3±0.4	1.3±0.4	0.2	-0.01 to 0.06
P-LDL-C(mmol/L)	2.9±1.0	3.1±1.9	0.7	-0.1 to 0.2
P-Lipoprotein (a) (nmol/L)	96 (31-238)	47 (19-188)	**<0.001**	13.5 to 49.5
Pro-calcific markers				
P-FGF23 (ng/mL)	1.8 (0.5-9.2)	3.1 (0.7-14.1)	0.2	-1.5 to 0.3
P-Phosphate (mmol/L)	1.1±0.2	1.2±0.2	0.1	-0.01 to 0.0001
P-Calcium (mmol/L)	2.3±0.1	2.3±0.1	0.9	-0.03 to 0.02
P-PTH (pmol/L)	11 (8.1-17)	13 (7.9-20)	**0.01**	-2.8 to -0.4
Anti-calcific markers				
P-$1.25(OH)_2D_3$ (nmol/L)	64±27	70±29	**0.04**	-10.3 to -0.1
P-Fetuin-A (g/L)	1.0±0.3	1.0±0.2	0.1	-0.1 to 0.01
Inflammatory markers				
P-IL6 (pg/mL)	2.0 (1.2-3.3)	2.1 (1.3-3.3)	0.2	-0.6 to 0.1
P-CRP(mg/L)	3.1 (1.4-6.2)	3 (1.4-6.0)	0.8	-0.5 to 0.4
P-Albumin (g/L)	37.3±3.2	36.4±4.5	0.05	-0.01 to 1.7

Note: Data presented as mean±SD or median (25th-75th percentile).

AAC= abdominal aortic calcification; mGFR= measured glomerular filtration rate; P= plasma; HDL-C= high-density lipoprotein cholesterol; LDL-C= low-density lipoprotein cholesterol; FGF23= fibroblast growth factor 23; PTH= parathyroid hormone; IL6= interleukin 6; CRP= C-reactive protein; 95%CI= 95% confidence interval.

Relationships between delta AAC score and baseline triglycerides

Delta AAC score showed a positive significant relationship with baseline levels of plasma triglycerides ($p=0.01$).

Discussion

Muscle mass and myostatin in CKD

At baseline, the prevalence of sarcopenia was 14%. We showed that muscle mass was positively related to mGFR and physical function was positively related to muscle mass. Myostatin showed a strong positive relationship to both muscle mass and physical performance.

The relationship between body composition and kidney function has been studied before, but eGFR was used to represent kidney function in these studies [175-177]. Since the estimation of eGFR is dependent on muscle mass, as it is based on plasma creatinine, both changes in muscle mass and in kidney function could affect eGFR. Thus the use of eGFR could lead to an inaccurate relationship between body composition and kidney function. We are the first, to our knowledge, to show a significant relationship between muscle mass and kidney function using DEXA and mGFR.

The upregulation of muscle myostatin has been suggested to be a major reason for muscle wasting in patients with CKD and inflammation, and the upregulation has been reported to depend on a pathway from the activation of signal transducer and activator of transcription 3 (Stat3) to CCAAT/enhancer-binding protein δ [178]. Although myostatin is a myokine that negatively regulates muscle growth [50], we found that myostatin was positively related to muscle mass and physical performance in these non-dialysis patients with CKD. However, in this study both plasma myostatin and the inflammatory status of our patients were within the ranges found in healthy subjects [179]. In fact, myostatin has been described to be normally regulated in a non-inflamed environment [180]. This could explain why we did not find a negative relationship between myostatin and muscle mass. The relationships between myostatin, muscle mass and muscle strength have been studied previously. However, the results have varied depending on the subjects studied [53,181,182]. It is fair to state that the relationships hitherto described between plasma myostatin and muscle mass or physical performance are not robust or clear.

Effects of exercise training on muscle mass and plasma myostatin

Firstly, after 12 months of exercise training, we found that muscle mass increased in the balance group and was maintained in the strength group. Fat mass decreased in both groups and the prevalence of sarcopenia was maintained in both groups. These results indicated that exercise training could stop muscle wasting and could even promote anabolism in these patients. Simultaneously, exercise training also reduced fat mass. In a previous study, 4 months of aerobic exercise alone did not significantly decrease fat mass in patients with moderate to severe CKD, [149]. With a longer intervention period of 12 months, we showed that exercise alone could lead to a decrease in fat mass. This is interesting and of potential clinical importance as previous investigators reported an association between adiposity and pro-inflammatory cytokines [183]. Thus, exercise training might affect inflammation and oxidative stress, although this was not apparent in the present study in which CRP and IL6 were analyzed and were unchanged.

Secondly, we found that the levels of plasma myostatin increased significantly in both groups. Despite the significant increase after 12 months, the levels of plasma myostatin remained within the normal range [179], as did the inflammatory status as measured with CRP, IL6 and plasma albumin. Consequently, plasma myostatin can still be regarded to be normally regulated [61,178] with the increase in myostatin due to the increase in muscle mass, since myostatin is mainly expressed in skeletal muscle [50]. These results could also be explained by the "accelerator-brake" model, in which myostatin and insulin-like growth factor-I (IGF-1) act as counter-regulatory molecules for muscle hypertrophy [184]. Thus, exercise training leads to muscle growth partly due to increased levels of IGF-1, the accelerator, that in turn increase the "brake" function of myostatin. In strength training muscle is stretched more intensively than during balance training, which could have triggered a greater "brake" response, explaining the greater increase in the levels of plasma myostatin in the strength group.

AAC and GFR

At baseline, we showed that the AAC score was negatively related to GFR.

The relationship between kidney function and AAC score has been studied previously, but the results were not in agreement. These contradictory results mainly depended on different methods employed to assess the AAC score: computer tomography [185,186] and X-ray [92,187]. Additionally, all these studies used AAC score as a categorical variable. In our study, we treated AAC score as a continuous variable instead of dichotomizing it, and used both mGFR and eGFR. However, we did not find a significant relationship between delta AAC score and baseline GFR so we have no evidence of a causal effect of GFR on AAC.

AAC and cardiac structure

In study 3 we showed that the AAC score was positively related to LVM, which has also been reported previously in patients with CKD stage 3 [187]. Left ventricular hypertrophy has been shown to be a clinical manifestations of medial layer calcification in CKD [81]. We also found that the AAC score was positively related to LAV and LAV index, which are measures of left atrial dilation and also predictors of mortality in patients with CKD [188]. Thus, the relationships between AAC and left atrium size provides interesting information about the pathophysiology of arterial vessels' calcification in CKD.

AAC and markers of arteriosclerosis

At baseline, among the calcific- and inflammatory markers we have investigated, AAC score was only significantly related to phosphate. Higher serum phosphate, even within the normal range, has been suggested to be associated with more plaques and greater risk of all-cause mortality, both in patients with CKD [189] and subjects with normal kidney function [190]. However, we did not find a causal relationship between plasma phosphate and the AAC score.

After 12 months of exercise training, the increase in AAC score was positively related to baseline levels of triglycerides, indicating that hypertriglyceridemia might contribute to arteriosclerosis in these non-dialysis dependent patients with CKD.

The dysregulation of the FGF23 - klotho endocrine axis is suggested to be one of the underlying mechanisms leading to vascular calcification in CKD [84]. However, the relationship between FGF23 and vascular calcification remains controversial [191-193].

Effects of exercise training on AAC and markers of arteriosclerosis

The effects of 12 months of exercise training on arteriosclerosis and inflammation were not as beneficial as those on muscle mass. After 12 months, the levels of lipoprotein (a) decreased by 51%. However, the AAC score increased, and the calcific- (FGF23, phosphate, calcium, and fetuin-A) and inflammatory markers (IL6, CRP and albumin) were all unchanged.

The increase of AAC score indicated that 12 months of regular low to moderate intensity exercise training could not stop the progression of vascular calcification in these patients with non-dialysis dependent CKD stages 3 - 5. Of special note, however, is the finding that age had the strongest relationship to the progression of AAC, a circumstance which cannot be affected. An important weakness of this

study is the lack of a sedentary control group. Another problem is that there are no studies reporting on the natural course of AAC in this group of patients. Thus, we do not know whether the progression of AAC was attenuated by the exercise training compared with the natural course. Moreover, it is also possible that an attenuation of vascular calcification requires a longer period of exercise training or that low to moderate intensity exercise training is an insufficient stimulus.

Exercise training has previously been shown to be effective in reducing levels of plasma triglycerides, LDL-C, while simultaneously increasing levels of plasma HDL-C [194,195]. However, few studies have shown that exercise training could reduce lipoprotein (a) [196,197]. Our study showed a 51% decrease in lipoprotein (a), albeit within the normal range, after 12 months of exercise. This result could well convey a positive effect of exercise training on the intimal vascular layer [81].

We also investigated the effects of exercise training on some pro-calcific and anti-calcific markers. The levels of plasma FGF23 were elevated around 100-fold above normal at baseline [102] and remained stable after 12 months despite a decrease in GFR. Unfortunately, due to methodological difficulties, we were not able to assay plasma klotho so that we could not follow the changes in the whole FGF23 - Klotho axis after exercise training. Plasma phosphate and calcium were maintained within the normal range both at baseline and after 12 months. Fetuin-A, an inhibitor of vascular calcification, was also unchanged after exercise training. During the natural course of CKD, FGF23 and other pro-calcific markers increase [102,198] and anti-calcific markers, like fetuin-A, decrease as GFR declines [107,115]. All these changes are associated with higher risks of morbidity and mortality [23,105,107,115]. Therefore, although exercise training did not reduce FGF23 or increase fetuin-A, it might have been effective in maintaining levels of these markers, which might also have a beneficial effect for these patients.

Although exercise training has been shown to be effective in reducing CRP and IL6 in previous studies [147,149], these results were not corroborated in our study, which had a longer period of intervention and a larger sample size.

Conclusions

General conclusion

To summarize, 12 months of self-administrated exercise training, either strength or balance training both in combination with endurance training, could prevent sarcopenia, maintain and even increase muscle mass in patients with non-dialysis dependent CKD. Although exercise training did not stop the progression of arteriosclerosis, it greatly reduced the levels of lipoprotein (a) and might have contributed to maintain the levels of calcific- and inflammatory markers. Hypertriglyceridemia and aging emerged as longitudinal predictors of arteriosclerosis in these patients. Further studies on the role of myostatin in patients with CKD and the progression of AAC during the natural course of CKD are required.

Specific conclusions

Study 1

- The prevalence of sarcopenia was 14%. Lower muscle mass, especially appendicular skeletal muscle, was significantly related to lower GFR, and physical performance was positively related to muscle mass in non-dialysis dependent patients with CKD stages 3 to 5.

Study 2

- 12 months of either strength or balance training both in combination with endurance training exercise training seemed to be effective in preventing sarcopenia and maintaining muscle mass, there was also an increase in plasma myostatin in non-dialysis dependent patients with CKD stages 3 to 5;
- Higher levels of plasma myostatin, albeit still within the normal range, were related to more muscle mass and better physical performance, but these relationships were attenuated after 12 months of exercise training.

Study 3

- AAC score was negatively related to GFR in non-dialysis dependent patients with CKD stages 3 to 5;
- AAC score was positively related to pulse pressure, plasma phosphate, and LVM, LAV, and LAVI; and was negatively related to plasma albumin.

Study 4

- Exercise training did not prevent the progression of AAC, but reduced the levels of lipoprotein (a) and might have contributed to unchanged levels of calcific- and anti-inflammatory markers in patients with non-dialysis dependent CKD stages 3 to 5;
- Hypertriglyceridemia and aging emerged as longitudinal predictors of arteriosclerosis in these patients.

Future Perspectives

- To investigate the effects of exercise training on bone density and some markers of bone metabolism.

- To measure some myostatin signalling pathway related markers, like activin A, GDF 11 and follistatin, in the RENEXC patients to investigate the comprehensive response of the myostatin signalling pathway after exercise training.

- To perform some survival analyses in the RENEXC cohort to investigate:
    - the relationship between survival and baseline body composition (muscle mass, fat mass and sarcopenia);
    - the relationship between survival and the studied calcific- and inflammatory markers.

Popular summary

The ability of the kidneys to eliminate waste products decreases in chronic kidney disease (CKD). When kidney function deteriorates, waste products accumulate. Patients with CKD usually develop symptoms like high blood pressure, anaemia (low blood count), loss of appetite and skeletal muscle mass, muscle weakness, fatigue and reduced physical working capacity. Also, CKD increases the risk of heart and blood vessel disease. These problems may happen slowly over a long period of time. CKD can be caused by diabetes, high blood pressure, autoimmune disease, etc. Early detection and treatment can often keep CKD from getting worse. However, CKD is usually progressive and eventually leads to kidney failure, which requires dialysis or a kidney transplant to maintain life.

Heart and blood vessel disease in CKD

Heart and blood vessel disease is the leading cause of death in CKD. Malnutrition due to low intake of both calories and protein, described as protein energy malnutrition, and inflammation are two important factors causing heart and blood vessel disease in CKD. These two factors interact with each other and both are involved in the process of vascular disease. Therefore, the syndrome, Malnutrition - inflammation - atherosclerosis (MIA) has been used to describe this situation. MIA syndrome is highly prevalent in patients on dialysis. But few studies have been done in patients who are not dialysis dependent and have less than about 50% of their kidney function left.

Exercise training in CKD

Exercise training has cardiovascular benefits for patients with CKD, like improving overall endurance, muscular strength and health-related quality of life. It is generally recommended as a regular treatment in international nephrological guidelines, aiming for at least 30 minutes of exercise 5 times per week. However, most clinical trials with exercise training were performed in patients on dialysis and with relatively small numbers of patients and a short exercise period. There is a sparsely of data in non-dialysis dependent patients.

Muscle wasting in CKD (Study 1)

Protein energy malnutrition with muscle wasting is present in a large proportion of patients with CKD. We showed that a lower muscle mass was related to worse kidney function in patients with a kidney function lower than 50% of the expected norm. This suggested that muscle wasting started well before end-stage renal failure requiring dialysis. Additionally, we also showed that a greater muscle mass was related to a higher physical performance in these patients. This meant that loss of muscle also leads to lower physical function. Therefore, an intervention preventing muscle wasting should be initialized in the early stages of CKD.

The effects of exercise training on muscle mass in CKD (Study 2)

Twelve months of self-administered exercise training, either strength or balance training both in combination with endurance training, resulted in maintained muscle mass in the strength group and an increased muscle mass in the balance group. Simultaneously, the fat mass decreased in both groups. Plasma myostatin, a myokine which is a negative regulator of muscle growth, increased in both groups after exercise training, but remained within the normal range. This might be due to the fact that myostatin is normally regulated in the non-inflamed state, like in our well-treated patients. Therefore the observed increase in myostatin might be caused by the increase in muscle mass, which is where myostatin is synthesized. Moreover, higher levels of plasma myostatin were related to greater muscle mass and better physical performance, but these relationships were attenuated after exercise training. So the role of myostatin on muscle mass and physical performance in patients with CKD needs further study.

Vascular calcification and markers of arteriosclerosis in CKD (Studies 3 and 4)

Abdominal aortic calcification (AAC) score, is an easy way to measure vascular calcification using lateral lumbar X-ray. AAC is an important predictor of heart disease and a useful measure to detect arteriosclerosis.

In study 3, we found that a greater degree of calcification in the abdominal aorta was strongly related to worse kidney function and higher levels of plasma phosphate. Besides, the AAC score was also related to an enlargement of the left ventricle and the left atrium in the heart.

In study 4, we found that the increase of AAC score was positively related to baseline levels of plasma triglycerides. This suggested a causal relationship between AAC and triglycerides. However, the AAC score was not found to be related to any of the measured calcific- and inflammatory markers.

The effects of exercise training on arteriosclerosis in CKD (Study 4)

Although exercise training did not stop the progression of vascular calcification, it reduced the levels of lipoprotein (a), which is a lipoprotein contributing to atherosclerosis. Additionally, the levels of cholesterol and triglycerides were unchanged after exercise. Normally, during the natural course of CKD, "the bad cholesterol" LDL-C and triglycerides increase as kidney function declines, simultaneously "the good cholesterol" HDL-C decreases. Therefore, our results suggested that exercise could be beneficial in preventing atherosclerosis, which is one type of arteriosclerosis.

Some calcific- and inflammatory markers were also unchanged after exercise. "The bad markers" which promote inflammation and calcification, like interleukin 6, C-reactive protein (CRP) and fibroblast growth factor 23 (FGF23), would increase as kidney function declines. Contrarily, "the good markers" which inhibit inflammation and calcification, like albumin, fetuin-A, and vitamin D, would decrease as kidney function declines. However, the levels of both the "bad and the good ones" were maintained after exercise training, which could indicate that exercise might have long-term benefits on inflammation and vascular calcification in these patients.

Conclusion

In these four studies, we showed that muscle mass and vascular calcification were both strongly related to kidney function, even though we did not see causal relationships. Plasma triglycerides had a causal relationship with AAC. Twelve months of self-administered exercise training could maintain and even increase the muscle mass in these patients. Although exercise training was not able to attenuate vascular calcification, it might be effective in reducing the levels of lipoprotein (a) and maintaining the levels of some related calcific- and inflammatory markers. Future studies with a sedentary control group are needed to evaluate the exact effects of exercise training.

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