Predictors of Muscle Function in Hemodialysis Patients

By

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Abstract

Decreased skeletal muscle function (MF) is ubiquitous in hemodialysis (HD) patients and linked to functional decline. Serum vitamin D (25-OHD) and habitual physical activity (PA) are decreased in HD and linked to reduced MF in other populations. The associations between 25-OHD, PA, and MF were investigated in 81 stable HD patients. PA intensity was quantified using accelerometry, MF using handgrip strength dynamometry, 25-OHD via serum measures, and dietary and supplementation of vitamin D intake via three-day food records. MF correlated with PA (r =0.411, p = 0.003) when controlled for body mass (BM) and with 25-OHD (r =0.298, p = 0.023) when controlled for BM, age, and sex. Both MF (r=0.285, p=0.025) and 25-OHD (r=0.314, p=0.005) correlated with vitamin D supplementation. MF remained correlated with supplementation after controlling for 25-OHD (r=0.269, p=0.037). These findings should be further explored in interventional studies to assess how their manipulation influences MF in HD.
"Education is what remains after one has forgotten everything she learned in school."

*Albert Einstein*
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1,25-(OH)2D 1α-25-Dihydroxyvitamin D = Calcitriol = Active Vitamin D
24,25-(OH)2D 24,25-Dihydroxyvitamin D = 24-Hydroxycholecalciferol = Inert Vitamin D
25-OHD 25-Hydroxyvitamin D = 25-Hydroxycholecalciferol = Calcidiol = Intermediate Vitamin D

3DDMR Three Day Diet & Medication Record
ADL Activities of Daily Living
Alb Albumin
BM Body Mass
BMI Body Mass Index
Ca Calcium
CKD Chronic Kidney Disease 1
CKD-MBD Chronic Kidney Disease Mineral Bone Disorder 2
COPD Chronic Obstructive Pulmonary Disease
DM Diabetes Mellitus
DV Dialysis Vintage 3
DRI Dietary Recommended Intake
GFR Glomerular filtration rate
ESRD End Stage Renal Disease 4
eGFR Estimated Glomerular Filtration Rate
FGF-23 Fibroblast Growth Factor 23
HD Hemodialysis
Hgb Hemoglobin
HTN Hypertension
Kt/V K=dialyzer clearance, t= time, V=volume 5
KDOQ/GI Kidney Disease Outcomes Quality/Global Initiative
MET Metabolic Equivalent
MIS Malnutrition Inflammation Score 6
MHC Myosin heavy chain
nPCR Normalized Protein Catabolic Rate 7
O2 oxygen
PTH Parathyroid Hormone
PD Peritoneal Dialysis
PO4 Serum Phosphate
PA Habitual Physical Activity
RRT Renal Replacement Therapy
SGA Subjective Global Assessment 8
Tx Kidney Transplantation
Type I Type I Muscle Fibre (slow twitch)
Type II Type II Muscle Fibre (fast twitch)
UVB Ultraviolet B rays
VDR Vitamin D receptors
VITD-S Vitamin D Supplements (including cholecalciferol (D3) and ergocalciferol (D2))
VO2max maximal oxygen consumption

1 Estimated Glomerular Filtration Rate <60mL/min for greater than three months
2 Disarrangement of calcium, phosphorus, vitamin D, parathyroid hormone and FGF-23 that can lead to a cascade of poor health outcomes and tissue damage in particular skeletal, vascular and cardiopulmonary systems
3 Number of years a patient has been receiving dialysis treatment
4 eGFR <15mL/min or stage five chronic kidney disease where renal replacement therapy may need to be considered
5 A ratio of the pre- and post-dialysis serum urea concentrations that is used to measure dialysis adequacy
6 A modified Subjective Global Assessment that uses objective measures as well as clinician’s subjective assessment of a patient to estimate level of malnutrition and inflammation in hemodialysis patients
7 Reflects the daily protein intake in maintenance hemodialysis and is calculated using following formula nPCR (g/kg/24hr) = 0.22 + (.036 x intradialytic rise in blood urea nitrogen (g) x 24)/(intradialytic interval time (hr))
8 A validated bedside tool used by clinicians to assess signs and symptoms of malnutrition by using short interview muscle tissue deposits questions followed by a physical assessment of adipose and skeletal muscle tissue deposits
Chapter 1

Introduction

1 Brief Introduction to Chronic Kidney Disease: Select Manifestations

I am interested in what determines muscle function (MF) in those affected by chronic kidney disease (CKD). I will provide a brief overview of CKD, with emphasis on patients receiving hemodialysis treatment (HD). Furthermore, I will describe how MF may change with CKD, and potential predictors of muscle dysfunction in CKD patients will be discussed. I will be focusing on some specific features of CKD that might have possible associations with muscle dysfunction in HD, including vitamin D and habitual physical activity (PA) levels.

1.1 Chronic Kidney Disease

Kidneys play important homeostatic roles including metabolic waste removal, blood pressure control, hormone production, and the regulation of acid-base balance, electrolytes and osmolarity [1]. Each kidney houses approximately one million functional units known as nephrons. Nephrons filter incoming blood via a cluster of specialized capillaries known as the glomerulus (plural, glomeruli). In general, renal function is considered normal when > 60 mL of blood per minute is filtered through the glomeruli \{Glomerular Filtration Rate (GFR)\} in absence of systemic disease or other evidence of kidney disease [2]. GFR is usually estimated (eGFR) and is based largely on the serum concentration of creatinine and is compared to
population means matched for age and sex [1]. CKD has five stages and is generally considered moderate when eGFR is <60 mL.min\(^{-1}\) for greater than three months, however specific stage classifications are defined using National Kidney Foundation Guidelines [2]. At eGFR of 15 mL.min\(^{-1}\) or less, kidney disease is referred to as End Stage Renal Disease (ESRD) [2]. ESRD may be the point at which kidney function no longer meets the metabolic demands of the body and renal replacement therapy (RRT) may be required [1]. RRT can be one of two forms; a) a human kidney transplantation, or b) the use of artificial kidneys in the form of either peritoneal dialysis (PD) or hemodialysis (HD) [1]. HD is the most common treatment amongst these options in the developed world [3] and was the treatment the subjects of this study received during the data collection for this thesis. HD treatment is often quite intense, consisting of an average of 12 hours of in-centre treatment each week divided in three, four-hour sessions (every other day) [1]. In addition to HD, these patients typically need to be on many medications and to follow a very restrictive dietary regimen [4].

1.2 Select Manifestations of Chronic Kidney Disease

CKD patients suffer from a number of metabolic and endocrine abnormalities including; uremia, anemia, wasting, CKD-Mineral Bone Disorder (CKD-MBD), uremic myopathy, and muscle atrophy [3]. Patients with ESRD, particularly receiving HD, display greater manifestations of these comorbidities [3]. In the case of uremia, metabolic (uremic) toxins build up resulting in a state of infirmity and is characterized by lethargy, poor appetite, nausea, and weight loss [1]. Anemia is caused by reduced erythropoietin production by the kidney, and poor iron status (often due to poor dietary intake and appetite) [5]. Anemia causes fatigue, poor concentration, reduced activity-tolerance, and inflammation in HD patients [5]. Anorexia or wasting occurs in response to decreased energy intake due to reduced appetite, salivary changes,
polypharmacy (define this), dietary restrictions, under- or over-dialysis clearance (define this) and increased catabolic state (especially during dialysis treatment) [4, 6]. Furthermore, absorption, circulation, metabolism and storage of many micronutrients are altered either directly because of the decrease in kidney involvement (lack of vitamin D activation by the kidneys) [7], metabolic toxin overload (serum retinol accumulation) [8], or side effects of medical treatments (such as removal of ascorbic acid and folate during blood filtration by dialysis) [9]. Another disease/condition involving bone-mineral metabolism, CKD-MBD, is characterized by reduced excretion of phosphate, increased parathyroid activity and hyperplasia (of what?), increased circulating Fibroblast Growth Factor 23 (FGF-23), decreased serum calcium (Ca) and decreased activation of vitamin D by the kidneys, leading to serum and tissue mineral derangements, soft tissue calcification and bone frailty [10] (Figure 2.2). Skeletal muscle dysfunction often referred to uremic myopathy in CKD, is defined as a reduction in skeletal muscle mass, muscle quality, and muscle performance [11-12]. Along with lower PA levels, skeletal muscle dysfunction often contributes to lower exercise tolerance [13], reduced physical function [14] and possibly premature disability [15] and mortality [16].

1.2.1 Skeletal Muscle: Dysfunction and Decline in Physical Activity

Uremic myopathy and muscle atrophy are manifested as muscular weakness [17]. Muscle wasting, limited endurance capacity, poor exercise tolerance and accelerated fatigability are common symptoms among HD patients likely because of uremic myopathy however in most cases these symptoms have poorly understood aetiologies [18] (Figure 1.1). Interestingly, patients who eventually receive a kidney transplant and no longer require HD seem to fully recover from the symptoms of myopathy and return to their usual level of PA [18]. Muscle biopsies of patients with uremic myopathy show structural deterioration such as fibre atrophy (Type II b, fast twitch), fibre splitting, internalized nuclei, fibre degradation, and altered
distribution of fibre types [19-21]. Poor muscle capillary oxygen (O$_2$) supply, reduced capillary to fibre ratio, mitochondrial metabolism alteration, mitochondrial damage, accelerated decreased intracellular pH with exercise, increased intramuscular lipid droplets, loss of Z-band distribution and loss of myofilaments have also been reported in this population [12, 17-18, 22-25]. These structural and metabolic abnormalities of skeletal muscle in uremic myopathy have been speculated to contribute to physical function abnormalities in dialysis patients [26]. However the distinct tapering of PA levels [27-28] are not fully explain by the observations mentioned above [29].

Evidence of deterioration in functional capacity with progression of CKD includes decreased ability to independently perform activities of daily living (ADLs) [29], poor exercise tolerance [30], lower habitual PA [31], and suboptimal skeletal MF [32]. The decline in physical function of CKD patients is greatest just prior to the start of RRT and within the first three months of dialysis treatment [3]. Most HD patients stabilize on dialysis after three months of treatment [33]. However, with passing time on dialysis, known as dialysis vintage (DV), and particularly beyond the first five years, overall health deteriorates at an accelerated rate when compared to age-matched sedentary counterparts [33]. One study estimates a three percent decrease in PA with every month spent on HD [15]. Poor vitamin D status is prevalent amongst CKD and particularly dialysis patients [34-35] and has been linked to MF and overall physical function in the elderly population [36-39] however, very limited research exists in this area.

1.2.2 Vitamin D: Prevalence of Nutritional Deficiency

Under normal metabolic conditions in the human body, Vitamin D undergoes several enzymatic and non-enzymatic transformations [40]. The most common circulating form of this nutrient, calcidiol or (25-OHD) can be analyzed in a serum sample using an
electrochemiluminescence immunoassay [40]. Vitamin D status is classified based on serum 25-OHD levels since this is the most reliable index of vitamin D storage in muscle, adipose and other tissues [40]. Health Canada defines serum 25-OHD levels below 27.5 nmol.L\(^{-1}\) as vitamin D deficiency and less than 37.5 nmol.L\(^{-1}\) as “inadequate” for bone health [41]. There is much debate in the literature on establishing deficiency and insufficiency levels [42]. Although the serum level thresholds for deficiency versus insufficiency may differ between laboratories and guidelines, it is generally accepted that “sufficient” serum levels are those greater than 75 nmol.L\(^{-1}\) [42]. Vitamin D sufficiency allows for its optimum function, in both endocrine bone-blood-mineral metabolism (classic functions) as well as autocrine and paracrine functions of several organ system tissues and cells (non-classic functions) [42].

CKD patients are known to have inadequate serum 25-OHD levels in all stages of CKD, in both pre-dialysis [35] and dialysis [34]. Dialysis patients have the highest prevalence of inadequate serum 25-OHD levels in the CKD population [34]. Given that kidneys are the major organ responsible for activation of 25-OHD to calcitriol or 1-\(\alpha\)-25 Dihydroxyvitamin D (1,25-(OH)\(_2\) D) for endocrine use [43], one would naturally expect a linear relationship between decreased renal function (renal mass, including proximal tubules) and serum 1,25-(OH)\(_2\)D. Theoretically, an elevation in 25-OHD may also be expected since this compound is not being utilized as a substrate for enzymatic activation in the kidneys. However low circulating levels of 25-OHD are prevalent, in CKD, and particularly in HD patients for reasons not clearly understood [34]. Lower vitamin D-rich food intake (such as fish and milk), less outdoor activity (hence lower sun exposure), uremic state (effecting the skin’s natural ability to utilize ultra violet B (UVB) rays to activate intrinsic pre-vitamin D) and protein losses (perhaps including protein-bound vitamin D) have all been speculated as underlying contributors to lower levels of 25-OHD [7, 34, 43-46], however these have not been demonstrated in clinical or physiological studies.
Although inadequate 25-OHD levels are endemic [42], CKD patients around the world have even lower levels than their respective country’s population’s pervasiveness of this biomarker [7]. The HD patients’ subgroups have the lowest serum levels of 25-OHD amongst the CKD population around the world (70-100% are insufficient or deficient) [7].

In addition to 25-OHD as a substrate for endocrine 1,25-(OH)₂ D production, there has been evidence that suggests its involvement in autocrine and paracrine functions of other organ systems outside of mineral metabolism [40, 42-43, 47-48]. Furthermore, in addition to 1,25-(OH)₂ D approximately 37 other metabolites of vitamin D have been discovered in the recent years and their roles have not been fully identified yet [49] and may further emphasize the importance of local cellular control in metabolizing vitamin D. Although many of the functions of these newer metabolites remain to be revealed, some have been speculated to have biological activity [50], despite being considered "inert" in past [50-53]. One such metabolite formerly thought as an inactive “catabolite” of 1,25-(OH)₂D, is 24,25-dihydroxyvitamin D (24,25(OH)₂D) [42]. This metabolite, has been shown to influence bone healing [50] as well as act as an effective agent to treat hypercalcemia in HD patients [54]. Further, there is evidence which suggests that 24,25(OH)₂D acts on specialized bone and intestinal cell receptors for regulation of calcium [50, 55-57].

The discovery of Vitamin D receptors (VDR) in more than 60 cell types in the body over the past three decades has also shed light on other possible functions of 25-OHD, outside of its classic functions [42-43]. VDRs present on cell and nucleic membranes, with affinity for both 25-OHD and 1,25-(OH)₂D indicate intracellular and genomic functional capacity of vitamin D in many cell types [42]. Since 25-OHD is the most common circulating form of vitamin D metabolites [40, 58], it is speculated that maintaining optimal serum levels of this metabolite
ensures adequacy at the cellular level of both 25-OHD and 1,25-(OH)₂D, as many cells have been found to possess 1,25-hydroxylase for extra-renal and intracellular activation of 25-OHD to 1,25-(OH)₂D [40, 58-60]. Reinhold Vieth, a leading researcher of Vitamin D, describes how inadequate levels of circulating 25-OHD limit non-classic functions, particularly crucial paracrine and autocrine functions of many tissue cells, such as cell type differentiation [61], including differentiation into muscle fibres [62]. The conversion of 25-OHD by the kidneys for endocrine use tends to take precedence over 25-OHD non-classic functions at lower (insufficient) circulating levels of this vitamin [45]. In the CKD population, this further creates a paradox between the physiological mechanisms of the disease and prevalence of 25-OHD insufficiency. Serum 25-OHD levels are lower in CKD, as opposed to being higher, when compared to those individuals without kidney disease, despite the lower rate of production of 1,25-(OH)₂D by the renal proximal tubules of those with CKD [40]. Vieth [61] further argues the importance of differentiating nutritional vitamin D, as indicated by 25-OHD levels in the serum and its diverse functions that at times may be entirely different from its metabolic derivative 1,25-(OH)₂D as 25-OHD represents a nutrient, where by its deficiency in the diet will cause a deficiency disease [61]. This is an important distinction to declare in this thesis, because in the nephrology community, calcidiol and calcitriol may be collectively referred to as “vitamin D”. Since in the classic metabolic pathway of vitamin D, this nutrient (either from ingestion or intrinsic activation by UV rays) is the parent metabolite leading to the final product as 1,25-(OH)₂D, in clinical practice and sometimes in scientific literature, it is referred to as “vitamin D” in all stages/forms along the metabolic pathway [47]. Historically, calcitriol or 1,25-(OH)₂D” was used as a medicine to treat secondary hyperparathyroidism [49, 63] and to this day continues to be the most common reason why most nephrologists prescribe it for CKD patients [64-65], while vitamin D deficiency levels are for the most part not deemed a major concern [7]. One of
the aims of this thesis will be to seek relationships between vitamin D and MF irrespective of the endocrine end-product 1,25-(OH)₂D. Therefore serum 25-OHD will be used as a surrogate of nutritional vitamin D status rather than its endocrine derivative 1,25-(OH)₂D that has been the focus in CKD.

1.3 Role of Vitamin D in Muscle Function and Structure

Sufficient vitamin D level is important to optimal MF [62]. This vitamin has been shown to assist in the proliferation and differentiation of fast twitch skeletal muscle cells (type II fibers) [62], increase muscle leucine, inorganic phosphate, and ATP content, while reducing serum phosphate [66], and enhance calcium regulation [67]. Higher vitamin D levels have also been associated with enhanced muscle fibre contractility [68], lower fat infiltration in muscle [69] and reduced myopathy [70]. These functions have been hypothesised to be linked to vitamin D receptor-mediated activities and non-receptor-mediated activities [7, 71]. Skeletal muscle cells in particular have two types of VDRs; membrane receptors and nuclear receptors [62]. At the cellular level, 25-OHD is converted to intracellular 1,25(OH)₂D in the cytoplasm of cells producing 1-alpha-hydroxylase. This type of 1,25(OH)₂D that serves autocrine functions, such as those mentioned above, involving cell division and differentiation [62]. Nuclear VDRs are 1,25-(OH)₂D-specific and bind to nuclear receptors that result in changes in gene transcription [72]. This change in gene transcription of mRNA subsequently results in de novo protein synthesis which may be observed in the morphology of type II fibres in variation of myosin heavy chain (MHC) isoform [62]. Intra and inter-cellular skeletal muscle fibre regulation of its calcium and phosphorus content however may or may not be mediated by VDR’s and therefore as some literature suggests 25-OHD affects muscle cells via VDR pathways as well as non-VDR pathways [7]. Use of 1,25-(OH)₂D and its analogs, have been a focus in the clinical care of
dialysis patients [64]. Since renal activation of 25-OHD to 1,25-(OH)\(_2\)D is markedly reduced and 1,25-(OH)\(_2\)D has a traditional role on parathyroid gland suppression over 25-OHD [64], the importance of adequate 25-OHD levels have been largely neglected [7]. Therefore treatment of nutritional vitamin D inadequacy, detected by levels of 25-OHD, has not been routine practice in most HD units and remains a concern given this vitamin’s multifaceted functions [7].

The HD population presents an important cohort for studying vitamin D and its relationship to muscle dysfunction because of two major points as listed below. Firstly, the HD patients have very limited to no ability to activate vitamin D to 1,25-(OH)\(_2\)D intrinsically in the kidneys. Instead they are often supplemented with this hormone or its analogs but not necessarily given nutritional vitamin D supplementation [7]. HD patients are known to be vastly insufficient in vitamin D and its etiology is not fully explained by the diseased state [73]. Since circulating levels of 25-OHD are low and often untreated, diminished non-classic functions of 25-OHD may become more apparent in this group, such as those related to MF and structure. Secondly, HD patients are known to have lower functional ability, muscle strength, and habitual PA when compared to other age-matched sedentary controls [11, 28]. Muscle health is an important component of overall physical health and physical ability to complete daily living tasks [74]. In the disablement model, pathology, impairments, functional limitations, and disability are clearly linked and can be applied to skeletal muscle organ systems [74-75]. HD patients have been reported to score very low on the Physical Function Scale Scale (~50%), even when compared to other chronically ill populations and have similar functional capacity to those with Chronic Obstructive Pulmonary Disease (COPD) [76]. The relationships between MF and vitamin D as well as PA levels have been illustrated in non-HD populations although these findings have not always been consistent [48].
In a recent review of literature, Girgis and colleagues reported several studies that link serum 25-OHD with tests of MF including; handgrip strength, Time Up and Go (TUG), knee extension, 6-minute walk test, gait speed and walking sway [48]. The reported findings have been mixed, where some studies found a positive relationship with serum 25-OHD levels and MF tests while other studies found no relationship [48]. Studies reporting that no relationship between the 25-OHD, and various MF tests where often conducted in younger and healthier groups, particularly those with higher 25-OHD levels [48]. Meanwhile an inverse relationship was reported in the review by Girgis et al. and other studies between serum 25-OHD levels and rate of muscle dysfunction (including fall risk) in the frail elderly groups [36-37, 48]. Vitamin D supplementation has improved MF in some groups [77] however not always [78]. Interventional studies using resistance training in combination with vitamin D therapy have also produced inconsistent results [38, 79]. Some studies have reported improvement in the vitamin D group, and others reported improvement in the exercise group and vitamin D group or no significant differences among supplemented and non-supplemented groups [39, 78-79]. One contributing reason for the mixed results may have been methodological variation. For example one study used vitamin D and calcium-fortified foods (possibility of nutrient-nutrient interactions influencing bioavailability and low dose of vitamin D (400 iu per day)) while a second study used calcium and vitamin D only (800 iu per day) and yet a third study used calcium and a high dose monthly vitamin D (90 000-150 000 iu per month) of vitamin D supplementation [39, 78-79]. There is evidence that a vitamin D supplementation-intervention may improved muscle strength [80]. A systematic review of vitamin D supplementation and muscle strength, showed no effect of vitamin D supplementation in vitamin D replete adults (considered to have serum 25-OHD levels >25nmol.L⁻¹) [70]. However, the authors indicated a limited number of studies demonstrate an increase in proximal muscle groups’ strength in adults with vitamin D deficiency.
(defined by this article as serum 25-OHD levels <25nmol.L⁻¹) [70]. Furthermore, there is less than a handful of studies that have used vitamin D as a therapeutic nutritional intervention in CKD, and particularly in the HD population [64-65, 80]. One study found a positive association between serum 25-OHD and MF tests in peritoneal dialysis patients (PD) only after correction of deficiency [80].

1.4 Summary

Poor skeletal MF and low PA levels are common in HD patients. Vitamin D status has been linked to both of these features in human and animal models. Few studies have investigated the relationship among habitual PA, MF, and vitamin D status in dialysis patients, and the findings have been inconsistent. Methodological variations as well as uncontrolled confounders present challenges in comparing results between previous studies. Therefore, the objective of this thesis will be to investigate possible links between low vitamin D levels (including dietary intake, nutritional supplementation and serum levels of vitamin D) and skeletal muscle dysfunction and reduced PA in HD patients while accounting for possible confounders, such as dietary intake, malnutrition, vitamin D-related serum markers, age, sex, presence of diabetes, and vintage. Some of the factors investigated in this work are novel or poorly understood, such as the role of vitamin D in HD patients’ MF as well as the corresponding PA levels. If MF is found to be associated with vitamin D levels and or PA, then these modifiable factors should be further investigated in interventional studies aimed at treating muscle dysfunction in dialysis patients.
Chapter 2
Review of Literature

2 Chronic Kidney Disease Prevalence, Complications, and Potential Modifiable Contributors

2.1 Prevalence of Chronic Kidney Disease

CKD is associated with significant morbidity and mortality, including high rates of cardiovascular disease and deaths, as well as high rates of dialysis-dependent ESRD [81]. CKD is widely prevalent, and its rates are growing worldwide. In Canada alone, the population of patients with CKD has tripled over the last two decades to an estimated two million people [82]. Approximately 40,000 of these individuals have ESRD, and these figures are projected to double by 2018 [83].

CKD can occur as a result of many inherited or acquired disorders. The leading causes are diabetes, renal vascular diseases (including high blood pressure) and glomerulonephritides (inflammatory diseases of the glomeruli) [82]. In Canada these chronic illnesses collectively account for approximately two-thirds of newly diagnosed CKD [81, 83]. Diabetes is the largest and fastest growing cause of CKD since approximately 30-50 percent of diabetics develop CKD over a lifetime and 20-40% of all dialysis patients are diabetic [84]. Worldwide diabetes prevalence of 240 million is expected to swell to 380 million people by 2025 [82] and this subsequently increases projected CKD prevalence worldwide. The causes of the diabetes epidemic include increase of the aging population, unhealthy eating habits, increase in adiposity
and sedentary lifestyles [85]. As the frequency of CKD continues to increase in Canada and worldwide, so will the prevalence of ESRD and the attendant need of RRT is inevitable. Current RRT strategies are limited to kidney transplantation and dialysis with hemodialysis being the most commonly used form of RRT [86]. Canada and US are amongst the largest providers of HD in the world, where approximately 80% of ESRD patients are treated with HD [82].

2.2 Complications of Chronic Kidney Disease

In addition to certain death from prolonged and untreated ESRD, patients with CKD suffer from numerous other complications. These include a markedly reduced quality of life owing to a multitude of factors. Many CKD patients suffer from fatigue [18], poor sleep patterns [87], depression [88] and poor nutritional status [33, 89-90]. These conditions contribute to poor physical function, decreased treatment compliance, and lower quality of life. Patients with poor physical function have lower PA levels than their non-CKD sedentary counterparts [29] and this contributes to lower bone mineral density and muscle power [22]. The reasons for this lack of activity have been attributed to several factors including anemia [29], skeletal muscle atrophy [91], cardiovascular deconditioning [91], persistent uremia [29], increased fatigue during exercise [18] and shifts in fluid balance with dialysis treatment [29].

2.3 Physical Activity and Chronic Kidney Disease

According to World Health Organization estimates, 6% of all deaths globally can be attributed to physical inactivity [82]. This makes it the fourth largest modifiable risk factor for mortality worldwide [82]. The American College of Sports Medicine, American Heart
Association, Canadian Society of Exercise Physiology, and the World Health Organization recommend a minimum of 150 minutes of moderate-vigorous intensity of PA per week to prevent heart disease and early all-cause mortality [92-93]. Further suggestions include participation in 30 minutes per day of PA on most days of the week, and in bouts of 10 minutes or longer that can include formal exercise or activities of daily living (ADL) [92]. Poor chronic disease outcomes have been demonstrated to inversely relate to regular PA in prospective observational studies [31, 92, 94-99]. There is substantial evidence that certain chronic illnesses including cardiovascular disease, thromboembolic stroke, hypertension, type 2 diabetes mellitus, osteoporosis, obesity, colon cancer, breast cancer, anxiety, and depression demonstrated benefit from regular PA [100]. Although life-long higher levels of PA correlate with better health and prevention of many chronic illnesses, we understand relatively little about this relationship and its understanding mechanisms in the CKD population as compared to other chronic diseases such as cardiovascular disease [29]. HD patients are generally amongst the least fit of CKD patients and are 15-57% less active than age-matched sedentary adults [28]. Observational studies have reported significant associations between physical function, aerobic capacity limitations, and increased mortality and morbidity rates in patients with CKD [15, 29, 31, 96]. PA has been shown to be extremely low in some dialysis patients as indicated by low scores (below the fifth percentile compared to healthy sedentary norms) on self-reported PA questionnaires [30]. Dialysis patients also have reduced skeletal muscle oxidative potential (46% less than age-matched sedentary cohort) [18], higher levels of muscle atrophy (18% less contractile tissue in cross-section of dorsiflexor muscle of HD patient compared to age and sex-matched sedentary control) [11, 80, 101-102] and reduced cardiopulmonary fitness (reduced predicted exercise capacity by 20-50% compared to sedentary-aged matched norms that improved to only 10% reduction in transplant patients in one study) [29]. Low PA levels in HD patients have been
associated with reduced physical function [29]. For example, Johansen and colleagues have reported that HD patients require greater than 50% of maximal oxygen consumption (VO$_{2\text{max}}$) to perform simple ADL such as light house cleaning or cooking [29] and have been shown to have low Short Physical Performance Battery scores (5-6 out of 12) [13]. Poor physical function often represents poor quality of life. Painter et al. demonstrated that HD patients scored lower on the Medical Outcomes Study Short Form [103]. Lower PA has also been linked to lower physical fitness with a resulting VO$_{2\text{max}}$ of 40-50% of age-matched non-dialysis counterparts) [104].

Reduced PA in HD patients has also been associated with nutritional status, where a linear relationship between spontaneous PA (measured by number of steps taken in a day) has been linked with a measure of lean tissue (bioelectrical impedance phase angle; p-value=0.002), and serum Alb (p-value=0.01). PA levels have also been inversely related with level of inflammation, age, dehydration and anemia [18, 22, 26, 29, 31, 80, 87, 104-107]. Dialysis vintage has also been shown to influence PA levels, where on Maximum Activity Score of the Human Activity Profile has been demonstrated to be decreased at a rate of approximately 6 points per each year of receiving HD (p-value=0.025) [104]. Although several pathological mechanisms for reduced PA in HD patients have been speculated, attempts to improve PA and exercise tolerance after correction of metabolic abnormalities via medical treatment of CKD have generally been unsuccessful. For example, despite the correction of anemia (with the use of recombinant erythropoietin therapy), HD patients continue to have low exercise tolerance levels [104], which likely also contribute to their lower overall habitual PA. Similarly, the theory that retention of uremic toxins cause reduced activity was questioned after a direct correlation between PA and increased dialysis (to clear uremic toxins) could not be demonstrated [104].

Habitual daily PA and exercise training differ from each other. PA is the overall bodily movement that is produced by the contraction of skeletal muscle throughout the day and that has
the potential to substantially increases energy expenditure [108]. Exercise, is a subset of PA, and
is described as planned, structured, and repetitive bodily movement done to improve or maintain
one or more components of physical fitness [108]. Only a few studies have examined correlates
of habitual PA levels (rather than exercise training) in HD patients [28, 30-31], and these studies
have several methodological differences in PA measurements (use of accelerometers,
pedometers or physical activity questionnaires), making it challenging to compare their results.
One cross-sectional study measured PA using pedometers in 60 HD patients, and found a
correlation between number of steps and degree of anemia and malnutrition, but not adequacy of
dialysis or inflammation [31]. In several studies, Johansen and colleagues have reported low PA
levels in HD patients compared to healthy sedentary age-matched controls and the general
population. In these studies PA was associated with age and serum Alb levels, but not sex, BMI
or lean body mass [11, 26, 28-30]. Another study found a positive correlation between serum 25-
OHD and self-reported PA [109]. Several studies have demonstrated exercise interventions to be
beneficial in physical function, improving quality of life, and dialysis efficacy in hemodialysis
patients [14, 91, 103-104, 110-115], although these improvements did not increase exercise
capacity to that observed in non-dialysis dependant CKD and age-matched sedentary populations
without kidney disease [13-14, 27, 97, 110-114]. A recent meta-analysis of 36 studies of exercise
training intervention for adults with CKD by Heiwe and Jacobson revealed that regular
exercising significantly improved health outcomes in CKD (dialysis and non-dialysis dependant)
as well as increased habitual PA in this population [116]. The types of exercise training used in
these studies differed in intensity (high or low), duration (20-110 minutes per sessions),
frequency, intervention duration (2-18 months) and, exercise mode (ranging from cardiovascular
training, mixed cardiovascular, resistance training, resistance-only training, and yoga) [116].
Heiwe and Jacobson concluded the results of these publications showed that regular exercise
significantly improved physical fitness and walking capacity, cardiovascular dimensions, some nutritional parameters and health-related quality of life in the CKD population [116]. Therefore although overall PA has been shown to increase as a result of exercise interventions, habitual PA has not been well studied with objective and reliable measures in CKD [29].

2.3.1 Accelerometers

Recently, accelerometers have provided researchers and clinicians the opportunity to objectively quantify PA, particularly in terms of intensity [117]. Accelerometers have proven to be more accurate in estimating movement intensity as compared to questionnaires [118-124] and have been validated in hemodialysis patients [16] and older adults [117, 122, 125-126]. Accelerometers are small, portable devices that measure the accelerations of movement. They are worn on the body (usually at the hip) in order to measure the rate and magnitude of movement in up to three planes (vertical, mediolateral and anteroposterior). When the sensor within the device is exposed to acceleration, the device responds by storing a movement count in a specified time interval (i.e., one second, ten seconds, or one minute) called an epoch. The data collected are therefore a series of counts representing the intensity of each specific time interval [64]. When the device is static (i.e., there is no detected movement at the point at which the device is attached on the body), the device simply stores a “zero” count. Counts are linearly related to movement intensity; higher count values are therefore associated with more intense movement for a given time interval. Consequently, accelerometer counts can be used to describe the frequency, intensity, and duration of PA as well as time spent in sedentary status.

When assessing habitual PA behavior in adults, accelerometers are typically set at a sampling interval of 60 seconds, which summarizes all registered movement during this time period, producing one “count” value every 60 seconds. Recent technological advances in the
current leading accelerometry-based PA measurement devices (i.e., ActiGraph; www.theactigraph.com) have lead to an increase in memory capacity, making it possible for devices to be worn continuously for seven per weeks at a time [127]. Capturing the intensity of PA is important to determine if a study group or subject is meeting their recommended PA needs, as outlined at the beginning of section 2.3. Higher intensity activities, particularly those in the moderate-vigorous range, have been linked to chronic disease prevention and reduced all-cause mortality [92]. Intensity of PA is usually represented by the number of Metabolic Equivalents (METs) one uses to perform a certain physical task. For example, 1 MET would be equivalent to the amount of energy expended while sitting quietly, and is generally translated to 1 kilocalorie of energy expenditure per kilogram of body weight per hour [92]. Through calibration research (i.e., having individuals wear accelerometers while engaging in activities of varying intensity and measuring O₂ consumption), accelerometer counts can be equated to METs. For example, Freedson and colleagues have developed a formula to calibrate METs with PA expenditure using O₂ consumption during treadmill exercises at multiple intensities. The ActiGraph accelerometer analysis software (ActiLife; www.theactigraph.com) then uses this formula to estimate PA intensity from count values [128]. For example: in adults, 1 to 2 METs are indicative of sedentary behaviour and are associated with an ActiGraph GT1M accelerometer intensity threshold of less than 100 counts per minute. Setting this threshold therefore permits the calculation of time spent sedentary (i.e., occupational sitting, driving a car or watching TV) [93, 128]. Light intensity PA (i.e. 2-3 METs) is associated with an ActiGraph GT1M accelerometer intensity threshold of 100-1951 counts per minute, and would be equivalent to doing light household cleaning and cooking [93]. The most recent ActiGraph accelerometer software (Version 6.0) however categorizes this group into subcategories, equating light intensity activity with an intensity threshold of 100-759 counts per minute and “lifestyle” activity with an intensity
threshold of 760-1951 counts per minute, the rationale being that the original category of “light’ may be too broad. Moderate intensity PA (i.e., 3 to 6 METs) is associated with an ActiGraph GT1M intensity threshold of 1951-5724 counts per minute; this would include activities such as vacuuming, car washing, brisk walking, or bicycling for pleasure. “Vigorous” PA (i.e., 6-9 METs) is associated with an ActiGraph GT1M intensity threshold of 5725-9498 counts per minute; this would include activities such as light jogging, or hard gardening. Lastly, ActiGraph GT1M accelerometer counts that are >9499 are indicative of very vigorous activity (i.e., fast running or playing competitive sports).

PA has been measured by several studies using accelerometers [15, 28, 129-130]. Johansen and colleagues [28] found habitual PA, measured by accelerometers, in the hemodialysis population to be below age-matched controls, and this gap widened with increases in age [28]. Matsuzawa et al [129] found that higher levels of accelerometer-measured PA were inversely associated with all-cause mortality over a seven year period on HD [129]. They stated that the hazard ratio for all-cause mortality per 10 min per day increase in PA to be 0.78 (95% confidence interval, 0.66-0.92; P=0.002) [129]. In the non-dialysis CKD population there has been a log correlation between accelerometer-measured PA and kidney function, where total and light physical activities were found to be positively associated with better kidney function and slower progression of CKD [96]. It is clear that PA is of great importance in CKD. Consequently, there is great promise in using a reliable and objective measure like accelerometry to describe the PA behavior of HD patients. Obtaining information on the overall daily intensity of PA, and the amount of time patients spend being sedentary and in light, lifestyle, and moderate-vigorous PA, would allow comparative measures to the general population, and help determine if these patients are meeting their recommended daily PA needs.
2.4 Muscle Function and Chronic Kidney Disease

The major function of human skeletal muscle is to produce force, which is the capacity to develop tension against external stimuli in order to allow voluntary movement [131]. Muscle strength, specifically isometric strength, is an expression of muscular force, or the individual's capacity to develop tension against an external resistance without any change in the muscle length [131]. Therefore a measure of muscle strength or force can be used as a proximal determinant of MF [131], however others have defined MF as muscle power (force produced for a given amount of time) [74] or muscle endurance (sustained repeated force per amount of given time) [132] or as performance tasks (the ability to complete functional tasks in a given time, commonly used in elderly populations) [133-134]. MF has also been described in terms of its influence on systemic metabolic activity, such as increasing insulin sensitivity and normalizing serum lipids [135]. In this thesis however, MF will be referred to in terms of isometric muscle strength, specifically using upper body strength as measured by hydraulic handgrip dynamometry (Figure 2.1).

Muscle strength is usually measured in specific muscle groups [131] and can be representative of overall strength for most populations [136]. Optimal muscle strength is multifactorial, and dependent on qualitative and quantitative measures of skeletal muscle in an individual [136-139]. Aging for an example, is a universal determinant of muscle strength with qualitative and quantitative measures of skeletal muscle in both sexes that differ from a young population [131]. In most healthy individuals muscle strength peaks in the third decade of life and although it slowly declines after ages 30-35, its effects on activities of daily living remain
relatively unchanged into the fifth decade of life [131]. From the fifth decade onward, muscle strength as well as muscle mass loss is accelerated [133-134] but the decline in muscle strength is not fully explained by the reduction of muscle mass [139]. Age-related decline of muscle strength does not affect all muscle groups equally [139-140]. Lower extremity muscles are affected more by the aging-related decline of muscle power and strength [74] and are dependent on muscle mass, muscle quality, and muscle phenotype changes [137]. Furthermore metabolic properties, neuromuscular control, and capillary-fibre ratio also affect muscle strength [138, 141]. Nutrition and PA also play an essential role in MF, strength, quality and mass, and are especially important as they are easily modifiable [23, 136, 142]. Good muscle health is critical to functional capacity particularly in elderly [143], chronically ill populations [144], and especially in groups with low functional capacity such as HD patients [21].

Verbrugge and Jette [75] link pathology, impairments, functional limitations, and disability in their Disablement Model of describing how chronic illness can lead to disability and mortality in the susceptible population [75]. More recently, Reid and Fielding have applied this model to skeletal muscle power in the elderly population [74]. They describe how muscle pathology (change in fibre type, fibre atrophy, reduced rate of muscle activation, etc.) can lead to overall impairment (reduced velocity of movement, force, and overall power output) that manifests in functional limitation (such as prolonged time of stair-climb, or chair-rise) which then leads to disability, ultimately changing the social and environmental role of the individual in the society [74]. Muscle dysfunction is a common feature of CKD, and generally attributed to muscle atrophy (decreased muscle mass), myopathy (neuromuscular disorders in which the primary symptom is muscle weakness due to dysfunction of muscle fiber) or a combination of both [11]. Abnormalities in muscle mass, MF, muscle morphology and response to exercise intervention have been observed in ESRD patients, in particular those receiving HD treatment.
This muscle dysfunction is often found in CKD patients with eGFR<25 L.min⁻¹ and dialysis patients, and is described as uremic myopathy [148].

### 2.4.1 Uremic Myopathy

Uremic myopathy is often reflected in reduced muscle strength, generalized fatigue and decreased ability to perform ADL [11]. Campistol reported prevalence of uremic myopathy to be in 50% of dialysis patients [148]. It was stated that uremic myopathy’s functional and structural pathogenesis might attributed to three groups of factors: 1) Organic or structural abnormalities related to uremic state, 2) Muscle-mitochondria alterations, and 3) Skeletal muscle functional factors [148]. Johansen et al. found several metabolic parameters linked to skeletal muscle dysfunction in HD patients that are categorized below under the categories described by Campistol [18].
Skeletal Muscle Strength

Muscle Function

Muscle Quality

Muscle Quantity

Muscle Force:
Capacity to develop tension against external stimuli

Muscular Endurance:
Capacity to repeat or maintain voluntary muscular contractions over time

Muscle Power:
Capacity to produce a given amount of force in the shortest period of time

Muscle Strength (isometric):
Maximal voluntary force produced against an external resistance without change in muscle length

Figure 2.1 Schematic Overview of Muscle Function:
Skeletal muscle organ system is highly plastic and its function is influenced by many factors [141]. Muscle function in this thesis is defined by force, measured through isometric strength; however others have defined MF by its power or endurance [74, 131].
2.4.1.1 Uremic State

Uremic state abnormalities are generally linked to presence of uremic toxins, or middle molecules, that are not effectively removed by dialysis and can be toxic to skeletal muscle tissue [148]. For example, high levels of ouabain present in ~50% of HD patients can alter enzymatic action of Na-K-ATPase, and can alter intracellular calcium and therefore impair muscle contractility [148]. Other factors contributing to uremic myopathy are insulin resistance, general acidosis, and decreased aerobic glycolysis as well as protein wasting (increased protein degradation and impaired protein synthesis) and malnutrition (particularly dietary protein deficiency) [148-158].

2.4.1.2 Muscle-Mitochondria Alterations

Mitochondrial abnormalities have been credited to oxidation enzyme alterations, excess lactic acid production (50% higher in HD patients than controls), and carnitine deficiency (a protein involved in fatty acid oxidation by the mitochondria) [148]. Johansen and colleagues noted more metabolic disturbances in the HD group at the end of exercise testing compared to controls (lower phosphocreatine and pH, and higher inorganic phosphate, and phosphate to creatinine ratio) [18]. They also found that oxidative potential was markedly lower in HD patients, indicating that they had an impaired ability to produce energy aerobically as exercise proceeded [18]. Given the importance of O₂ availability and carbon dioxide removal, anemia and low blood flow to skeletal muscle during exercise have also been associated with uremic myopathy [18]. However, even after erythropoietin therapy a disproportionate response between serum hemoglobin (Hgb) concentration (60% improvement) and exercise performance (only 30% improvement) in HD patients have been reported [26, 148].
2.4.1.3 Skeletal Muscle Function and Structure

Skeletal muscle architectural abnormalities have been described as reduced capillary to fibre ratio, fibre atrophy and distribution (affecting type II and IIb which are fast twitch with higher anaerobic metabolism than type I fibres), fibre splitting and necrosis, myofilament abnormalities, and increased intramuscular fat droplets [17, 19-20, 145-146, 148]. Some of these abnormalities have been shown to be reversible with exercise intervention in HD patients [159]. Resistance training in advanced CKD for example, has been shown to promote skeletal muscle hypertrophy, increasing size and number of type I, IIb and IIx muscle fibres [159]. Interestingly, aerobic training has resulted in improved muscle strength and power as well as decreasing fatigability in this group, similar to strength exercises [159]. These muscular adaptations generally are not seen with aerobic exercise in a healthy population. Muscle fatigue was also negatively correlated with oxidative potential among dialysis subjects but not controls [18]. Changes in central activation ratio were also correlated with muscle fatigue in the dialysis subjects but not in controls [18].

2.4.2 Response to Exercise Training

Kosmadakis and colleagues speculated that since HD patients’ skeletal muscles are highly deconditioned, they are more responsive to any exercise stimuli and can benefit from improving muscular function even from low intensities of stimuli provided through aerobic training [159]. In general, results from strength training interventional studies in CKD and HD have been limited and mixed [12, 26, 29, 101]. Chan et al. found that participants in a resistance training program (in comparison to a control group) exhibited significant improvements in knee extensor strength as well as self-reported leisure-time PA, physical function and ADL, after low intensity, progressive resistance training twice a week for 24 weeks [101]. Meanwhile, Johansen et al. found increased strength in knee extension with anabolic steroid treatment but not strength
training alone [107]. A review of literature by Kouidi et al revealed all forms of exercise training were effective in rehabilitating physical fitness of HD-dependant ESRD patients to a certain degree, even if they did not fully recover their muscle strength to normal levels [91]. Performing these exercises (a combination of resistance and cardiopulmonary training) in supervised outpatient programs, home exercise rehabilitation programs, and exercise rehabilitation programs during the first hours of the HD treatment in the renal units enhanced these patients’ physical fitness through both central and peripheral adaptations [91]. Exercise training in HD patients has been shown to increase aerobic capacity, cause favourable left ventricular functional adaptations, reduce blood pressure in patients with hypertension, increase cardiac vagal activity, suppress incidences of cardiac arrhythmias and modify other coronary risk factors [91]. Kouidi et al concluded exercise training had beneficial effects in some CKD-induced muscle structural and functional abnormalities [91], however physiological mechanisms of these effects have still not clearly been identified [70, 91, 115, 160].

2.4.3 Handgrip Strength and Dynamometer

In order to assess muscular health, muscular function or dysfunction might be measured. Three commonly used measures of muscle strength in humans are handgrip strength, knee flexion/extension, and peak expiratory flow for respiratory muscles [161]. Handgrip strength measured by dynamometer is the most widely used method in clinical and research settings [161]. Reduced muscle strength has been shown to be objectively measured using dynamometers and had been validated in the HD population [137]. Handgrip dynamometers are commonly used to assess muscle strength, particularly in clinical settings because they are non-invasive, compact, easy to use and have been validated in many patient populations [12, 32, 136, 140, 162]. Hydraulic dynamometers have been recommended for use in the elderly population and the chronically ill since they can be used while the patient is seated [140]. Handgrip dynamometers
assess perceived maximal isometric force of approximately 36 upper body muscles that are used for gripping [140]. This information can then be compared to expected values and standards established by epidemiological studies such as the National Health and Nutrition Examination Survey (NHANES) and Canada Health Measure Survey (CHMS) [140, 142, 163] to assess muscle strength in relation to population norms. The major limitations of this tool are its reliance on only a select group of upper body muscles as a measure of strength, variation in protocol for assessment (where to grip, angle of limbs and remaining motionlessness for the duration of the test), alignment, as well as dependence on subject’s perception of “maximal” force produced. Therefore, to use this method as a surrogate of overall muscle strength, the known confounders must be controlled. Leal and colleagues have suggested that handgrip dynamometers are a reliable tool for estimating MF in the CKD population [32]. They concluded that there were significant correlation between MF estimated by dynamometers and variables used in the assessment of muscle mass, nutritional status, and prediction of clinical complications [32].

2.5 Vitamin D in Chronic Kidney Disease

Dietary and ultraviolet light-derived vitamin D represent the two natural sources of vitamin D [47]. Dietary sources are further subcategorized to D2 and D3 in some publications, indicating its plant or animal origin, prior to its metabolism by the liver [40]. However, all of these natural sources will be collectively referred to as vitamin D in this thesis. The liver converts all sources of vitamin D into 25-Hydroxy-Cholecalciferol vitamin D or calcidiol (25-OHD) [47], which is the most prevalent metabolite of vitamin D in the circulation [40]. Typically, the last step in the metabolism of vitamin D is by renal 1,25-hydroxylase in the proximal tubules of kidneys which converts 25-OHD to calcitriol (1,25-(OH)2D). Calcitriol is often referred to as “activated vitamin D” with several endocrine functions (Figure 2.3 a). The
main endocrine function of 1,25-(OH)₂D is to regulate serum and bone calcium dynamics. Hence 1,25-(OH)₂D is often referred to as being a “calcemic” hormone. Since renal 1,25-hydroxylase activity is the primary enzyme for conversion of 25-OHD to endocrine 1,25-(OH)₂D, low serum 1,25-(OH)₂D levels are commonly seen in CKD as renal 1,25-hydroxylase activity is reduced [46]. Other tissues (such as smooth muscle, cardiac muscle, and skeletal muscle) also possess 1,25 hydroxylase, known as extra-renal-1,25-hydroxylase [42]. This may be of particular importance to HD patients since these extra-renal sources of 1,25-hydroxylase might contribute to some circulating 1,25-(OH)₂D, when its precursor 25-OHD would be available at sufficient levels [42, 164].

Calcemic actions of vitamin D products including 25-OHD and 1,25-(OH)₂D, have been known as the “classic functions of vitamin D” [42]. Non-calcemic effects have been the subject of much medical research in the recent years, and vitamin D has been named a pleiotropic hormone that orchestrates an expanding number of important cellular functions via VDRs [42]. VDRs are found on virtually every cell throughout the body [62], an indication for vitamin D’s potential multifaceted effects. Normal 25-OHD levels are needed in order to allow for local production and distribution of 1,25-(OH)₂D in paracrine and autocrine activity (Figure 2.3 b). Theoretically, from a physiological perspective, 25-OHD levels in CKD should be similar to that of the general population or even elevated, since its main use by the renal 1,25-hydroxylase is diminished. However, for reasons that remain obscure, 25-OHD levels are commonly lower than the general population with advancing CKD [7, 71]. It also appears that paracrine and autocrine 1,25-(OH)₂D functions are reduced in CKD [43, 71]. Recent finding of 25-OHD levels’ correlation with overall mortality and survival in dialysis patients suggest important ramifications of reduced 25-OHD levels [165].
2.5.1 Vitamin D in Chronic Kidney Disease Mineral Bone Disorder

CKD-associated Mineral-Bone Disorder (CKD-MBD) represents another major manifestation of CKD. CKD-MBD is characterized by low serum calcium, high serum phosphate, low active vitamin D, and often high Fibroblast Growth Factor 23 (FGF-23) levels, and sometimes evident in bone demineralization and soft tissue calcification. The primary cause of CKD-MBD appears to be reduced GFR leading to impaired renal phosphate excretion [166-168]. This elevation in serum phosphate leads to a cascade of endocrine events, including increased parathyroid hormone (PTH) expression, decreased renal 1,25-hydroxylase expression, and decreased serum calcium [166-168] that may directly or indirectly influence MF (see below, section 2.6) [39, 54, 63-64, 169-170]. Evidence for CKD-MBD is apparent at early stages of CKD. When compensatory regulatory mechanisms can still maintain serum phosphate levels to within the normal range, the FGF-23 (which is a more sensitive marker of the bone-mineral disarrangement) is usually elevated if phosphate intake is increased even at early stages of CKD [171] (Figure 2.2). Phosphate binders, vitamin D receptor sensitizers and calcitriol analogs are often used to normalize some of these derangements, but the role of serum 25-OHD in CKD-MBD has not been fully explored [64-65].

Calcitriol analogs are often used to suppress elevated PTH levels in CKD patients with secondary hyperparathyroidism [64-65]. National and international clinical guidelines, including Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Outcomes Global Initiative (KDOGI) do not, however, specify supplementation with nutritional Vitamin D. Furthermore Vitamin D status is not routinely measured in dialysis patients and nutritional or pharmaceutical assessment of these patients do not routinely address intake from either dietary or supplemental sources of this nutrient, despite its well documented deficiency.
Several perturbations seen in CKD-MBD, including elevated serum phosphate levels, PTH and FGF-23 as well as a decrease in 25-OHD and 1,25-(OH)₂D, have each been independently associated with increased mortality rates [172-173]. Prolonged CKD-MBD is also associated with significant morbidity, including, increased cardiovascular complications (such as vascular calcification, peripheral vascular disease, and congestive heart failure), accelerated bone and muscle atrophy, and accelerated progression rates to ESRD [167-168, 174]. FGF-23 has been shown to be a major determinant of CKD-MBD as well as affecting vitamin D metabolism.

FGF-23 is a phosphatonin (phosphate-regulating protein) that appears to play a critical role in the development of CKD-MBD [175]. Produced by osteocytes and to a lesser degree osteoblasts in bone, FGF-23 belongs to the family of fibroblast growth factors [166]. FGF-23 levels rise in parallel with declining renal function long before a significant increase in serum phosphate concentration can be detected [168] (Table 2.1). FGF-23 acts on its receptor complex, Klotho-FGFR1c (fibroblast growth factor receptor 1 c-splicing form), in the distal convoluted tubule to repress renal phosphorus resorption in the proximal tubule and suppress renal synthesis of 1,25-(OH)₂D in the mitochondria [176]. Klotho-FGFR1c is also expressed in the parathyroid glands [177]. FGF-23 acts on the receptor complex in the parathyroid glands to decrease PTH gene expression and secretion. In CKD, both FGF-23 and PTH are increased, implying resistance of the parathyroid glands to FGF-23 (Table 2.1). A decrease in the number of parathyroid Klotho-FGFR1c complexes in both experimental CKD models and in ESRD patients has also been demonstrated [166, 178-179]. Klotho protein and gene are putative aging suppressors [180-181]. Membrane-bound Klotho also acts as co-receptor for FGF-23, while soluble Klotho functions as an endocrine substance [182]. Pathology in Klotho-knockout mice include osteopenia and soft tissue calcification that resemble that seen in CKD-MBD, as well as shortened lifespan, and senescent changes in the heart, lungs, thymus, gonads, skin, muscles,
hearing, and motor neurons [181]. Even though both FGF-23 and PTH affect vitamin D, calcium and phosphate changes in serum and tissue in CKD, their influences differ in activation/deactivation of metabolic pathways (Table 2.1). PTH increases activation of 25-OHD into 1,25-(OH)₂D whereas FGF-23 decreases it. Therefore FGF-23, Klotho and PTH are important cofactors in determining vitamin D’s role in CKD-MBD.

Treatment strategies of CKD-MBD have generally not been very successful [171, 183]. They currently consist of a combination of dietary phosphate restriction and the use of phosphate binder medications to target specific serum phosphate levels, vitamin D analogs to treat secondary hyperparathyroidism and parathyroidectomy to manage non-suppressible (tertiary) hyperparathyroidism [184]. More investigations into the role of 25-OHD and its effects on CKD-MBD may lead to interventional studies in the treatment of CKD.

Table 2.1: Fibroblast Growth Factor-23 and Parathyroid Hormone: Secretion and Action Sites

<table>
<thead>
<tr>
<th></th>
<th>FGF-23</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of production:</td>
<td>Bone Cells</td>
<td>Parathyroid Cells</td>
</tr>
<tr>
<td>Principle stimulus:</td>
<td>Hyperphosphatemia</td>
<td>Hypocalcemia</td>
</tr>
<tr>
<td>Principle Regulator of:</td>
<td>PO₄</td>
<td>Ca or PO₄</td>
</tr>
<tr>
<td>☻ Effect on urinary Phosphorus:</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>☻ ◼ Effect on 1,25(OH)₂D:</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

Note: In ESRD, urine production diminishes as patients stabilize on HD often resulting in elevated PTH and FGF-23 due to unresponsiveness of nephrons. ◼ Elevated PTH and FGF-23 have an additive effect on declining renal tubular active vitamin D production. Figure adapted from Reference [185] FGF23: Fibroblast Growth Factor-23, PTH: Parathyroid Hormone, Ca: Serum Calcium, PO₄: Serum Phosphorus, 1,25(OH)₂D: activated vitamin D
2.6 Possible Links between Vitamin D, Muscle Dysfunction and Physical Inactivity in CKD

PA and MF are significantly altered in the CKD population. Vitamin D has been shown to play an important role in muscle hypertrophy, type II (fast twitch) muscle fibre growth, and function [17-18, 186]. Although vitamin D deficiency has been linked to muscle dysfunction in the elderly population, its role has not been well described in uremic muscle atrophy. Muscle atrophy patterns in both ESRD and elderly patients are similar, however, and consist mainly of type II muscle fibre atrophy and motor-neuron degeneration [17-18, 186]. In a cross-sectional study of 25 pre-dialysis ESRD patients, Boudville and colleagues found suboptimal levels of 25OHD were associated with reduced quadriceps muscle strength and increased falls-risk [73]. They suggested that 25-OHD may be more important than the active renal metabolite 1,25(OH)₂D for muscle strength and therefore might be the primary choice of supplementation in ESRD [73].

Although relationships between serum 25-OHD and muscle quality, muscle power and muscle morphology have been studied in the elderly [36], investigators have only recently examined these relationships in ESRD patients [30, 187]. An interventional clinical trial by Taskapan and colleagues showed significant improvement in muscle strength and size when PD and CKD patients who were deficient or insufficient in serum 25-OHD were treated with nutritional vitamin D [80]. This study was notable for revealing that 25-OHD supplementation alone (not in combination with exercise nor use of 1,25(OH)₂D) was sufficient to result in muscle size and function improvement in CKD patients where as others in the past had only shown larger muscle size to be correlated with 1,25(OH)₂D prescription [80, 188]. Earlier work by Jean et al [164] also demonstrated improvement in serum 1,25-(OH)₂D levels in HD patients.
who had received 6 months of 25-OHD supplementation [164]. This indicated that once adequate stores of 25-OHD were reinstated, extra-renal 1,25-hydroxylase may actually contribute to endocrine production of 1,25-(OH)_{2}D in the absence of sufficient 1,25-hydroxylase activity, compensating for the diminished serum 1,25-(OH)_{2}D levels that would result from reduction in renal mass of CKD patients [164].

In 2009, Ravani et al [165] reported a strong correlation between serum 25-OHD (but not 1, 25-(OH)_{2}D) and five-year mortality rates in 168 CKD patients (stages 2-5) [165]. They also found baseline 25-OHD levels directly and significantly correlated with GFR [165]. In 2010, Anand and colleagues assessed 196 patients who were new to dialysis [109], and found 98% of these patients had deficient or insufficient levels of serum vitamin D [109]. There was a significant correlation between the PA questionnaire scores and serum vitamin D [109]. These results were similar to those found in an aging population [36], suggesting that the relationship between PA and serum vitamin D levels may be related to nutritional status and skeletal muscle atrophy [109]. However, Petchey et al found vitamin D was independently associated with aerobic capacity in CKD patients, but not with muscular strength or physical function [189]. Therefore further research is needed to identify possible role of vitamin D in MF, and PA level in the HD population.
In progressing chronic kidney disease, renal phosphate excretion is impaired, resulting in increased serum phosphate levels and subsequent elevation of fibroblast growth factor-23 (FGF-23) secretion from osteoblasts. High FGF-23 levels augment phosphate excretion and decrease circulating calcitriol (1,25-(OH)₂D) levels, leading to increased parathyroid hormone levels resulting in secondary hyperparathyroidism. Figure adapted from reference [168].
Figure 2.3a: Vitamin D Metabolism: Classic Functions

Normally, vitamin D from diet or and its endogenous formation by exposure of pre-vitamin 7-dehydrocholesterol in skin to ultraviolet B rays is transported to the liver and converted to 25-OHD and then further metabolized in the proximal tubules cells of the kidney to 1,25-(OH)2D.

In CKD, 1,25-hydroxylase expression is reduced due to decreased renal mass. However since several other cells (including skeletal muscle cells) express 1,25-hydroxylase intercellularly, 25-OHD is activated for paracrine and autocrine uses of the tissue locally.
Figure 2.3b: Vitamin D Metabolism: Non-Classic Functions (specific to muscle cells)

In CKD, renal 1-25-hydroxylase expression is reduced due to decreased renal mass. However since several other cells (including skeletal muscle cells) express 1-25-hydroxylase intercellularly, 25-OHD is activated for paracrine and autocrine uses of the tissue locally. Both 1-25-(OH)₂D and 25-OHD are involved on VDR and non-VDR-mediated skeletal cellular function. At 25-OHD <75 nmol.L⁻¹ non-classic functions are compromised. VDR; Vitamin D Receptor
2.7 Possible Links between Vitamin D Cofactors and Muscle Dysfunction

Markedly elevated FGF-23 levels are seen in CKD in comparison to other groups. Since FGF-23 inhibits renal 1,25-hydroxylase activity [185], it may also prove to be a relevant marker in muscle atrophy that is seen in CKD. A recent study of osteomalacia (adult form of rickets or soft bones, often developed because of a lack of vitamin D that causes severe bone pain and muscle weakness) and FGF-23 found muscle strength and spontaneous activity in hypophosphatemic (Hyp) mice were enhanced after injection with FGF-23 antibodies [190]. In osteomalacia and rickets, unlike CKD and HD, patients are often hypophosphatemic, and it is difficult to generalize the benefits of this research to myopathy in CKD patients [191]. However, there are indications that FGF-23 may affect MF directly via 1,25-hydroxylase suppression and indirectly by influencing phosphate regulation of muscle tissue [175, 190]. Furthermore, aging phenotypes are present in experimental models of elevated FGF-23 levels and low klotho expression, including similar skeletal muscle atrophy similar to that observed in CKD [176, 192-193]. These physiological factors involving klotho and FGF-23 may also be relevant in reduced habitual PA level of CKD, as seen in the aging population. Reduced klotho expression is associated with both poor renal function and aging morphology in animal models [194]. However, evidence of these relations are scarce in humans [194]. Therefore FGF-23 will be controlled for in this study.

Elevated PTH has been shown to have catabolic effects on skeletal muscle [195], an effect that would be further amplified in therapy-resistant (tertiary) hyperparathyroidism where PTH levels are extremely elevated for long periods of time [11]. In addition to its direct effect on
skeletal muscle [195], PTH produces an increase in intracellular calcium and has been shown to cause osmotic fragility of erythrocytes, myocardial dysfunction, cardiac hypertrophy, peripheral neuropathy, glucose intolerance, increased aldosterone secretion, decreased serum testosterone levels, increased circulating levels of prolactin, abnormalities of the immune system, and disturbances of lipid metabolism [196]. Many of these hormonal and metabolic disturbances augmented by hyperparathyroidism may also contribute to further muscle catabolism in CKD patients.

Lastly, the combined effects of elevated FGF-23, elevated PTH, decreased 25-OHD and low 1-25(OH)₂D levels in ESRD could be synergistically affecting skeletal MF and structure. Currently, serum FGF-23 and 25-OHD are not routinely measured in clinical practice for HD patients. Typically, serum PTH levels are used as surrogate of intrinsic 1-25(OH)₂D activity in regulation of the parathyroid glands. Prescription of calcitriol and its analogs are used to treat hyperparathyroidism rather than any other vitamin D related dysfunction. Phosphate and calcium levels, are part of routine blood work. These markers along with others are often measured monthly in HD units and are used as markers of CKD-MBD, to aid in medication dose alteration, medical dietary interventions and dialysate solute concentration adjustment. Since FGF-23 has been shown to be a better indicator of CKD-MBD as well as a marker of morbidity and mortality in most CKD patients (often better than phosphate or phosphate-calcium product), it might prove to be pertinent in the HD population in determining muscle dysfunction as demonstrated in experimental uremic animal studies mentioned above. Since FGF-23 levels in HD patients are not well studied this thesis will help to characterize FGF-23 levels in a stable HD population. Further investigation of these markers, in combination with vitamin D and PTH, may shed light on muscle atrophy and physical inactivity seen in the CKD population, a possibility that warrants further investigation.
2.8 Other Possible Contributing Factors to Muscle Dysfunction: 

Age, Vintage and Nutritional Status

The average age at which adult patients started HD in 1990 was 55 years. This rose to 65 years by 2009 [83]. Given the previously described link between advanced age and muscle atrophy, it has been suggested that this is an important factor contributing to the decline in MF and PA levels observed in the CKD population [197]. Therefore, aging itself might be a contributing factor to muscle dysfunction, physical dysfunction, reduced PA, and therefore should be considered in this cohort.

Dialysis vintage (DV) might be another factor influencing clinical outcomes such as functional capacity and nutritional status, and should be considered when examining data from these patients [33]. Chertow and colleagues [33] found that DV was related to nutritional status in HD patients, with DV of more than 5 years associated with a significant decline in all measured nutritional parameters [33]. Therefore, since DV may be associated with one or more variables of interest in this study, it will be considered as an independent variable.

Nutritional status of dialysis patients is also an important contributor to physical function. Currently, most clinicians use Alb alone or in conjunction with normalized protein catabolic rate (nPCR) and BM to assess nutritional status of dialysis patients. Dwyer and colleagues [89] surveyed 1,387 HD patients enrolled at baseline in the Hemodialysis Study (HEMO study) in 15 sites across the US, and found under-nutrition to be prevalent in this population [89]. They found that appetite, age and years on dialysis were associated with the physical functioning component of the survey used [89]. After controlling for other demographics and comorbidities, they found score of seven points or more (out of 100 scores for all components) on the physical functioning
component that related to nutritional parameters, while dialysis dose did not significantly alter the score [89]. Furthermore patients with CKD (particularly those on HD) are required to eat restricted diets, are frequently anorexic owing to a variety of factors including constipation, nausea, hypotension and medication side effects, and have high levels of systemic inflammation [198]. These factors augment rates of malnutrition and therefore reduced MF and PA levels seen in CKD patients [198]. Therefore, given the importance of nutritional markers, several serum nutritional markers such as Alb, hemoglobin (Hgb), and normalized protein catabolic rate (nPCR), in addition to a more comprehensive bedside assessment (Malnutrition Inflammation Score) as well as a nutritional intake analysis will be used to characterize the cohort in this study.

2.9 Gaps in Research and Clinical Practice, and Study Rationale

MF measured via handgrip strength, habitual PA levels and vitamin D levels are significantly reduced in the CKD population and independently related to mortality [165, 199-200]. Pathophysiological mechanisms underlying these features are poorly understood and there have been links made between these factors in the literature in other populations [36-37, 39]. HD patients are often among the most physically deconditioned in the CKD spectrum [3]. Given importance of MF in this population and potential common correlates of decreased PA and poor vitamin D status of CKD, and specifically HD patients, a cross-sectional study to investigate relationships between MF, vitamin D and PA is warranted. To date, correlations between PA, MF, serum vitamin D and vitamin D intake (dietary and supplemental intake) have not been studied in a stable HD population. The primary aim of this study will be to investigate the
possible links between MF, PA level and serum 25-OHD in a stable hemodialysis population from two satellite units of outpatients-units in Toronto, Canada.

MF will be represented by a single measure of handgrip strength and measured using a hydraulic handgrip dynamometer. This measure will be used as the dependent variable in univariate and multivariate correlation analysis in this cross sectional study. Habitual PA will be measured via uniaxial accelerometry worn 24-hours over a seven-day period. Mean count per minute over wear time of device will indicate PA intensity and will be used as a single measure of PA in this study as an independent factor in univariate and multivariate correlation analysis of the dependent variable. Vitamin D levels will be assessed via a one-time measure of serum 25-OHD within the same week of the handgrip and accelerometer measure. This value will also be used as an independent variable in univariate and multivariate correlation analysis of the dependent variable.

If an association between MF and the independent variables exists, this might lead to further studies examining the pathophysiology of poor physical function and exercise tolerance in this population. This study might further characterize dialysis patients. The findings of this study might potentially provide new evidence on which to base further investigation to improve standard of care provided to CKD patients. For example findings may be used to:

A) Identify the need to include a serum vitamin D measure with monthly or quarterly blood work to regularly screen and assess vitamin D status

B) Providing rationale to tailor vitamin D treatment to patients on HD

C) Support a rationale to pursue a randomized control trial including exercise and a vitamin D intervention for this patient population to enhance MF in HD
D) Investigate novel relationships that may exist among MF and FGF-23, vitamin D, phosphorus, PTH, calcium and PA in HD patients

E) Supporting the need for PA and MF assessments to be routine clinical care of HD patients

To my knowledge, only a limited number of studies have looked at vitamin D status and PA in these patients in relation to MF. This study will be unique in design in that it will include many confounders of MF, such as nutritional status, dietary intake and DV in combination with objective measures such as serum analysis, accelerometer-measured PA behaviour and handgrip dynamometer readings.

2.10 Objectives and Hypotheses

2.10.1 Primary Objectives

Objective 1: To examine the relationship between MF and PA in HD patients.

MF and physical movement are naturally interconnected. An individual’s level of PA can influence muscle integrity and function, and vice-versa. I therefore hypothesize that a cross-sectional analysis of MF and habitual PA will demonstrate a direct positive relationship in stable HD patients and both factors will be reduced in this group.

Objective 2: To examine the relationship between MF and vitamin D in HD patients.

Since both vitamin D and MF decline in dialysis patients and vitamin D has been shown to have a physiological effect on muscle morphology and function, it is reasonable to speculate
that serum vitamin D levels influence muscle strength. I hypothesize that muscle strength and serum vitamin D levels will demonstrate a direct positive relationship in stable HD patients.

Lastly, I also hypothesize that even after adjusting for potential confounders such as poor nutritional status, poor dietary intake, serum abnormalities (as in clinical markers used for adequacy of dialysis and medical treatment of these patients such as Alb, electrolytes, and urea/creatinine clearance etc.), comorbid conditions and advanced age, there will be a positive correlation between vitamin D status and MF, as well as between MF and habitual PA.

2.10.2 Secondary Objectives

To characterize habitual PA patterns in a stable hemodialysis population and predict relationship of PA intensity to nutritional status, dietary intake, analysis of monthly blood work and a novel marker of physiological importance in this population, FGF-23. Mean count per minute over wear time of device will indicate PA intensity and will be used as the dependent variable in the univariate and multivariate correlation analyses.

To characterize vitamin D profile of a stable HD population and predict relationships of serum 25-OHD to supplemental vitamin D, dietary intake, nutritional status, markers of monthly serum clinical biomarkers and a novel marker of physiological importance in this population, FGF-23. In addition 25-OHD will be used as the dependent variable in the univariate and multivariate correlation analyses.
Figure 2.4: Schematic overview of primary relationships to be investigated in this thesis
Solid arrows represent primary relationships and dashed arrows represent possible confounders.
MIS: Malnutrition Inflammation Score, nPCR: normalized Protein Catabolic Rate, 3DDMR: 3-day diet & medication record, Ca: serum calcium, PO₄: serum phosphate, FGF-23: fibroblast growth factor, PTH: parathyroid hormone, Alb: albumin, Hgb: hemoglobin, DV; Dialysis vintage, DM; Diabetes Mellitus
Chapter 3
Methods

3 Procedures, Timelines and Data Collection

3.1 Overview of Screening, and Enrollment

One hundred and eighty patients from two satellite hemodialysis units were screened for this study. Exclusion criteria were: presence of active malignancy, diagnosis of active bone disease (under treatment with estrogen replacement therapy, bisphosphonates therapy or and diagnosis of osteoporosis or Page’s disease), acute illness such as infections and active antibiotic therapy, on dialysis < three months, uncontrolled hypertension, uncontrolled diabetes (> 3 events of hypoglycemic or and hyperglycemic event with serum blood glucose <4 or >14), or use of mobility devices (had to be independently mobile), recent (< three months) major surgery or hospital admission and stay > three days. This information was reviewed in the medical chart (physician notes and diagnoses listed).

After screening, study procedures were explained to those interested, including record keeping for all ingested foods and medication for three days, wearing an accelerometer for seven days, keeping an activity log for seven days, blood sample collection, health data collection from the medical chart and handgrip strength measure. Eighty one stable HD subjects were enrolled and upon signing the informed consent form. Participants were fitted for an accelerometer around the hip and were given instructions on how to wear the device for seven days as well as how to keep an activity log. Record keeping of a three-day food and medications was explained and an example was given by a dietitian-assisted 24-hour food recall. Written instructions were
also reviewed and given to participants (Appendix III). Participants were informed that they would be contacted via telephone daily for seven days to be reminded to wear the accelerometer, log wear time and keep records of what they ingested throughout the study duration in order to improve compliance. Medical charts were reviewed to collect relevant information, including date of birth, start of dialysis date, dry weight, height, comorbidities, presence of diabetes, medication list and history of recent (< three months) weight loss or poor dietary intake. The information was confirmed through informal interview at bedside with each patient.

3.2 Timelines

Participants were recruited on dialysis shifts. The first day of dialysis in the week for all participants (either a Monday or a Tuesday, depending on dialysis shift) is referred to as day one of the study, where study procedures were explained, accelerometers were provided and examples were given on how to record data. The following day (non-dialysis day), participants’ accelerometers were set to start measuring and recording PA data at midnight (see section 3.3.6 for accelerometer and setting details). On this day, all participants were also contacted by phone to ensure they were wearing the accelerometers, and recording food and activity logs. Blood samples were collected at the regularly scheduled monthly blood work, which was day three of the study for all. On day four, participants were called to remind them to keep record of their accelerometer wear time. On day five physical assessments were completed while patients continued to wear the accelerometer. Day 6 of study was similar to day four, and patients were simply contacted to ensure they were wearing the accelerometer and keep keeping a log of non-wear time. Day 7 was the last day of the study and patients were contacted via phone to be reminded to continue to wear the accelerometer and record any non-wear time, record the last day of 3DDMR and to return the accelerometer and all records on the next HD treatment (see
timelines below). The total duration of the study was one week, followed by an additional day in the subsequent week when accelerometers and records were collected. The study was designed in a way so that no additional visits outside of the regular dialysis time were required. All data were collected consecutively within a three-week span, in October 2011 (Figure 3.1). Timelines for data collection according to monthly blood work are shown in Appendix II.

Figure 3.1: Study Time Lines- Overview of Study

Synopsis of Study Timelines (over the course of three weeks). Study timelines were set according to dialysis schedule and monthly blood work. There were three dialysis groups, divided according to dialysis shift (morning, noon or afternoon). ACC; accelerometer, 3DDMR; three-day dietary and medication list
3.3 Data Collection

3.3.1 Medical Chart

Electronic and hard copy medical records were reviewed at two stages, first to screen and recruit suitable participants and a second time after recruitment to collect pertinent medical information for each participant. At the screening stage, medical history for conditions mentioned in section 3.1 were noted and those patients were excluded from recruitment. At the second stage, which was after recruitment for the study, the latest physician-driven dry weight was recorded as well as height, age, dialysis start date and presence of diabetes diagnosis.

3.3.2 Serum Collection

Blood samples were collected just before start of dialysis treatment for each participant on day three of the study by a trained HD nurse and according to the Nephrologist’s medical order as per standard hospital procedure. Using best practice venopuncture and sterile needles, blood was drawn from either internal jugular central venous catheter or the bloodline connected to the arteriovenous fistula, whichever was the surgical access created for dialysis treatment and placed into six pre-labeled sterile test tubes that were labeled with each participant’s hospital number (two 3.5 ml, two 5.0 ml, two 8.0 ml). Samples were shipped immediately after collection for analysis to The Scarborough Hospital central laboratory or affiliated locations, depending on serum analysis ordered (Table 3.1). Samples were centrifuged and aliquoted for commercial assays (see Appendix VII) that were used to determine serum level of each biomarker. Glycated Hgb levels were used to determine average three month serum glucose control and to describe baseline information of the group. Serum calcium level was corrected for Alb using the following formula: Corrected Ca = [0.8 x (normal Alb - patient's Alb)] + serum Ca level, where
“normal albumin” was $40\text{g.L}^{-1}$. Results were then manually entered and electronically available under each patient’s electronic medical charts at The Scarborough Hospital. These were electronically transferred to the master spreadsheet for the study and each entry was recoded with a unique study number that replaced all identifiers (such as name, date of birth and address) which were removed to protect the identity of participants.

Table 3.1: Sites for Sample Analysis of Biomarkers

<table>
<thead>
<tr>
<th>Serum Measure</th>
<th>Site of Sample Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcium, phosphorus, parathyroid hormone, hemoglobin, albumin, iron, ferritin, transferrin, total iron binding capacity, glycated hemoglobin and random blood glucose</td>
<td>The Scarborough Hospital Central Laboratory, Toronto, Ontario</td>
</tr>
<tr>
<td>25-Hydroxy-Cholecalciferol vitamin D</td>
<td>Mount Sinai Hospital, Toronto, Ontario</td>
</tr>
<tr>
<td>fibroblast growth factor-23</td>
<td>University Health Network, Toronto General, Toronto Ontario</td>
</tr>
</tbody>
</table>

Serum calcium, phosphorus, parathyroid hormone and fibroblast growth factor were measured as markers of CKD-mineral bone disease and because they are pertinent to vitamin D metabolism and regulation. Serum 25-OHD was used as a marker of vitamin D status and no other serum metabolites of vitamin D were tested. Iron, ferritin, transferrin, total iron binding capacity (TIBC) and Hgb were used to assess anemia. TIBC was also used as marker of inflammation and malnutrition in the Malnutrition Inflammation Score assessment. Glycated Hgb and random blood glucose was used as an indicator of glycemic control.
3.3.3 Muscle Strength assessment

On day five of the study, just prior to dialysis treatment initiation, participants were instructed on how to use the handgrip dynamometer (Jamar Hydraulic Handgrip Dynamometer J00105, Lafayette Instruments, Lafayette, Indiana, USA,) as per guidelines set by Roberts et al [140]. Handgrip strength was used as a surrogate of overall MF as it has been validated in this patient population [32]. A grip strength test was first demonstrated by using the device once in front of the participant. Hand dominance and arteriovenous fistula access was noted. Dominant arm was used for the majority of subjects to perform this test. In cases where the dominant arm was used for dialysis fistula access, the non-dominant arm (non-access arm) (total of 5 participants) was used, whereas in cases where patients had a central venous access, the dominant arm was used. Each participant sat on an armchair (same chair used for all participants) with their back flat against the backing of the chair and their legs bent at the knees at an angle to the floor. Their forearm was placed on the armrest and bent at the elbow at 90° and parallel to the armrest. The adjustable handle of the dynamometer was fitted to the second knuckle on the hand for each individual so that it would fit comfortably in his or her hand. The dynamometer was placed in the participant’s hand ensuring the wrist was hanging freely and parallel to the armrest, but not touching the armrest or any other object. The participant then was asked to squeeze as hard as they possibly could in one attempt. After a 30 second rest they were asked to repeat the test one more time. Dynamometer readings were noted in nearest kilograms and the highest measure out of the two attempts was reported as a single reading of handgrip strength.
3.3.4 Nutritional Status

A modified Subjective Global Assessment (SGA) tool, validated in dialysis patients [201], was used to assess nutritional and inflammation status of each participant. This tool, called Malnutrition Inflammation Score (MIS) [201], consists of two sections. The first section has been adapted from the original SGA [202], which assesses the patient’s nutritional status using a patient interview about appetite, intake, weight loss, acute illness and gastrointestinal symptoms in combination with a physical assessment of muscle and fat stored at several body sites as well as fluid retention (see Appendix III for a sample MIS). The second section of the MIS is based on more objective data such as number of chronic comorbidities, serum Alb level, total iron binding capacity (TIBC) and body mass index (BMI). The score for each participant was given out of 30, zero indicating no malnutrition and inflammation state and 30 indicating severe malnutrition and inflammation state. Once the assessment was completed for each participant, it was recorded as a single score of malnutrition and inflammation out of 30.

3.3.5 Dietary Intake

A standard three-day diet and medication record (3DDMR) was given to each participant on day one of the study (see Appendix II for example) with specific instructions on how to record intake. Participants were instructed to keep record of what they ingested over 3 predetermined days during the seven days of the study (one dialysis day, two non-dialysis days consisting of a weekend and a weekday)) and were contacted via phone to be reminded of these days early on the day of recording. Three-day food records for three typical days in a week is standard in nutritional analysis [203-204] and therefore was used in this study. The dates for which diet records needed to be completed were marked on each participant’s handout along
with their unique study number. Participants were asked to record food and beverages in household measures and this was demonstrated to patients using food models and measuring cups. They were also instructed to be as descriptive as possible with their recording regarding type of food items, condiments and cooking methods (ex. frying versus steaming). They were also asked to specify whether they used butter, margarine, fortified soy or other dairy foods. Prior to start of self reported 3DDMR, a 24-hour food recall interview by an experienced renal dietitian was used to demonstrate recording food intake on the three-day food record. Participants were also asked to record any medication and or supplements they were taking. The recorded list was then compared to the patient’s medication list to identify discrepancies relevant to the study and clarified with participants as to what actually was ingested by them. Written instructions and examples of measures were given with each booklet (see Appendix II). All medication and supplemental sources of vitamin D were noted in medication records, diet records and verbally confirmed for each participant (Table 3.2). Dietary records were then entered into a computer diet analysis software (Axxya Nutritionist Pro™, Version 2.5, 2006, Stafford, TX) and three-day-mean values for vitamin D, calcium, phosphate, protein and daily energy were extrapolated for each participant. Incomplete food records (those that did not include at least one dialysis and one non-dialysis day), lack of recording for portion sizes of each food item reported and diet analysis energy intake less than 1000 kcal.day\(^{-1}\) or greater than 10,000 kcal/day were considered inaccurate and excluded (total of 16 excluded). Three-day average dietary intake of protein and energy were used to assess protein energy intake and as a quality assurance measure to estimate validity of food records. Three-day average dietary intake of calcium, phosphate and vitamin D were used to assess food sources and compared to daily recommendation of these nutrients as they pertain to serum levels and vitamin D metabolism.
Table 3.2: Vitamin D and Calcitriol Sources Recorded

<table>
<thead>
<tr>
<th>Supplemental Vitamin D (non-food sources) Included in the Study (24%)</th>
<th>Calcitriol analogs Included in Study (72%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over-the counter vitamin D (including D3 and D2)</td>
<td>One Alpha</td>
</tr>
<tr>
<td>Calcium + vitamin D</td>
<td>Rocaltrol</td>
</tr>
<tr>
<td>Fish oil/cod liver oil</td>
<td>Calcijex</td>
</tr>
<tr>
<td>Omega 3 oil</td>
<td>Calcitriol</td>
</tr>
<tr>
<td>Multivitamin (containing vitamin D)</td>
<td></td>
</tr>
</tbody>
</table>

3.3.6 Physical Activity

Uniaxial accelerometers (ActiGraph GT1M® 7164, Pensacola, FL) were used to objectively measure habitual PA intensity in participants. This device measures and records vertical acceleration as “counts,” providing an indication of the intensity of PA associated with movement [205] that is recorded in one-minute epochs for up to one week. Prior to distribution of accelerometers to participants, devices were preset to start measure movement versus non-movement at 00 hours of day two of the study and run for seven consecutive days and settings were programmed according to NHANES [96]. Accelerometers were tagged with a unique identifying number and matched the unique study ID number of each participant. Upon collection of the device at the end of the study, the numbers were matched to ensure uploaded accelerometer data were matched to the appropriate subject. Once accelerometers were collected from participants, they were immediately connected to a laptop equipped with the ActiLife 6.0 (Pensacola, FL) data analysis software and uploaded information for each participant was automatically converted onto an excel spreadsheet. A valid day was defined as 10 (600 minutes in 24 hours) or more hours of monitor wear time; data from respondents with five or more valid days (2 = dialysis days, 3 = non-dialysis days) were retained for analyses. These criteria were in line with large-scale accelerometer-measured PA surveillance studies in the US (NHANES) that used the same device [96] as well as Canada (CHMS) [93]. At the start of the study, participants
were instructed on how to wear the accelerometer and keep a log book for wear time and daily activities for seven consecutive days. This encompassed three dialysis days and four non-dialysis days for all participants. Accelerometers were fitted around the waist of each participant with an adjustable elastic belt on the right hip and were worn for seven days. All participants were independently mobile and did not use a wheelchair, a walker or other walking aids. Each participant was instructed to wear their accelerometer for 7 days, 24 hours per day and only remove it before coming into contact with water (such as before taking a shower, bath, swimming or any other water activity). All participants kept a log of wear-time, recording the time, date and the activity for which they removed the accelerometer, as well as the date and time when the accelerometer was put back on. This log was then compared against recorded activity counts downloaded from the device to capture “true” wear time and non-wear time.

Wear time was determined by subtracting non-wear time from 24 hours. Non-wear time was characterized by at least 60 consecutive minutes of zero counts. However, it was recognized early on that these criteria might not be entirely appropriate for this population. For example: while on dialysis, patients could in fact be moving very little, and it is entirely possible that 60 consecutive minutes of zero counts could be recorded during this time. Therefore, while the 60 consecutive minutes of zero counts criteria were used to denote non-wear time, daily accelerometer profiles for each participant were manually compared to log books. Where a participant had recorded wear time on their log, but the device did not detect sufficient movement to register as wear time, the non-wear time was manually checked off to be accounted for as wear-time, overruling the preset non-wear criteria. Non-wear times logged by the participant were also counted as valid non-wear time in the analysis. Any records outside of these parameters were excluded (total of 10 were excluded).
The data were then presented in two ways for each subject. The first measure was the overall daily intensity of PA (i.e., mean counts). This was calculated by dividing total PA (counts; sum of all valid days) by total wear time (min; sum of all valid days). Mean counts (counts.min\(^{-1}\)) therefore represent the overall daily intensity of PA over the duration of the study. The second measure took into account the proportion of time (%) each individual spent in various intensity categories (i.e., % of day spent sedentary and in light, lifestyle, moderate, vigorous and very vigorous activity), calculated using the Freedson et al. accelerometer intensity thresholds (Table 3.3). Days were also coded as dialysis versus non-dialysis days for further comparison of whether any changes in the daily activity intensity profile occurred. As mentioned previously, these accelerometer intensity thresholds were obtained from calibration studies that equated accelerometer counts to measured activity energy expenditure [98, 206]. Counts per minute evaluated the raw data provided by the accelerometer without imposition of any external criteria other than determination of wear and non-wear time [98]. Mean counts per minute were calculated by dividing the sum of activity counts for a valid day by the number of minutes of wear time in that day across all valid days [98]. Percent of time spent in each activity threshold was calculated by summing all minutes of counts that met the intensity threshold for each intensity category, and dividing this value by the total amount of valid wear time (min) for each day (Table 3.3). An average was then taken for dialysis days and also for non-dialysis days.

Table 3.3: Accelerometer Counts Intensity Categories

<table>
<thead>
<tr>
<th>Physical Activity Intensity</th>
<th>Cut off</th>
<th>Counts per minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>0 to 99</td>
<td>99</td>
</tr>
<tr>
<td>Light</td>
<td>100 to 759</td>
<td>759</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>760 to 1951</td>
<td>1951</td>
</tr>
<tr>
<td>Moderate</td>
<td>1952 to 5724</td>
<td>5724</td>
</tr>
<tr>
<td>Vigorous</td>
<td>5725 to 9498</td>
<td>9498</td>
</tr>
<tr>
<td>Very Vigorous</td>
<td>9499 and above</td>
<td></td>
</tr>
</tbody>
</table>

\(\times\) (adapted from references [93, 121, 124, 128])
3.4 Statistical Analysis

All raw data were entered into an Excel spread sheet (Microsoft Office 2003, Silicone Valley, California) and were coded with participant ID. Simple descriptive statistics were calculated in Excel including, mean, median, standard deviation, and distributions according to age, sex and dialysis versus non-dialysis days (accelerometer data only). All data were then imported to SPSS 19.0 for inferential statistical analysis. Bivariate relationships were tested using correlation test for each pair of variables before selection for multiple regression model-building (see Figure 4.15 for all primary variables used). In the case of dichotomous variables such as sex (male/female) or vitamin D supplementation (yes/no) a special form of point-biserial Pearson Correlation was used, and the variables were assigned dummy values of 0 as default, and 1 as meeting condition set (ie Yes, for a vitamin D supplement). Male sex was set as the default 0, and female sex was counted as 1. Partial-correlation tests were used to further investigate persisting or veiled relationships between variables of interest controlling for potential confounders observed in the initial bivariate correlation tests mentioned above and or those documented in the literature. For example, BM is well-known to be associated with muscle strength and PA level therefore it was controlled for using partial correlation analysis. In the primary analysis partial-correlation tests were used to investigate relationship of 25-OHD with handgrip strength (while controlling for age, sex and BM) and to investigate the relationship between mean PA intensity and handgrip strength (while controlling for BM). Controlled variables used in the partial correlation analysis were chosen based on interactions on both variables being tested in this study with initial correlation analysis or as documented in the literature from other studies.
Stepwise, forward multiple linear regression analysis was completed to identify predictors for three outcome constructs: MF (handgrip strength), PA (mean accelerometer counts.min\(^{-1}\)) and vitamin D status (serum 25-OHD). Initial variables chosen to enter each model were taken based on bivariate relationships indicated above and correlations documented in other studies in similar populations. The Akaike Information Criterion Correction was used to test goodness of fit (initial entry or refusal of each variable) for each variable in relation to other variables and the model as a whole. A standard linear model was created including the effects of predictors with p-value <0.05, and removal of predictors with p-value >0.10. Final multiple regression formulas were reported with coefficients of predictors that yielded an effects of statistical significance (set at p-value <0.05).

Student T-tests or Mann-Whitney tests were used to compare means or median of groups where appropriate (i.e. normally distributed versus not respectively using Shapiro-Wilk normality test). Paired T-Test was used to compare percent time spent in each of the four categories of exercise intensity (sedentary, light, lifestyle, and moderate) on dialysis versus non-dialysis days (vigorous and very vigorous categories of mean activity intensities were eliminated since most participants did not score in either of these intensities). A one-way-ANOVA was used to test for differences between means of more than two comparable groups. ANOVA was also used to determine the differences in serum vitamin D levels across those supplemented eith vitamin D, those with adequate dietary vitamin D intake, and groups. Statistical significance was set at p \(\leq 0.05\). Sigma plot 11.2 (San Jose, California) was used to create scatter plots, box plots, bar graphs, and line graphs.
Chapter 4

Results

4 Sample Characteristics and Associations

4.1 Characteristics

4.1.1 General Characteristics

Eighty-one stable hemodialysis patients were recruited for this study. Mean age was 58.2 ±14.1 years, and mean dialysis vintage (HD Vintage) was 2.5 ±2.4 years and ranged from 1 year to 15 years. Approximately two thirds of participants were men (51 males versus 30 females) (Table 4.1). Body mass (BM) and body mass index (BMI) were normally distributed and within the healthy range for this age group (BM = 71.9 kg ±17.3 and BMI = 26.1± 4.7 kg.m⁻²). Thirty-two patients (40%) had a medical diagnosis of diabetes mellitus (DM). Vitamin D supplementation was reported in 19 (24%) participants, while 59 (70%) were on calcitriol (and its analogs, referred to collectively as calcitriol from hereon) and only 16 (20%) were on both.

Table 4.1: General Characteristics of Participants

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>N</th>
<th>Males Mean ±SD</th>
<th>Females Mean ±SD</th>
<th>Group Mean ±SD</th>
<th>Sex Differences P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>81</td>
<td>56.6±13.3</td>
<td>61.2±15.2</td>
<td>58.2 ±14.1</td>
<td>0.150</td>
</tr>
<tr>
<td>Hemodialysis Vintage (yrs)</td>
<td>81</td>
<td>2.5±2.0</td>
<td>2.6±2.9</td>
<td>2.5 ±2.4</td>
<td>0.790</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>81</td>
<td>77.1±16.3</td>
<td>63.5±15.9</td>
<td>71.9 ±17.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body Mass Index (kg.m⁻²)</td>
<td>81</td>
<td>26.6±4.8</td>
<td>25.7±4.5</td>
<td>26.1 ±4.7</td>
<td>0.390</td>
</tr>
<tr>
<td>Physical Activity (MC.min⁻¹)</td>
<td>69</td>
<td>130±83</td>
<td>105±57</td>
<td>119 ±76</td>
<td>0.706</td>
</tr>
<tr>
<td>Handgrip Strength (kg)</td>
<td>69</td>
<td>31.1±11.7</td>
<td>19.6±6.3</td>
<td>27.0 ±11.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes Mellitus Diagnosis</td>
<td>32</td>
<td>40%</td>
<td>37%</td>
<td>40%</td>
<td>0.770</td>
</tr>
<tr>
<td>Vitamin D Supplementation</td>
<td>19</td>
<td>28%</td>
<td>17%</td>
<td>24%</td>
<td>0.790</td>
</tr>
</tbody>
</table>

MC= Mean Count

* Median =2.0 years
4.1.2 Serum Characteristics

Blood samples were collected based on standard protocol detailed in 3.3.2. Hemoglobin (Hgb) and albumin (Alb) levels were within acceptable range for dialysis patients (mean 113.9 ±12.30 g.L⁻¹, and 33.8 ±3.30 g.L⁻¹, respectively; Table 4.2). There was a large variation in Fibroblast Growth Factor 23 (FGF-23) levels regardless of relatively acceptable range of serum phosphate (PO₄) and calcium (Ca) levels (mean 5421.4 RU.mL⁻¹ ±9012.7, mean 1.60 mmol.L⁻¹ ±0.05, and mean 2.39 mmol.L⁻¹ ±0.17, respectively). Mean Serum 25-Hydroxy-Cholecalciferol vitamin D (25-OHD) for the group was 42.4 nmol.L⁻¹ ±23.1 and in the insufficient range (28-75 nmol.L⁻¹) (Deficient=28% (<27.5 nmol.L⁻¹), Insufficient=62% (27.5-75 nmol.L⁻¹), Sufficient=10% (>75nmol.L⁻¹)). Parathyroid hormone (PTH) also varied widely amongst the patients with group mean 65.6 ±68.5 µg.L⁻¹. There was a significant correlation between FGF-23, PO₄, and Ca but not with FGF-23, PTH and 25-OHD (Appendix V-A to C).

Table 4.2 Mean Serum Levels of Select Biomarkers (N=81)

<table>
<thead>
<tr>
<th>Serum Biomarkers (normal distribution)</th>
<th>Males Mean ±SD</th>
<th>Females Mean ±SD</th>
<th>Group Mean ±SD</th>
<th>P-value</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxy Vitamin D (nmol.L⁻¹)</td>
<td>38.9±22.9</td>
<td>48.5±22.6</td>
<td>42.4 ±23.1</td>
<td>0.100</td>
<td>&gt;75</td>
</tr>
<tr>
<td>Hemoglobin (mg.L⁻¹)</td>
<td>114.0±13.1</td>
<td>113.3±11.2</td>
<td>113.9 ±12.3</td>
<td>0.954</td>
<td>110-150</td>
</tr>
<tr>
<td>Albumin (mg.L⁻¹)</td>
<td>33.9±3.5</td>
<td>33.6±3.1</td>
<td>33.8 ±3.30</td>
<td>0.690</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Calcium (mmol.L⁻¹)</td>
<td>2.39±0.16</td>
<td>2.49±0.16</td>
<td>2.39 ±0.17</td>
<td>0.370</td>
<td>2.20-2.56</td>
</tr>
<tr>
<td>Phosphate (mmol.L⁻¹)</td>
<td>1.62±0.54</td>
<td>1.57±0.44</td>
<td>1.60 ±0.05</td>
<td>0.693</td>
<td>0.80-1.78</td>
</tr>
<tr>
<td>Protein Catabolic Rate (g.kg⁻¹)</td>
<td>1.03±0.24</td>
<td>1.10±0.25</td>
<td>1.06±0.24</td>
<td>0.258</td>
<td>&gt;1.0</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.52±0.38</td>
<td>1.57±0.34</td>
<td>1.55±0.36</td>
<td>0.746</td>
<td>2.00-1.40</td>
</tr>
<tr>
<td>TIBC (µmol.L⁻¹)</td>
<td>345±258</td>
<td>320±73</td>
<td>332±349</td>
<td>0.765</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum Biomarkers (non-normal distribution) Quartiles</th>
<th>25th, 50th, 75th</th>
<th>25th, 50th, 75th</th>
<th>25th, 50th, 75th</th>
<th>25th, 50th, 75th</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast Growth Factor23 (RU.mL⁻¹)</td>
<td>563, 2483, 67927682333, 4090616, 2395, 5830</td>
<td>0.727</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Parathyroid Hormone (µg.L⁻¹)</td>
<td>22.3, 34.1, 78.9</td>
<td>21.5, 35.6, 80.121.3, 34.3, 79.8</td>
<td>0.968</td>
<td>3-20</td>
<td></td>
</tr>
</tbody>
</table>

Kt/V: Measure of dialysis adequacy driven from blood urea clearance pre and post dialysis treatment, TIBC: Total Iron binding Capacity used for Malnutrition Inflammation Score, RU; Relative Unit
4.2 Association between Variables

All variables intended to enter multiple linear regression models, were first correlated against each other in order to detect any potential interactions (Figure 4.15). This was performed as an exploratory means of detecting significant correlation between any two selected variables to be tested with the regression model (statistical significance set at p-value ≤ 0.05) for this study cohort. In addition, decisions to include each of the variables in the three linear multiple regression models were based on clinical relevance as supported by previous literature (Chapter 2.0). Stepwise, forward linear regression analysis was performed as described in Chapter 3.4. Statistically significant correlates are reported under each linear regression model of the three major sections of the study including; muscle strength, PA level, and vitamin D.

4.3 Muscle Strength

Mean handgrip strength was 27.0 ±11.4 kg for the group. However there was a significant difference between the two sexes, with men scoring higher than women (males 31.1 ±11.7 kg and females 19.6 ±6.3 kg, p-value <0.001; Figure 4.1).

4.3.1 Correlates of Muscle Strength

The predictors of muscle strength were tested individually with handgrip strength, using Pearson correlations, student t-tests (for sex), or Mann-Whitney tests (for vitamin D supplementation). There was a positive association between BM and handgrip strength, and an inverse association of age and serum calcium with handgrip strength (Figures 4.2- 4.4). Men and those on vitamin D supplementation also had significantly stronger handgrips than females and those not on a vitamin D supplement, respectively (Figures 4.1 and 4.5). PA tended to increase
with MF but it was not statistically significant (p-value=0.053). Calcitriol prescription did not correlate to muscle strength.

Figure 4.1: Handgrip Strength in Females versus Males
Handgrip strength was significantly greater in males (♂ 31.1±11.7 kg, ♀ 19.6 ±6.3 kg). *p-value <0.001

Table 4.3: Correlates of Muscle Strength

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.310</td>
<td>0.014</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.482</td>
<td>0.000</td>
</tr>
<tr>
<td>Body Mass</td>
<td>0.409</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td>-0.396</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin D Supplementation</td>
<td>0.285</td>
<td>0.025</td>
</tr>
</tbody>
</table>
Figure 4.2: Serum Calcium Levels against Handgrip Strength
Handgrip strength (kg) and serum calcium level (mmol.L⁻¹) were inversely related ($r = -0.396$).

Figure 4.3: Relationship of Age against Handgrip Strength
Handgrip strength (kg) and age (yr) were inversely related ($r = -0.310$).
Figure 4.4: Relationship of Body Mass against Handgrip Strength
Handgrip strength (kg) and body mass (kg) were directly related ($r = 0.409$).

Figure 4.5: Comparisons of Mean Handgrip Strength between Vitamin D Supplemented and Non-Supplemented groups
Mean handgrip strength (kg) difference between S ($28 \pm 3$ kg) and NS ($24 \pm 2$ kg) statistically significant. NS: No Supplementation, S: Supplementation, * p-value=0.028.
4.3.2 Partial Correlates of Muscle Strength

PA level partially correlated with muscle strength when controlling for BM. Muscle strength persisted to correlate with 25-OHD after sex, age, and BM were controlled for. MF correlated with vitamin D supplementation even after controlling for serum 25-OHD (Table 4.4). No other values had a statistically significant relationship with muscle strength.

Table 4.4: Partial Correlates of Muscle Strength

<table>
<thead>
<tr>
<th>Controlled Variables</th>
<th>Correlate</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass</td>
<td>Physical Activity</td>
<td>0.411</td>
<td>0.003</td>
</tr>
<tr>
<td>Age, Sex &amp; Body Mass</td>
<td>25-OHD</td>
<td>0.298</td>
<td>0.023</td>
</tr>
<tr>
<td>25-OHD</td>
<td>Vitamin D Supplementation</td>
<td>0.269</td>
<td>0.037</td>
</tr>
</tbody>
</table>

4.3.3 Multiple Regression Analysis: Predictors of Muscle Strength

Handgrip strength was used as the dependant variable in a stepwise-forward multiple regression model, where the predictive relationships of seventeen selected variables were entered (see Figure 4.15 for list of initial variables entered). The strongest predictor of handgrip strength serum calcium (p-value=0.006), followed by age (p-value=0.017) sex (p-value=0.022), BM (p-value=0.046), and vitamin D supplementation (p-value=0.048).

The variability of handgrip strength (HGS), this study’s measure of MF, was described via the following multiple linear regression formula:

\[
HGS (\text{kg}) = 73.2 + (-19.0(\text{Serum Calcium in mmol.L}^{-1})) + (-0.2(\text{Age in years})) + (6.3 \text{ (Sex)}) + (0.2(\text{BM in kg})) + (-5.6(\text{VITD-S})),
\]

where VITD-S: 0 = no vitamin D supplementation and 1= vitamin D supplementation, and where Sex: 0=Males and 1= Females (adjusted \(R^2=0.41\), p-value<0.0001)
4.4 Physical Activity

The overall daily intensity of PA (mean counts; counts.min⁻¹) was calculated by dividing total PA (counts; all valid days) by valid wear time (min; all valid days), resulting in a group mean of 119 ±76 counts.min⁻¹ (Table 4.1). Accelerometer thresholds used in the National Health and Nutrition Examination Survey (NHANES, 2010) were chosen to assess the percentage of time spent in each activity intensity category per day (see section 4.3.1 and 3.3.6 for details). Activity level was strongly and inversely correlated with age (p-value 0.000, r-value=−0.533). As age increased, the daily PA intensity profile of participants decreased (Figure 4.8).

4.4.1 Patterns of Physical Activity

On an overall group basis, the majority of time was spent sedentary (77%). Time spent in light as well as in lifestyle activity comprised less than one quarter of patients’ time (22%), and less than one percent of the day was spent in moderate activity. Not a significant percent of time was spent in vigorous or very vigorous intensity activity (Table 4.5).

Table 4.5: Time Spent in Physical Activity Intensity on Dialysis and Non-Dialysis Days

<table>
<thead>
<tr>
<th>Physical Activity Intensity</th>
<th>Percent Total Daily Time NDD (%)</th>
<th>Percent Total Daily Time DD (%)</th>
<th>Percent Total Daily Time Overall (%)</th>
<th>Activity Level Criteria (MC.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>76.2</td>
<td>78.6</td>
<td>77.4</td>
<td>0-99</td>
</tr>
<tr>
<td>Light</td>
<td>19.6</td>
<td>18.0</td>
<td>18.7</td>
<td>100-759</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>3.4</td>
<td>3.0</td>
<td>3.2</td>
<td>760-1951</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.6</td>
<td>0.4</td>
<td>0.5</td>
<td>1951-5724</td>
</tr>
<tr>
<td>Vigorous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5725-9498</td>
</tr>
<tr>
<td>Very Vigorous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9499 and above</td>
</tr>
</tbody>
</table>

DD: Hemodialysis Days, NDD: Non-Hemodialysis Days
MC.min⁻¹, arbitrary unit-less mean count of intensity per minute of movement
Figure: 4.6(a-d) Physical activity intensities of subjects on DD vs NDD
* Difference of mean percent time spent in each physical activity category based on DD versus NDD was statically significant (p-value<0.001). DD: Dialysis day, NDD: Non-dialysis days

PA levels were analyzed according to dialysis versus non-dialysis days in order to compare percent of time spent in each at of the PA intensities. These were calculated using paired T-test. The differences in time spent in sedentary, light and lifestyle activities on dialysis
versus non-dialysis days that were statistically significant but not for time spent in moderate intensity activity (Figure 4.6 a-d and Table 4.5).

### 4.4.2 Correlates of Physical Activity

Correlates of PA were individually tested using Pearson correlations or Student t-tests (DM versus non-DM groups) to investigate if the same markers individually related to the daily intensity of PA (mean counts.min\(^{-1}\)). Correlations were found to be statistically significant with serum Alb, DM, advanced age and a higher Malnutrition Inflammation Score (MIS) but not with dialysis vintage, sex, 25-OHD or BM. Alb and MIS relationships to PA were individually significant (Table 4.6) but these relationships were not evident in the multiple regression models, where age and DM may have had a much stronger correlation with PA.

![Physical Activity Intensity in Diabetics and Non-Diabetics](image)

**Figure 4.7: Physical Activity Intensity in Diabetics and Non-Diabetics**

Mean physical activity intensity (MC.min\(^{-1}\)) was lower in diabetics (91±60 MC.min\(^{-1}\)) as compared to non-diabetics (137±80 MC.min\(^{-1}\)). DM: Diabetes Mellitus, NDM: No Diabetes Mellitus

*p-value= 0.003*
Figure 4.8: Relationship of Age with Physical Activity
Daily intensity of PA (MC.min⁻¹) and age (yrs) were inversely related (r = -0.533)

Figure 4.9: Relationship of Malnutrition Inflammation Score with Physical Activity
Daily intensity of PA (MC.min⁻¹) and MIS (score/30) were inversely related (r=-0.332)
Figure 4.10: Relationship of Serum Albumin Level with Physical Activity
Daily intensity of PA (MC.min⁻¹) and serum albumin (mg.L⁻¹) were directly related (r=0.243).

Table 4.6: Correlates of Physical Activity

<table>
<thead>
<tr>
<th>Correlates</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.533</td>
<td>0.000</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.297</td>
<td>0.016</td>
</tr>
<tr>
<td>Malnutrition Inflammation Score</td>
<td>-0.331</td>
<td>0.007</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>0.243</td>
<td>0.049</td>
</tr>
</tbody>
</table>

4.4.3 Partial Correlates of Physical Activity

When PA was tested while controlling for BM, a partial correlation was found with muscle strength and serum Hgb. No other values had a statistically significant relationship with daily intensity of PA (Table 4.7).

Table 4.7: Partial Correlates of Physical Activity

<table>
<thead>
<tr>
<th>Controlled Variables</th>
<th>Correlate</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass</td>
<td>Hemoglobin</td>
<td>0.296</td>
<td>0.018</td>
</tr>
<tr>
<td>Body Mass</td>
<td>Muscle Strength</td>
<td>0.411</td>
<td>0.003</td>
</tr>
</tbody>
</table>
4.4.4 Multiple Regression Analysis: Predictors of Physical Activity

PA (mean accelerometer counts; counts.min\(^{-1}\)) was tested as the dependent variable to which predictive relationships of seventeen independent factors were tested using a stepwise-forward linear multiple regression analysis (see Figure 4.15 for candidate initial variables entered). Mean count PA was predicted by presence of DM diagnosis (p-value 0.022) and age (p-value 0.000) (Figure 4.7 and 4.8). No other factors were found to predict PA level in the model.

The regression equation to predict physical activity therefore was:

\[
PA (MC.min^{-1}) = 146 + (-2.971 (age in years)) + (-37.198 (DM)); \text{ where DM: 0= No Diabetes Mellitus diagnosis and 1= Diabetes Mellitus diagnosis (adjusted } R^2=0.37, \text{ p-value}<0.0001)
\]

4.5 Nutritional Status, Dietary Intake and Vitamin D

Self-reported 3-day diet and medication records (3DDMR), serum measures and physical examination, were used to assess the nutritional status of participants in three areas. Firstly, intake of nutrients were analyzed and compared to needs as per Dietary Recommended Intake’s (DRI) and KDOQI recommendations. Secondly, biochemical markers were measured and then compared to acceptable reference ranges. Thirdly, a bedside assessment, MIS, was completed to estimate clinical nutritional status, as well as skeletal muscle and adipose storage, according to procedures in sections 3.3.2 - 3.3.5.
Table: 4.8: Mean Dietary Intake of Select Nutrients from 3DDMR

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ±SD</th>
<th>DRI/ KDOQI Guidelines</th>
<th>Percent of DRI/Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal.day⁻¹)</td>
<td>1471 ±351</td>
<td>2160-2520¹</td>
<td>65%</td>
</tr>
<tr>
<td>Protein (g.day⁻¹)</td>
<td>74 ±25</td>
<td>93.6¹</td>
<td>80%</td>
</tr>
<tr>
<td>Vitamin D (µg.day⁻¹)</td>
<td>2.53 ±3.67</td>
<td>10.00</td>
<td>25%</td>
</tr>
<tr>
<td>Phosphorus (mmol.day⁻¹)</td>
<td>895 ±288</td>
<td>800</td>
<td>112%</td>
</tr>
</tbody>
</table>

¹ Calculated based on group mean body mass and recommendations of KDOQI guidelines for Hemodialysis patients (30-35 kcal.kg⁻¹ body mass for Energy and 1.3g.kg⁻¹ body mass for Protein requirements) DRI: Daily Recommended Intake, KDOQI: Kidney Disease Outcomes Quality Initiative

Dietary Analysis revealed lower than average energy, protein and vitamin D, while phosphate intake was comparable to standard requirements for HD patients at 112% (Table 4.8). Energy and protein intake were suboptimal to estimated requirements for the group. Energy was only 65% of recommendations while protein was 80% of recommendations. Alb, nPCR, Hgb and malnutrition scores however did not reveal recent weight loss, or suboptimal protein-energy intake, amongst the participants (Table 4.2). Vitamin D intake, on the other hand, was only 25% of daily dietary requirements. There was a large variation in dietary intakes of protein, energy, calcium and phosphorus, but vitamin D was consistently low, irrespective of other nutrients consumed. The majority of subjects (97%) lacked sufficient dietary vitamin D intake.

Malnutrition Inflammation Score (MIS) was used as a tool to assess the overall nutritional status of patients using both objective (i.e. BMI, serum Alb and TIBC) and subjective clinician judgment measures (i.e. muscle wasting, decreased appetite and decreased adipose tissue) to give a score out of 30 (0 being well nourished, and 30 severely undernourished) to each patient. The median value scored for the group was 5, indicating relatively low prevalence of malnutrition amongst the group.
Serum samples were used for select biochemical nutritional status assessment, including 25-OHD, PCR, serum Alb, serum Hgb, TIBC, and Hgb levels. TIBC, PRC, Alb and Hgb means were within acceptable range (Table 4.2). Mean Kt/V also suggested adequate dialysis dose for participants (Table 4.2). Mean serum vitamin D levels however, indicated suboptimal vitamin D status of participants (mean 42.4 ±23.1 nmol.L⁻¹). The majority of subjects (62%) had serum 25-OHD levels consistent with vitamin D insufficiency (50-75 nmol.L⁻¹) (Figure 4.11). Only 24% of participants were on any form of nutritional vitamin D supplementation and these individuals had higher serum vitamin D levels than those without supplementation, but nevertheless were still vitamin D insufficient.

Figure 4.11: Vitamin D Status of Subjects Recruited
Vitamin D status of study participants indicated that the majority of participants were deficient or insufficient in serum level of dietary vitamin D (Deficient=28% (<27.5 nmol.L⁻¹), Insufficient=62% (27.5-75 nmol.L⁻¹), Sufficient =10% (>75 nmol.L⁻¹).
Vitamin D supplementation had a statistically significant relationship with 25-OHD levels when comparing mean differences between groups who took a vitamin D supplement and those who did not. Those who met their daily requirement through dietary intake tended to have a higher mean 25-OHD versus those who did not meet their dietary requirement and did not take a supplement. However, this relationship was not demonstrated to be statistically significant (Figure 4.12). Those who did not meet their needs via dietary intake, but were on a vitamin D supplement, had a statistically significantly higher mean 25-OHD than those who did not take a supplement (Figure 4.12). Dietary vitamin D intake was inadequate for all patients who were on a supplementary form of vitamin D therefore an accumulative association of dietary and supplementary vitamin D on serum vitamin D could not be tested in this cohort (N=0).
4.5.1 Correlates of Serum Vitamin D

Both serum Alb and vitamin D supplementation positively related to serum 25-OHD (Table 4.9), while dietary intake of vitamin D and sex did not relate to 25-OHD levels. No other values had a statistically significant relationship with serum vitamin D.

Although the supplemented group did not meet sufficient cut off levels of >75 nmol.L\(^{-1}\) for vitamin D (mean 54.6 nmol.L\(^{-1}\) ± 23.3), those who took a vitamin D supplement had higher serum levels than those who did not (p-value=0.004) (Figure 4.14). Serum Alb level was also plotted against 25-OHD levels since it correlated with vitamin D status and was shown to directly relate with serum vitamin D level (Figure 4.13).

![Figure 4.13: Comparison of Serum 25-OHD in Vitamin D Supplemented and Not Supplemented](image)

Mean serum vitamin D level (nmol.L\(^{-1}\)) differences between supplemented and not supplemented groups inadequate NS: No Supplementation, S: Supplementation *p-value=0.004

<table>
<thead>
<tr>
<th>Table 4.9: Correlates of 25-OHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlates</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Vitamin D Supplement</td>
</tr>
<tr>
<td>Serum Albumin</td>
</tr>
</tbody>
</table>
Figure 4.14: Relationship of Serum Albumin Level and Serum 25-OHD level
Mean serum vitamin D level (nmol.L⁻¹) and serum albumin (mg.L⁻¹) were related (r=0.315, p-value =0.005).

4.5.2 Multiple Regression Analysis: Predictors of Serum Vitamin D

Serum 25-OHD was used as a dependent factor and tested in a stepwise-forward multiple regression against eighteen predictive variables (see Figure 4.15 for initial candidate variables). Four factors jointly predicted serum vitamin D status. They included vitamin D supplementation (p-value=0.003), dietary vitamin D intake (p-value=0.001), serum Alb (p-value=0.004) and male sex (0.005).

The regression formula to predict serum 25-OHD by the independent variables was as following:

\[
25\text{-OHD (nmol.L}^{-1}\text{)} = -19.96 + (5.32 (VTD-D)) - (15.87(VITD-S) (\mu g)) - (13.61 (sex)) + (2.14 \text{(sAlb) (mg.L}^{-1}\text{)}, \text{Where Sex: 0 = Male, 1 = Female and VITD-S: 0 = No Supplementation, 1 = Supplementation (adjusted } R^2=0.27, \text{ p-value}<0.001)
\]
Figure 4.15: Schematic Overview of Study Findings

Independent Variables
Tested for MF
1. Age
2. Sex
3. DM
4. HD-vintage
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. Dietary Energy
13. Dietary Protein
14. Dietary Vitamin D
15. MIS
16. Habitual Physical Activity
17. Supplemental D
18. Calcitriol

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for 25-OHD
1. Age
2. Sex
3. BM
4. Alb
5. Ca
6. PO$_4$
7. FGF-23
8. PTH
9. Dietary Energy
10. Dietary Protein
11. Dietary Vitamin D
12. Dietary Calcium
13. Dietary Phosphate
14. Supplemental Vitamin D
15. MIS
16. MF
17. PA

Independent Variables Tested for PA
1. Age
2. Sex
3. DM
4. HDV
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. HGB
13. Dietary Energy
14. Dietary Protein
15. Dietary Vitamin D
16. MIS
17. MF

Independent Variables
Tested for PA
1. Age
2. Sex
3. DM
4. HDV
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. HGB
13. Dietary Energy
14. Dietary Protein
15. Dietary Vitamin D
16. MIS
17. MF

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for MF
1. Age
2. Sex
3. DM
4. HD-vintage
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. Dietary Energy
13. Dietary Protein
14. Dietary Vitamin D
15. MIS
16. Habitual Physical Activity
17. Supplemental D
18. Calcitriol

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for 25-OHD
1. Age
2. Sex
3. BM
4. Alb
5. Ca
6. PO$_4$
7. FGF-23
8. PTH
9. Dietary Energy
10. Dietary Protein
11. Dietary Vitamin D
12. Dietary Calcium
13. Dietary Phosphate
14. Supplemental Vitamin D
15. MIS
16. MF
17. PA

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for PA
1. Age
2. Sex
3. DM
4. HDV
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. HGB
13. Dietary Energy
14. Dietary Protein
15. Dietary Vitamin D
16. MIS
17. MF

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for MF
1. Age
2. Sex
3. DM
4. HD-vintage
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. Dietary Energy
13. Dietary Protein
14. Dietary Vitamin D
15. MIS
16. Habitual Physical Activity
17. Supplemental D
18. Calcitriol

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for 25-OHD
1. Age
2. Sex
3. BM
4. Alb
5. Ca
6. PO$_4$
7. FGF-23
8. PTH
9. Dietary Energy
10. Dietary Protein
11. Dietary Vitamin D
12. Dietary Calcium
13. Dietary Phosphate
14. Supplemental Vitamin D
15. MIS
16. MF
17. PA

Legend:
Bold Variables indicate individual correlation
Underlined Variables indicate partial correlation

Independent Variables
Tested for PA
1. Age
2. Sex
3. DM
4. HDV
5. BM
6. Alb
7. Ca
8. PO$_4$
9. FGF-23
10. PTH
11. 25-OHD
12. HGB
13. Dietary Energy
14. Dietary Protein
15. Dietary Vitamin D
16. MIS
17. MF
Chapter 5
Discussion and Conclusion

5 Discussion

In this study, factors related to muscle strength, (a surrogate for MF), such as vitamin D, PA and other measures in patients’ standard clinical work up were investigated in stable HD patients. There were three key findings of this study. First, MF was partly correlated with both habitual PA and serum 25-OHD. MF was correlated with habitual PA, after controlling for BM, suggesting a relationship between decreased PA and muscle dysfunction in the HD population. Secondly, those with higher 25-OHD levels had stronger muscles, once the confounders of sex, age and BM were controlled and vitamin D supplementation was correlated with greater MF and serum 25-OHD level. Those who were supplemented with vitamin D had stronger handgrip strength, regardless of age, sex, or BM. Lastly, despite Vitamin D supplementation in 24% of the participants, the mean serum level for those supplemented remained within the insufficient range of this vitamin, although mean serum values were statistically higher than those not on any supplementation. Together these findings suggest links between poor MF, lower PA and vitamin D insufficiency in HD patients. These findings are clinically relevant for HD patients, since the potentially modifiable factors demonstrated in this thesis are currently not routinely addressed in hemodialysis centers and may need to be reconsidered.
5.1 Primary Findings: Muscle Function and its Key Correlates

5.1.1 Muscle Function and Vitamin D

In the current study, both muscle function (MF) and 25-OHD were markedly reduced in HD patients compared to the general population in Canada. Results of the Canadian Health Measures Survey (CHMS) conducted in 2007 through 2009 revealed a mean serum concentration of 25-OHD equal to 67.7 ± 2.4 nmol.L⁻¹ for the general population (optimal levels are >75 nmol.L⁻¹) [41]. Mean serum 25-OHD concentrations in the current study were 42.4 ± 23.1 nmol.L⁻¹, thus only 63% of population norms in Canada and 43% less than sufficient levels of 75 nmol.L⁻¹. The current observation of greater grip strength in male compared to female HD patients has previously been demonstrated [32] and was similar to that reported for the general population [12, 136-137]. Muscle strength of 63% of the males and 75% females in this study was considered suboptimal compared to only 30% in males and 33% in females of the Canadian population aged 50-59 based on the Canada Health Measure Survey [163]. Therefore there was an average of 33-45 % higher prevalence of poor muscle function in this study in comparison to the CHMS study (suboptimal reference for handgrip strength measure of two hands combined were <75kg for males and <44kg for females) [163].

Skeletal muscle dysfunction is widely prevalent in HD patients, and has debilitating effects on quality of life and physical function [29]. New discoveries in the past decade have revealed roles of vitamin D beyond bone-mineral metabolism and VDRs have been identified on many cells, including skeletal muscle cells which indicate the potential of paracrine and autocrine functions in muscle tissue [40, 42, 62]. Specifically, the role of vitamin D in cellular metabolism, gene transcription and phenotype expression of fibre types has been identified in
skeletal muscle [62, 68, 70]. In some studies individuals with vitamin D deficiency have been shown to regain lost MF (in forms of better strength, scoring higher physical functional tests, improved balance and reduced incidences of falls) [39, 73]. In a randomized double-blinded, controlled study of institutionalized elderly who were supplemented with vitamin D, lower extremity muscle strength and balance were improved after six months in the supplemented group (hip flexors by 16.4%, \( p = 0.0001 \) and knee extensors by 24.6%, \( p = 0.0007 \)) independent of PA and exercise training [39]. However, these relationships are not well demonstrated when examined in skeletal muscle of uremic patients [80, 188] although one study of 25 HD patients showed a positive correlation of serum 25-OHD levels and better quadriceps muscle strength, better Berg Balance Scale scores, and lower fall risk assessments [73]. It is also important to note that Boudville and colleagues [73] found no relationship between muscle strength, balance scales or fall risks with serum 1,25-(OH)\(_2\)D [73], since analogs of this compound are most often the medicine of choice when prescribing vitamin D for HD patients. The current study showed both serum 25-OHD and MF were positively correlated with vitamin D supplementation in 81 HD patients, while calcitriol analog intake did not relate to MF. These findings suggest that monitoring 25-OHD level and treating vitamin D deficiency are important in this population, and that 25-OHD may serve different functions than circulating 1,25-(OH)\(_2\)D. It has also been suggested, in this study and in others involving dialysis patients [80], that vitamin D deficiency may contribute to MF deterioration. Taskapan and colleagues [80] demonstrated that treating vitamin D deficiency with supplementation, resulted in serum 25-OHD values within the sufficient range, alleviation of some muscle dysfunction in Peritoneal Dialysis (PD) patients such that they took a significantly shorter time to complete the Timed Up and Go test, gait velocity test, the timed chair stand test and stair climb test [80].
Despite prevalence of suboptimal serum vitamin D levels in the majority of dialysis patients in this study (only 10% of participants had sufficient serum 25-OHD levels), vitamin D supplementation and 25-OHD level monitoring were not common practice for this group as shown with others [7, 44]. Others have shown that vitamin D supplementation might facilitate a hypertrophic effect in skeletal muscles of dialysis patients [188], but these studies have used calcitriol analogs, such as 1-alpha. These analogs might have similar effects as the endocrine 1,25-(OH)₂-D, but might not share a common mechanism of action with its precursor, 25-OHD. Since 25-OHD is the predominant form of vitamin D in serum and linked to a plethora of vital functions (including maintaining skeletal muscle integrity and function through several mechanisms [62, 66, 68, 70, 207-210]), it requires prudent monitoring in a population that is highly susceptible to its deficiency. The findings of this thesis on nutritional vitamin D (serum, diet and supplemental form) being related to MF (Tables 4.3 and 4.9) but no association between calcitriol intake and MF (Pearson correlation p-value= 0.97 and r-value=0.0051). This further suggests importance of addressing the nutritional needs of vitamin D in the HD population beyond calcitriol treatment. Optimizing MF by correcting serum vitamin D levels might prove to be an important intervention, in addition and perhaps separate from its metabolite, calcitriol. Improving vitamin D status may in turn also improve performance of activities of daily living and habitual PA through increases in muscular strength.

5.1.2 Muscle Function and Physical Activity

MF and habitual PA intensity were shown to be partially correlated in this study, once BM was controlled for (p-value =0.023, r-value=0.411). Sex also had a strong correlation with MF, and this was proportional to the differences in BM between the sexes which was also significant (Table 4.1). This is likely due to the fact that those with higher BM and of the male
sex are likely to have higher muscle mass that in turn would contribute to a stronger handgrip [136-137, 211]. Mean handgrip strength was 27 ±11 kg (compared to 74 kg for age 40-59 in the CHMS) and mean count for PA was 119±76 MC.min⁻¹ (compared to 305 MCmin⁻¹ for the 50-59 years age group in the NHANES), demonstrating this group’s muscle strength was only 36% of the age-matched population and their daily PA intensity reached only 40% of the age-matched population without kidney disease [93, 163], Even though MF and PA were below normal values for an age-matched healthy population those subjects with better MF had a higher intensity of daily PA once controlled for BM (p-value = 0.023). This relationship was likely due in part to a similar positive feedback loop of MF and PA described in sarcopenia, whereby a decline in the intensity of daily PA contributes to further muscle deterioration which in turn may pose a challenge to accumulating more intense PA throughout the day [212]. Sarcopenia, or the age-related lower relative muscle mass, is a common contributor of declining physical function and disability in the elderly. Sarcopenia and uremic muscle dysfunction share some phenotypes [146-147, 213]. Age was the strongest predictor of PA and therefore aging muscle in addition to uremic muscle may further debilitate physical movement in older compared to younger patients on dialysis. Johansen and colleagues found similar results where activity count was lower for HD patients compared to sedentary controls, and the gap widened between the groups with increasing age [28].

Declining lean BM, including muscle mass, with increasing age after 40 has been shown to be common in the general population [143, 186]. Sarcopenia is estimated to affect 5-13% of those aged 60-70 years and this increases up to 40% in those 80 years of age and above [212]. Some of the underlying mechanisms in sarcopenia include denervation of motor units, decrease in capillary density, increase in intramuscular fat [186] and a net conversion of fast type II muscle fibres into slow type I muscle fibres with resulting overall muscle power loss [186].
Uremic muscle dysfunction is comparable in many ways to sarcopenia and includes fibre type changes and neuromuscular myopathy [146]. Disuse atrophy is another contributing factor in sarcopenic and uremic populations, and is indicated by the low habitual PA [30, 213-214]. Indeed, PA (i.e., the daily intensity of PA) was lower in this study in comparison to age-matched peers [28]. It is therefore possible that uremic muscle dysfunction may be accelerated in older HD patients in this study, and may have limited higher intensity of PA to be attained throughout the day.

The factors identified in the current study further contribute to the affirmation of poor MF and low PA that might relate to poor physical function, even in a relatively healthy and stable population of HD patients. Moreover, it is important to note that this relatively large cohort of HD patients was made of a heterogeneous group of adults in terms of age, sex and cultural background, therefore reducing likelihood of confounders such as all subjects being of old age, that might have been present in a more homogenous sample of HD patients. The positive correlation of vitamin D with muscle strength (through serum as well as dietary supplementation) was another unique feature of this study that to my knowledge has not been reported in HD patients. These primary findings point to possibilities of modifying influential factors, such as exercise/PA and vitamin D supplementation in order to augment muscle strength and contribute to enhanced physical function in the HD group.
5.2 Secondary Findings: Predictors of Physical Activity and Vitamin D

5.2.1 Predictors of Physical Activity

PA presented as mean count of accelerometry per minute over the duration of study, was predicted by age and presence of diabetes mellitus (DM). Group daily minutes-by-minute mean counts of DM patients (91±60 MC.min\(^{-1}\)) were significantly reduced in comparison to non-diabetic patients (137±80 MC.min\(^{-1}\)) (p-value= 0.028). A reduction in the intensity of daily PA might have been due to additive degenerative neuromuscular effects of DM, such as diabetic neuropathy and diabetic microvascular disease [215-218]. DM has been shown in other studies to reduce blood supply to the working muscle, resulting in lower fatigability tolerance, and reducing PA [219-220]. Another factor that was not controlled for but may be suspected to have had an effect is that those who had diabetes were less willing to be active since there is an overabundance of evidence supporting those with lower habitual PA levels at a higher risk of developing DM [94-95, 99, 221-222]. Other contributing factors that may have reduced PA and physical function in diabetics include subclinical left ventricular dysfunction, reduced oxidative capacity and skeletal muscle mitochondrial damage [223-225], and might have played a role in the lower daily PA intensity of diabetics in this study compared to their non-diabetic counterparts (Figure 4.7).

An interesting observation in this study was how sex was not associated with PA levels. Others who have quantified PA in the HD population have made similar observation [27-28, 30]. In the general population there is a distinct difference in PA levels between the sexes throughout all life stages with men consistently being more active than women [93, 98]. Similarly, women
are consistently less active in the chronically ill community dwelling adults [226-227], but these
differences are not evident in HD patients [28, 31]. In this study as in other HD studies [28, 31],
there were no statistically significant differences in PA levels between the sexes. Despite there
being a correlation of both sex and BM with muscle strength, sex differences did not carry over
to correlate with PA levels. Although average PA (as measured by mean count per minute over
wear-time) was higher in men compared to women, these differences were not statistically
significant (males 130 MC.min\(^{-1}\) vs females 105 MC.min\(^{-1}\)). One possible reason for this
observation might be the to reduced statistical power caused by the low number of female
participants as compared to males in this study (30 females vs 51 males). Another more likely
speculation however could be the fact that since the overall PA level counts were very low
compared to healthy sedentary population, the sex differences become trivial in this group.

PA patterns were also studied to estimate amount of time spent in different PA
intensities. Overall, the subjects spent the majority of their time in sedentary intensity (77% or
1080 minutes.day\(^{-1}\)). This finding revealed the current subjects spent double the amount of time
in sedentary behaviour compared to other age matched Canadians who spend an average of 580
minutes per day being sedentary [93]. The PA values reported here are higher than those reported
in other HD studies [28]. This study was unique as it described PA patterns of a large cohort of
stable Canadian HD patients population using the gold standard method for quantifying PA
measures. Accelerometers can easily and objectively estimate PA levels that can be compared to
in reference with national and international PA levels to reveal relative differences and
similarities in this HD group compared to others. For example, Kutsuna and colleagues have
suggested a minimum of 50 minutes of engagement in PA per day to prevent deterioration of
walking ability in this population [15]. The subjects of this study spent 19% of their time (274
minutes per day) in light activity, similar to the general Canadian population (250 minutes per
day). They also spent an average of 3% of their days (43 minutes per day) in lifestyle-moderate activity (equivalent to walking at a slow and steady speed) which is just below the cut offs recommended by Kutsuna et al [15]. Further, the patients of the current study spent less than 1% of their time (~ 8 minutes per day) in moderate and negligible (less than 0.03%, and zero for most) time in vigorous and very vigorous intensity PA. The findings of this study might shed light on low habitual PA levels of HD patients being one of the speculated causes of the accelerated rate of functional decline in the HD population in comparison to their age-matched sedentary counterparts. Kosmadakis et al have suggested a training regime similar to ones for the general population [92]. However given the severe inactive state of most dialysis patients, the authors recommend initiating exercise at a lower intensity level in ten minute increments fewer than five days a week. They suggest to gradually increase intensity and frequency, as tolerated, to meet recommendations of 30 minutes of moderate-high intensity of activity on most days a week [159] to maximize health benefits in this group.

The negative health effects of sedentary behaviour have been well documented in the general population [92] and dialysis patients [15, 31, 96] and have been reviewed in this thesis (section 2.3). Independent associations observed between health outcomes and sedentary behaviour in addition to those seen with a lack of overall PA involvement at moderate-vigorous intensity PA levels have been documented [228-234]. In this patient population, reducing sedentary time might be just as important if not more important, than increasing actual exercise. The HD patients are more physically deconditioned in comparison to sedentary healthy counterparts [28] and most other chronically ill groups [97] and may not be able to attain recommended moderate-vigorous intensity PA. Even in the general population, only less than 5% of the population has been shown to reach the moderate-vigorous intensity PA in 10-minutes bouts [93, 98]. Therefore it may be more realistic to set goals of decreasing inactivity in this
group, even if those activities are going to be low in intensity. For example, in-center dialysis time itself (~12 hours weekly on a reclining chair), contributes to overall sedentary time of HD patients. Clinical trials using stationary bikes and other forms of exercise interventions during dialysis have shown an overabundance of qualitative and quantitative health benefits for these patients [103, 110, 112-115]. Standardizing the use of exercise intervention during HD might be of great benefit to HD patients and should be explored further in clinical settings.

There was a significant difference in the amount of time for three out of the four PA intensity categories accounted for on dialysis versus non-dialysis days (Figure 4.6). On dialysis days subjects scored a higher percent of their time in sedentary time (79% versus 76%). They also spent a lower percentage of time on dialysis days in light intensity counts (18% versus 20%) and a lower percent of their time in lifestyle intensity (3.0% versus 3.4%). There were no statistically significant differences in percent of time spent in moderate intensity on dialysis versus non-dialysis days (0.4% versus 0.6%). Therefore, the patients of this study were slightly more active on non-dialysis days (spent 24% of their time performing any intensity of PA higher than sedentary), compared to dialysis days (spent 21% of their time in any intensity of PA higher than sedentary). Information on habitual PA level differences between dialysis versus non-dialysis days using accelerometers has only been reported only by one other group [130]. Majchuzak and colleagues used triaxial accelerometers in 20 stable HD patients and measured PA during waking hours for seven days [130]. They showed dialysis versus non-dialysis total activity counts were less active on dialysis days versus non-dialysis days [130], similar to higher the present study. Both studies revealed that HD population overall has a much higher than average time of inactivity on all days even in comparison to other sedentary but healthy groups [93, 98]. Majchuzak and colleagues only reported on total acceleromety count, and energy expenditure rather than activity intensity [130]. In the current study, the results were analyzed
further to indicate intensity levels that might be more meaningful in terms of health benefits of PA and comparable to other national reports [93, 98]. The lower intensity PA levels were roughly comparable to lower energy expenditure estimated by the Majchuzak group on dialysis versus non dialysis days [130], indicating dialysis treatment itself maybe a barrier to achieving more intense PA in the HD population. Furthermore, the current study measured 24-hour activity levels, compared to only waking hours and used an activity log to compare “true” wear-time compared to the study previously mentioned where this was a limitation [130]. Lastly the current study was in a larger cohort of stable HD patients (81 versus 20), reducing chances of type two statistical errors.

5.2.2 Predictors of Vitamin D

Serum 25-OHD levels in this study were predicted by dietary vitamin D, intake of a vitamin D supplement, serum Alb, and sex. Serum mean levels were found to be insufficient for the group, confirming findings of others who have shown prevalence of vitamin D deficiency in all stages of the CKD [7, 34, 43-44, 46, 165, 235]. Although most of these factors have been reported in other studies, the emphasis has almost exclusively been placed on supplemental vitamin D, while dietary vitamin D has not been assessed in most HD studies [7, 109, 165]. This study used a three day-diet record to quantify and estimate dietary sources of vitamin D consumption in this HD group. This showed group mean vitamin D intake from food to be only 25% of DRI recommendations. Vitamin D intake was associated with higher dietary calcium intake, but not that of phosphate intake. These factors might have clinical implications given the high rates of hypovitaminosis D, combined with the prevalence of hyperphosphatemia in the CKD population and suggests reconsideration of renal dietary guidelines. Generally, the most common source of vitamin D in the Canadian diet is fortified milk [236]. In the dialysis
population however, recommendations of milk are lowered to 25% (from 500 mL.day\(^{-1}\) to 125 mL.day\(^{-1}\)) of that recommended in Canada’s Food Guide to Healthy Eating [237]. The clinical justification is in attempt to limit dietary phosphate intake, as it can become a uremic toxin which is not efficiently removed by the dialysis process [238]. Dietary restriction of phosphate is the first line of therapy in hyperphosphatemia which is common in the dialysis population [238]. A recent analysis of dietary phosphate sources in the American diet by Noori and her colleagues, however have shown phosphate sources in the diet differ in their bioavailability in the gut [239]. The authors indicate that “organic” phosphate naturally occurring in foods in comparison to those “inorganic” phosphates added to processed food have a much lower absorption rate in the gut. The patients in this study had a mean serum phosphate levels within an acceptable clinical range (1.60 ±0.05 mmol.L\(^{-1}\)). Interestingly, the percentage of milk consumption recommended to dialysis patients, exactly matched the percent daily value of dietary vitamin D intake in this study (25%). This low intake of vitamin D was also reflected in the low serum level of vitamin D observed for this group (only 10% of patient had optimal levels) and has been reported in other studies to be low as well [34]. For example Del Valle et al reported only 33% of their subjects in the optimal 25-OHD range [34]. Even in light of vitamin D supplementation (24% were on some form of vitamin D supplement), mean serum levels of HD patients were below clinically acceptable levels, although they were statistically higher than those not on any form of vitamin D supplementation (Figure 4.12). These low levels are associated with high mortality and morbidity in the dialysis and non-dialysis population [165, 240-241] however it continues to not be a part of routine clinical monitoring. Given the findings of this study on vitamin D, calcium and phosphate and MF and along with the knowledge of their important interactions in endocrine pathways that may be disturbed in CKD, there is a need for further dietary and pharmacological
interventional studies that need to address discrepancies in current scientific findings and clinical practice.

5.3 Other Findings and Observations

5.3.1 Serum Calcium

Serum calcium was shown to be inversely correlated to MF in this study (p-value=0.001, r-value=0.396). This was a novel finding of this study. Serum calcium levels were within the acceptable reference range for this study’s patients with mean serum calcium of 2.39+/-0.17 mmol.L^{-1}. Physiologically, calcium ions are involved in multitude of cellular functions in the skeletal muscle tissue [242]. Vitamin D supplementation was positively associated with MF in this study and in other organs and tissue, vitamin D is often linked to calcium metabolism [243], hence vitamin is considered to be a “calcemic” compound. Although there are many studies that suggest a link between vitamin D and skeletal MF [39, 48, 66, 69-70, 73, 78-79, 207], there is limited information about the potential interactions of elevated serum calcium alone on MF. Two studies using animal models have demonstrated vitamin D’s influence on calcium metabolism at a cellular level, one of which was conducted under uremic conditions [67, 208]. In the first study, Matthews et al found that fragmented sarcoplasmic reticulum from skeletal muscle of rabbits with experimentally-induced uremia had defective calcium ion transport with impairment in all parameters, including initial rate of uptake, storing capacity and concentratability [67]. Local administration of 1-25-(OH)₂D (low and high dose) in vivo was found to improve impaired calcium transport by the sarcoplasmic reticulum in these samples [67]. At a low dose, of 1-25-(OH)₂D improved storing capacity, while the higher dose, in addition to improving the storing capacity, also corrected concentratability and initial rate of calcium uptake [67]. From these
results they concluded that active calcium transport in the sarcoplasmic reticulum is impaired by uremia and that this defect is responsive to the administration of 1,25-(OH)_{2}D [67]. In the second study conducted by Giuliani and Boland, both 25-OHD and 1,25(OH)_{2}D at physiological concentrations exerted direct effects on calcium flux in cultured vitamin D-deficient chick soleus muscle and myoblasts [208]. They demonstrated that isotopic desaturation curves of soleus muscle pre-labelled with $^{45}$Calcium, indicated that the action of 25-OHD was localized in a slow-exchangeable calcium pool where it stimulates net Ca uptake [208]. Giuliani and Boland also found that these effects were accompanied by changes in growth and differentiation of the cultures, suggesting a direct and local involvement of 25-OHD and 1,25(OH)_{2}D3 on muscle fibre development and calcium regulation [208], however whether these findings can be applied to human skeletal muscle remains entirely speculative.

These two findings suggest important paracrine and autocrine roles for 25-OHD_{3} and 1,25(OH)_{2}D_{3} in skeletal muscle involving calcium regulation [67, 208]. A theory behind the negative correlation of serum calcium and MF found in the current study, might be involve lower vitamin D levels prevalent in this group. Since uremic conditions seem to impair calcium metabolism of skeletal muscle, higher serum calcium levels, which are often a side effect of calcium-based phosphate binders use in HD patients, might promote an overabundance of calcium surrounding the myocytes. This overabundance might strain the tissue’s existing suboptimal regulation of the ion which is worsened by inadequate vitamin D levels.

5.3.2 Hemoglobin, Malnutrition Inflammation Score, and Albumin

Reduced Hgb and Alb levels, poorer nutritional status, and higher inflammation state were all associated with accumulating less mean counts of PA intensity throughout this study. Lower Hgb levels have been seen of as result of lower PA in aging [244] as well as
hemodynamic deconditioning as a result of hypokinesia in healthy adults [245]. Although lower Hgb levels are expected in the CKD population, the majority of participants in this study received regular erythropoietin injections as well as iron therapy. Despite therapeutic measures to improve anaemia, some continued to have persistently lower Hgb levels than the ideal. Although mean serum Hgb was within an acceptable range for the group (Table 4.2) there was a correlation with lower PA intensity when controlled for BM (Table 4.8). BM and Hgb levels have been shown to be related in aging population [246] and PA and Hgb are interrelated in dialysis patients and other groups [26, 247]. Hgb and exercise capacity are interrelated due to the oxygen-carrying capacity of red blood cells that depend on sufficient Hgb production [248-250]. Low Hgb levels have also been reflected in improved physical functioning where an average 10.5% increase in the Karnofsky functional score after erythropoietin therapy initiation in dialysis patients had been reported [26]. However, no relationship was found between handgrip strength and Hgb in this group. Therefore this study shows Hgb plays a part in PA level of dialysis patients, although this relationship was relatively weak (p-value=0.018, r-value=0.296) and was not deemed significant in the presence of other factors in the linear multiple regression model (p-value=0.08, coefficient=1.15±0.63).

MIS scores indicated that on average the participants in the study were relatively well nourished; with a mean scores of 5 (out of the 30 maximum possible points) scale of malnutrition inflammation score (MIS). However, as indicated by MIS and serum Alb levels, even marginal presence of malnutrition and inflammation seemed to have an association with overall PA intensity. This correlation was not as strong for Alb (p-value=0.049, r=0.243) as it was for the MIS (p-value=0.007, r=−0.332). This finding suggests that MIS might be a better predictor of habitual PA, since it is a more in-depth tool to assess nutritional status than Alb alone. Interestingly, this was contrary to observations in this study with vitamin D status and Alb. In
In this study, Alb correlated with 25-OHD and significantly predicted its serum levels in the multiple regression formula while MIS did not have an association with 25-OHD levels. These differences might be hypothesized to be due to vitamin D’s relationship with protein stores indicated by Alb (that might also indicate levels of vitamin D carrying proteins) versus malnutrition and inflammation that represents systemic poor nutritional status, and or inflammatory state. Alb is a common and diverse protein carrier in the serum and the second most important carrier proteins for 25-OHD [43, 47] therefore it is reasonable to postulate lower serum levels may also indicate lower vitamin D carrying potential.

Inflammation [251] and malnutrition [150, 252] have a catabolic effect on muscle mass [151], can also alter energy expenditure [253-254] and reduce PA [255] in dialysis and other populations [256]. Reduced energy and nitrogen intake through diet (negative nitrogen balance) or increased use by the cells (as in case of inflammation) alter protein stores, particularly through catabolism of skeletal muscle [152, 257]. Jeejeebhoy describes this caloric and nitrogen deficit reduction leading to reduced muscle mass and function, in both human and animal models [152]. However, these features often manifest as reduced limb muscle circumference and reduced muscle contraction and relaxation capabilities, have been demonstrated to be reversible in the case of muscle dysfunction (and to a lesser degree muscle mass) after re-feeding the subjects with required energy and protein to restore nitrogen balance [152]. Furthermore, inflammation may also affect microcirculation in the aging skeletal muscle [258], hence further reducing blood supply needed for active muscle. Thus, even though the overall level of malnutrition and inflammation was relatively low in this study’s stable hemodialysis cohort, low Alb, low Hgb and malnutrition and inflammation, even at low levels, appeared to be correlated with participants’ having less intense PA. These factors further suggest the importance of nutritional
status to both vitamin D status (indirectly through association with Alb) and PA (through association with MIS, Alb and Hgb) in HD patients.

5.3.3 Fibroblast Growth Factor-23

FGF-23 was analyzed in this study because it has been shown to be an important factor in vitamin D activation, is a better predictor of CKD-MBD than serum phosphate or PTH levels, is related to aging phenotypes, and is also an independent predictor of mortality in CKD patients [175-176, 178, 185, 192, 259]. FGF-23 has not been well studied in the HD population. This study’s participants were found to have a wide range of serum FGF-23 levels, from low to extremely high (range 168 to 49,478 RU.L⁻¹). FGF-23 correlated with serum calcium as well as serum phosphate levels, especially when serum phosphate levels were above clinically acceptable guidelines (Appendix V). This is a unique finding, since dialysis patients are generally expected to unanimously have elevated levels of FGF-23 due to reduced renal mass [173]. Elevated FGF-23 levels are thought to be a result of decreased responsiveness of renal-phosphate excretion feedback mechanism (Figure 2.1) and reduced renal mass present in the dialysis population, amongst other possible causes [52, 260]. Serum phosphate level is the primary stimulator of FGF-23 [261], and as confirmed in this study, levels of these two biomarkers were strongly correlated (p-value<0.0001, r-value= 0.55). FGF-23 also correlated with calcium but did not correlate with 25-OHD or PTH (Appendix V) contrary to other findings in CKD patients [259]. FGF-23 is involved in activation of serum vitamin D (Table 2.1 and Figure 2.2) via suppression of 1,25 hydroxylase [259] and combined vitamin D and cinacalcet therapy for secondary hyperparathyroidism have been shown to lower FGF-23 level in stable HD patients [262]. This is an important factor as FGF-23 levels independently predict mortality in HD patients [172, 263]. No relationship was found between 25-OHD or vitamin D
supplementation and FGF-23 in this study and did not relate to other measures of the study including MF, or PA. FGF-23 is a potent regulator of phosphate and vitamin D since it decreases expression of 1,25-hydroxylase and increases expression of 24,25-hydroxylase. [264]. Calcitriol injection has also been shown to increase FGF-23 expression in mice [264]. It might be postulated that calcitriol analog intake might have an influence in the FGF-23 levels in humans, given the mechanism of action (up regulating FGF-23 and down regulating 25-OHD), however no statistically significant relationship were found in the current study between FGF-23, calcitriol prescription, and 25-OHD.

This study contributed to further identifying FGF-23 levels in a stable group of HD patients and as with serum phosphate levels, longitudinal measures, might be more clinically informative than cross-sectional analysis of this important marker. Since FGF-23 requires transmembrane protein klotho to enter target cells via FGF-23 receptor, α-Klotho plays an important role in regulation of calcium and phosphate homeostasis [166]. Klotho gene polymorphism has been also associated with CKD patients’ disease progression and survival [265]. Given the emerging evidence of importance of FGF-23, along with its cofactors in morbidity and mortality of CKD patients, further long term studies of HD patients that would combine analysis of its klotho with FGF-23 may be able to better characterize the wide variation of this important marker in the HD group.

5.4 Limitations

A major limitation of this study was its short duration and cross-sectional nature. These factors combined could have potentially led to misrepresenting PA, nutritional intake, or serum measures. Since measures were taken only at one given point of time and were not repeated for
the same participants, it is difficult to assess if the serum levels measured represent usual readings for each patient or an unusual reading (off days or week of eating habits or medication non-compliance etc) compared to their historic blood results patterns. Some measures that had a longer half life (such as Hgb and Alb) were less susceptible to this potential limitation compared to others (serum calcium and phosphate).

5.4.1 Muscle Strength

There was only a one-time assessment and a single indicator of muscle strength used. Handgrip dynamometers are subject to human error in methods and interpretation of maximal effort by the participant [140]. Furthermore, although it is used as an indicator of overall MF, it has been argued that since it only represents function in select upper body muscle groups, it may not be a true representative of overall muscle strength for some participants. An example of this would be in persons with hand joint disorders or hand and arm muscle injury in the hand tested. Participants that voiced these concerns were excluded from the handgrip strength readings, however if the participant did not voice issues with using the device, readings were included in the study. Although eliminating subjects allowed safe use of handgrip dynamometer assessment in this study, screening may have inadvertently resulted in a “healthier” subset of HD patients and decreased study finding’s generalizability. Nevertheless, the substantially lower MF compared to population norms in this subset of HD patients is sobering. Use of other more sophisticated equipment and strength measures for more than one muscle group may provide more global measures of muscle strength while additional tests such as Time Up and Go, 6 minute walk test or Times Stair Climb would have been able to provide additional functional capacity information.
5.4.2 Vitamin D

Serum vitamin D levels are influenced by exposure of bare skin to ultraviolet rays. This factor was not controlled for in this study, although seasonal timing was considered to be representative of relatively lower sun exposures within latitude and all participants’ serum measures were taken within a three-week span. Skin colour may have potentially been another confounder in measuring serum vitamin D levels driven from sun exposure in the sample since participants were of heterogeneous ethnicities. The Canadian Health Measures Survey (CHMS) reported a statistically significant difference in 25-OHD concentrations of white versus non-white participants, with the latter being lower [41]. However, the higher consumption of milk in the white-group compared to other ethnicities confounded this finding [41]. This study did not control for race or skin colour, however milk consumption was low among all reported dietary intake records. Furthermore, in this study, Vitamin D supplementation was recorded as a dichotomous variable, only indicating presence or absence of any vitamin D supplementation. Actual doses and sources of the supplement varied within the group which may have had a direct impact on quantifying serum levels and some differences observed in the outliers (for example 25-OHD of one subject was 128nmol.L⁻¹ since this subject was on three types of vitamin D supplements).

5.4.3 Physical Activity

The accelerometer data indicated a very low group mean count (MC.min⁻¹) and this was likely due to the large range (38-404 MC.min⁻¹) reported. There are two other possible limitations with this low count, firstly, subjects were asked to wear the monitors continually, therefore capturing sleep-time as well as waking hours. This method was chosen over the more commonly used method of “wake-hours only” wear-time to increase compliance of device wear and to normalize differences in waking hours between the three different dialysis shifts.
Secondly, activity logs were used to validate MC.min\(^{-1}\) against a secondary measure describing activities. Subjects were asked to record device on/off time throughout the study and these records were used to manually override devices non-wear time detection in case of discrepancy. Those subjects with extremely low counts (such as those below 90’s MC.min\(^{-1}\)), may have had periods of time when they simply had taken the device off and forgot to write this information down in the log therefore falsely lowering their overall count.

5.4.4 Outliers

Another limitation was presence of outliers in some key measures. Mean serum values for most biomarkers were within clinical expectations for the group, however there were a few outliers in serum vitamin D (range 10 -128 nmol.L\(^{-1}\)) and FGF-23 (168-49 476 RU.mL\(^{-1}\)) or PA (38-404 MC.min\(^{-1}\)) that may have influenced the results and analysis of the study outcomes. These values likely statistically weakened indicated relationships bivariate correlations, although when used in linear multiple regression model building, the values were automatically trimmed and replaced by statistical software to reduce influence on accuracy of the model.

5.4.5 Bias and Selection

Since participants were not blinded to the study outcome measures of food intake and PA, they may have intentionally underreported intake and attempted to increase PA for more desirable accelerometer data. Although the diet analysis suggested under-reporting, PA data (i.e., daily mean counts; counts.min\(^{-1}\)) were not above those reported in other stable HD patients. Furthermore, dietary intake of energy did not correlate with PA level or energy expenditure estimated by accelerometry in this study, indicating that dietary analysis did not reflect actual energy intake by the subjects. Protein stores, MIS and MF did not relate to protein and energy intake in the 3DDMR. As indicated by nPCR, mean Alb level, mean BMI and MIS, the
participants were likely in protein-energy metabolism equilibrium when compared to clinical standards [151]. These results allude to possible underreporting in the three-day diet analysis. Mahabi and colleagues estimated their underreporting to be in the range of 30-50% less than that of actual energy consumption from foods ingested [266]. Therefore, the actual group mean energy intake might have been closer to 1900-2200 kcal.day\(^{-1}\) which is closer to target recommendations for the group.

Furthermore, since subjects were recruited from two satellite outpatient HD units (generally more stable than in-centre dialysis patients) and several exclusion criteria were used in the screening and recruitment process, the cohort may represent a healthier and higher functioning group compared to other studies conducted in-center or in-patient dialysis units. Although these factors may have limited the generalizability of this study’s findings to other HD groups, low MF, vitamin D levels, and low PA levels observed were remarkable. These findings can further contribute to understanding of known deconditioning in this group, even in a relatively healthy and stable cohort.

5.4.6 Statistical Power

The number of variables initially entered as potential predictors of 25-OHD, MF, and PA were relatively large compared to sample size and likely reduced statistical power. The ideal N for each variable tested has been suggested to be six subjects [267] requiring a sample of approximately 92-102 participants. Since the sample size for this study was only 81, this potentially increased the chances of type II statistical errors occurring, where weaker relationships were not detectable in the model in presence of stronger independent variables.

Post-hoc power calculations for the three models used were:
1. **Muscle Strength as Dependent Factor, Tested with 18 Independent Variables**
   
   Model $R^2 = 0.25$, Observed statistical power = 0.83994493

2. **Physical Activity as Dependent Factor, Tested with 17 Independent Variables**

   Model $R^2 = 0.25$, Observed statistical power = 0.85464053

3. **Serum Vitamin D as Dependent Factor, Tested with 17 Independent Variables**

   Model $R^2 = 0.25$, Observed statistical power = 0.85464053

   As demonstrated in the power calculations above, each model’s statistical power is greater than 80%, translating to detecting four out of five relationships that may have existed within each model between the dependent and independent variables. This level of power is considered to be sufficient to reject the null hypothesis according to Fox and Maters [267] when p-values are equal or lesser than 0.05.
5.5 Conclusion

This study confirmed that decreased MF, a lower daily intensity of PA and lower vitamin D status are prevalent in stable HD patients. Two major findings of this study were association of poor MF with reduced PA intensity and low vitamin D levels (serum and supplemental). In addition, high serum calcium levels were shown to negatively correlated MF in these subjects which is a novel finding in patients with renal disease. These findings linking modifiable factors to poor MF can be further investigated in intervention studies, and have the potential to improve MF in stable HD patients through correction of serum 25-OHD and increasing activity.

The null hypotheses of this thesis were rejected. I hypothesized that MF would be associated with PA in HD patients and this was demonstrated to be true when controlled for BM. I also hypothesized that MF would be associated with 25-OHD levels and this was also demonstrated once controlled for known confounders of age, sex and BM.

Muscle dysfunction has debilitating effects on physical function and quality of life, and it is associated with increased mortality. Findings of this study may justify a randomized controlled trial testing MF of three HD groups; 1) vitamin D supplemented group, 2) increased PA (perhaps by use of accelerometers or pedometers) group, and, 3) vitamin D supplemented and increased PA group against an age-sex-matched HD controlled group. If a contributory relationship between MF and PA as well as vitamin D supplementation were demonstrated in a future randomized controlled trial, the findings of that study could potentially contribute to improvement in wellbeing of HD patients.
References


Appendices
Appendix I-A: Ethics Approval- University of Toronto

UNIVERSITY OF TORONTO

PROTOCOL REFERENCE # 20945

September 26, 2011

Dr. Catherine Amara
FACULTY OF PHYSICAL EDUCATION AND HEALTH

Ms. Sara Mahdavi
FACULTY OF PHYSICAL EDUCATION AND HEALTH

Dear Dr. Amara and Ms. Sara Mahdavi,

Re: Administrative Approval of your research protocol entitled, "Nutritional Status, Muscle Function and Physical Activity in Stable Hemodialysis Patients: Is there a link?"

We are writing to advise you that the Office of Research Ethics (ORE) has granted administrative approval to the above-named research protocol. The level of approval is based on the following role(s) of the University of Toronto (University), as you have identified with your submission and administered under the terms and conditions of the affiliation agreement between the University and the associated TAHSN hospital:

- Graduate Student research - hospital-based only
- Storage or analysis of De-identified Personal Information (data)

This approval does not substitute for ethics approval, which has been obtained from your hospital Research Ethics Board (REB). Please note that you do not need to submit Annual Renewals. Study Completion Reports or Amendments to the ORE unless the involvement of the University changes so that ethics review is required. Please contact the ORE to determine whether a particular change to the University’s involvement requires ethics review.

Best wishes for the successful completion of your research.

Yours sincerely,

Daniel Sowom
REB Manager

OFFICE OF RESEARCH ETHICS
McMaster Building, 11 Queen's Park Crescent West, 2nd Floor, Toronto, ON M5S 3G8 Canada
Tel: 1 416 646-5727 Fax: 1 416 646-5765 ethics.research@utoronto.ca [http://www.research.utoronto.ca for research administrators/ethics]
To: Sara Mahdavi/ Dr. Tabo Sikaneta
From: The Scarborough Hospital Research Ethics Board (REB)
Date: September 19, 2011
Re: Nutritional Status, Muscle Function, and Daily Habitual Physical Activity in Stable Hemodialysis Patients. Is there a Link?
Study No: NEPH-49

This is to acknowledge that the full Research Ethics Board has approved

Protocol: Nutritional Status, Muscle Function, and Daily Habitual Physical Activity in Stable Hemodialysis Patients. Is there a Link?

Protocol Version Dated: Received by Research Department August 4, 2011

Adult Information & Consent Form Version Dated: Received by Research Department August 4, 2011

Health Canada No Objection Letter Dated: N/A

Reviewed By The Research Ethics Board On: September 19, 2011

Effective From: September 19, 2011 to September 18, 2012

The Research Ethics Board of the Scarborough Hospital agrees with the principles for ethical research found in the Tri-Council Policy Statement: Ethical Conduct For Research Involving Humans, the Declaration of Helsinki, the ICH Guideline for Good Clinical Practice, and the Code of Federal Regulations: Title 45, Part 46. The Research Ethics Board of the Scarborough Hospital adheres to the regulations found within these documents, as appropriate.

Should this study continue beyond the expiry date noted above, you must submit a request for re-approval prior to this date. Please advise the REB annually on the progress of your research. During the course of the research, any significant deviations from the protocol or any adverse events should be brought to the attention of the REB. If the study has been completed by this date, a Completion Form should be submitted.

L. Castagna, MD, FRCP (C )
Chairman, Research Ethics Board
The Scarborough Hospital
3050 Lawrence Avenue East
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Date 3.10.2011
Appendix II: Study Time Lines- Flow Diagram of Data Collection
Appendix III-A: Sample 3 Day Diet-Medication & Activity Record Instructions

Study ID:

Diet and Drug Log Instructions

What is the Diet and Drug Log?
This is just a form that you keep with you for a week and write down everything you, drink or take as medication.

Why write down everything I eat and drink?
This will help us study what nutrients you are having and if it is affecting your blood values. The medication and supplements you are taking may also affect these values so we will take a look to see what kinds of effects it have.

When do I need to write information down?
You should keep the log with you at all times so you can write down everything you take right away so you don’t forget. Take the log form with you everywhere you go.

What if I'm not sure about what's in my food when I eat out?
Do your best to ask what ingredients are and write down as much detail as possible. This will help us make the study of your diet more accurate. Take a look at the next page which gives examples of how you can estimate what you are eating.

What if I forget to take my medication?
You can write down that information or just leave the space blank.

Will this information affect the care I am receiving if I write down that I am not following my diet or medication order?
No. You should be honest when writing this information down because it will give us an idea of what is happening with you. This tool is not meant to evaluate your ability to follow your diet or medication but rather a representation of what you normally do. This information, as with any other collected in the study is confidential to study investigators and will not affect the care you receive from your health care providers.

Keep Your Log Book and A pen with you ALL THE TIME!

After one week, we will collect the accelerometer, and the log books. We will call and remind you to bring them to the dialysis unit with you. Once we have analyzed the data, you can ask us to see what your results were like.
Appendix III-A: Sample 3 Day Diet-Medication & Activity Record Instructions (continued)

Accelerometer Study Instructions

What is an accelerometer?

An accelerometer is a device that measures and records movement patterns. The accelerometers we use in project count the number of movements you make every few seconds, and these numbers can tell us how much you were moving (or physical activity levels) during the entire time that you are wearing the accelerometer. This is why it's really important to remember to wear your accelerometer during the week that you will have it. The device weighs and is roughly the same size as a small pager.

Why use accelerometers?

Information from the accelerometers can be downloaded to a computer for analysis. We will use this information to determine overall physical activity levels.

Are accelerometers safe to use?

There is no risk associated with the use of these devices.

What do I have to do with the accelerometer?

1. Wear the accelerometer snugly on the right side of your waist (above the hip) for 6 days.

2. Make sure that you haven't put it on upside down! You will know that it is the right way up if someone standing in front of you can reach the name “ActiGraph” of the device. The arrow on the white label should be facing up.

3. Put it on after you dry up from your shower & take it off when you go to shower or swim. You do not need to take it off when you go to sleep.

4. Do not let the accelerometer get wet. This will destroy the device.

5. COMPLETE YOUR LOGBOOK. If you have to take the accelerometer off for any reason during the day, write IN THE MIDDLE why you took it off and when you put it on again. We have provided space for you to do this two times each day.

Please wear it all the time except when you need to go swimming or when the device could get wet.

If you have questions, give us a call: 416-438-2911 Ext: 6886. We will call you each morning to remind you to wear the accelerometer and keep records.
Appendix III-B: Sample 3 Day Diet-Medication & Activity Record Form

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Day 3 Date: 2011/10/___</th>
<th>Keep this with you all the time.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount (eggs, the pressed)</td>
<td>Food details (e.g., cooking method, fresh, frozen, canned, etc.)</td>
<td>Time &amp; Place (where &amp; where did you eat)?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What kind? (e.g., bottle)</th>
<th>How many?</th>
<th>When and where?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Carbonate or Renu (what strength? 500, 750, or 1000 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium Carbonate + vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium Citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamins (what kind?)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 alpha hydrox</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega 3 oil, Cod liver oil, Fish oil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other**

- **Did this a typical normal day for you?** Yes / No
- **Did you wear your accelerometer today?** Yes / No
- **Did you go to a party today?** Yes / No
- **When did you go to bed last night?** " 

Go to page 3 for the accelerometer log [Page 4]

<table>
<thead>
<tr>
<th>Time accelerometer put ON in the morning</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
</table>

**The 1st Time I took the accelerometer OFF DURING the DAY**


**What did you DO when it was OFF?**

| Activity: | Activity: | Activity: | Activity: | Activity: | Activity: | Activity: |

**The 2nd Time I took the accelerometer OFF DURING the DAY**


**What did you DO when it was OFF?**

| Activity: | Activity: | Activity: | Activity: | Activity: | Activity: | Activity: |

**Time accelerometer put back ON DURING the DAY**


Keep this with you all the time and fill out daily.
## Appendix IV: Malnutrition Inflammation Score Form

### MALNUTRITION INFLAMMATION SCORE (M.I.S.)

**A. Patients' related medical history:**

1. **Change in end dialysis dry weight** (overall change in past 3-6 months):
   - 0: No decrease in dry weight or weight loss < 0.5 kg
   - 1: Minor weight loss (> 0.5 but < 1 kg)
   - 2: Weight loss more than one kg but < 5%
   - 3: Weight loss > 5%

2. **Dietary intake:**
   - 0: Good appetite and no deterioration of the dietary intake pattern
   - 1: Somewhat sub-optimal solid diet intake
   - 2: Moderate overall decrease to full liquid diet
   - 3: Hypo-caloric liquid to starvation

3. **Gastrointestinal (GI) symptoms:**
   - 0: No symptoms with good appetite
   - 1: Mild symptoms, poor appetite or nauseated occasionally
   - 2: Occasional vomiting or moderate GI symptoms
   - 3: Frequent diarrhea or vomiting or severe anorexia

4. **Functional capacity (nutritionally related functional impairment):**
   - 0: Normal to improved functional capacity, feeling fine
   - 1: Occasional difficulty with baseline ambulation, or feeling tired frequently
   - 2: Difficulty with otherwise independent activities (e.g., going to bathroom)
   - 3: Bed/Chair-ridden, or little to no physical activity

5. **Co-morbidity including number of years on Dialysis:**
   - 0: On dialysis less than one year and healthy otherwise
   - 1: Dialyzed for 1-4 years, or mild co-morbidity excluding MCC
   - 2: Dialyzed > 4 years, or moderate co-morbidity including one MCC
   - 3: Any severe, multiple co-morbidity (2 or more MCC)

**B. Physical Exam (according to SGA criteria):**

6. **Decreased fat stores or loss of subcutaneous fat** (below eyes, triceps, biceps, chest):
   - 0: Normal (no change)
   - 1: Mild
   - 2: Moderate
   - 3: Severe

7. **Signs of muscle wasting** (temples, clavicle, scapula, ribs, quadriceps, knee, interosseous):
   - 0: Normal (no change)
   - 1: Mild
   - 2: Moderate
   - 3: Severe

**C. Body mass index:**

8. **Body mass index: BMI = Wt(kg) / Ht^2(m):**
   - 0: BMI > 20 kg/m^2
   - 1: BMI: 18-19.99 kg/m^2
   - 2: BMI: 16-17.99 kg/m^2
   - 3: BMI < 16 kg/m^2

**D. Laboratory Parameters:**

9. **Serum albumin:**
   - 0: Albumin > 4.0 g/dL
   - 1: Albumin: 3.5-3.9 g/dL
   - 2: Albumin: 3.0-3.4 g/dL
   - 3: Albumin: < 3.0 g/dL

10. **Serum TIBC (total Iron Binding Capacity):**
    - 0: TIBC > 250 mg/dL
    - 1: TIBC: 200-249 mg/dL
    - 2: TIBC: 150-199 mg/dL
    - 3: TIBC: < 150 mg/dL

**Total Score = sum of above 10 components (0-30):**
Appendix V-A: FGF-23 versus phosphate
Overall serum phosphate levels positively correlated with FGF-23.

Appendix V-B: FGF-23 versus phosphate in normal range and above normal range (>1.45mmol⁻¹)
Serum phosphate levels below normal range (<1.45mmol⁻¹) did not correlate with FGF-23 while serum phosphate levels above normal range positively and strongly correlated with FGF-23.

FGF-23 = Fibroblast growth factor 23

Appendix V-C: FGF-23 versus calcium
Calcium levels above normal range positively correlated with FGF-23.
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<th>Camg3DAv</th>
<th>VitDug3DAv</th>
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Appendix VII- Serum Analysis Methods

**Serum 25-OHD**

This is a competitive electrochemiluminescent immunoassay (ECLIA) with three incubations. During the first incubation, the sample is incubated with pretreatment reagent 1 and 2 which releases bound 25-OH vitamin D from the binding protein. During the second incubation, pretreated sample is incubated with ruthenium labeled vitamin D binding protein, forming a complex. During the third incubation, streptavidin-coated microparticles and biotinylated 25-OH vitamin D are added. Biotinylated vitamin D competes with vitamin D from the patient sample for binding sites on ruthenylated binding protein. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin. The solid phase of streptavidin-coated microparticles is magnetically held in place at the surface of the electrode in the measuring cell. Application of a voltage to the electrode induces chemiluminescent emission that is inversely proportional to the amount of 25-OH vitamin D in the patient sample. Analytical measuring range was 7.50 – 175 nmol/L.

**FGF-23**

The samples were centrifuged at 1500 × g and 4°C for 10 min, and the supernatants were stored in aliquots at −80°C until further use. FGF23 was measured using the human carboxy-terminal FGF-23 assay. The interassay coefficients of variability of c-terminal FGF23 are 6.5% at 40 RU/ml and 7.5% at 175 RU/ml, respectively, with a lower detection limit of 3.0 RU/ml.

**Albumin**

Colorimetric assay was used with endpoint method; by sample and addition of R1 (buffer). Addition of R2 (substrate) and start of the reaction: At a pH value of 4.1 albumin displays a sufficiently cationic character to be able to bind with bromcresol purple (BPG), an anionic dyestuff, to form a purple complex:

\[
\text{pH 4.1, albumin} + \text{BCG} = \text{albumin BPG complex}
\]

The color intensity of the purple color is directly proportional to the albumin concentration and can be determined photometrically when using Roche Modular P800.

**Calcium**

Colorimetric assay with endpoint determination and sample blank; by sample and addition of R1 (buffer) addition of R2 (chromogen) and start of reaction:

Alkaline solution Calcium + o-cresolphthalein complexone = Calcium-o-cresolphthalein complex

The color intensity of the purple complex formed is directly proportional to the calcium concentration and is measured photometrically using when using Roche Modular P800.
Phosphorus

Endpoint method with sample blanking; by sample and addition of R1 (reagent blank) and addition of R2 (phosphate reagent) at start of reaction: Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula \((\text{NH}_4)_3[\text{PO}_4(\text{MoO}_3)_{12}]\) with ammonium molybdate in the presence of sulfuric acid. The complex is determined photometrically in the ultraviolet region (340 nm) when using Roche Modular P800.

Parathyroid Hormone (PTH)

Sandwich principle (electrochemiluminescence): First incubation: 50 µL of sample, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex. Second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. PTH is run on the Roche Modular E170.

Iron (Fe)

Colorimetric assay: Sample and addition of R1 (buffer/detergent); Addition of R2 (ascorbate/FerroZine) and start of reaction:

\[
\text{Transferrin-Fe-complex} + (\text{Fe}^3+ \text{ ascorbate}) + (\text{Fe}^3+) (\text{Fe}^2+) = \text{FerroZine} + \text{Fe}^2+ \text{ coloured complex.}
\]

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe\(^{3+}\) ions to Fe\(^{2+}\) ions which then react with FerroZine to form a coloured complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically when using Roche Modular P800.

Ferritin

Sandwich principle: First incubation: 10 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complex (Tris(2,2’-bipyridyl)ruthenium(II)-complex (Ru(bpy))) form a sandwich complex. Second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier using Roche Modular P800. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
**Transferrin**

Immunoturbidimetric assay: Anti-transferrin antibodies react with the antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point and increases sensitivity.

**Glycated Hemoglobin (HbA1c)**

This method uses TTAB* as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay. Consequently, in contrast to chromatographic methods, the metabolic state of diabetic patients having hemoglobinopathies or uremia can be determined using this assay. The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Sample and addition of R1 (buffer/antibody): HbA1c in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, complex formation does not take place. Addition of R2 (buffer/polyhapten) and start of reaction: The polyhapten react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically. Hemoglobin Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the preincubation phase of the above immunological reaction using Roche Cobas Integra 400+.

* TTAB = Tetradecyltrimethylammonium bromide

**Glucose**

Ultra Violet test: Sample and addition of R1 (buffer/ATP/NADP); Addition of R2 (HK/G-6-PDH) and start of reaction:

Glucose + ATP $\rightarrow$ HK® $\rightarrow$ G-6-P + ADP

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

G-6-P + NADP+ $\rightarrow$ G-6-PDH $\rightarrow$ gluconate-6-P + NADPH + H+

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and can be measured photometrically.