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PhD thesis

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## **Strength training in patients undergoing dialysis**

2012

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## **Acknowledgements**

For the financial support I thank Nutricia, Danish Kidney Association, The Becket Fund, Danish Society of Nephrology, Hillerød Hospital, Association of Danish Physiotherapists, The Lundbeck Fund, and The fund of Kaptajnløjtnant Harald Jensens and wife. Without these grants it would not have been possible to carry out the PhD project.

Then I would like to thank my three supervisors Adrian P. Harrison, Inge Eidemak and Jesper L. Andersen. They have supported and trusted my work with great enthusiasm and always been ready to suggest relevant solutions to problems. They have also made important contacts to other persons with special skills, who were able to improve the work. Besides very qualified supervision there has been plenty of fun during our meetings.

I thank LIFE, Copenhagen University, for the scholarship and I thank the administrative staff for all the help they gave me during the three years work.

From the department of nephrology on Rigshospitalet and Frederiksberg Hospital I thank Marianne Gulbek for her great work setting up and starting the oral glucose tolerance tests. I also thank the staff of the dialysis centres for their positive attitude and help during the study.

From Hillerød Hospital I thank Katrin Salomo and Malene S. Stage, Forskningsenheden, for their great support. From Department of endocrinology I thank Birger Thorsteinsson, Pernille Banck-Petersen, Tove Larsen and Charlotte Hansen for support and oral glucose tolerance tests. From Department of nephrology I thank Henrik Daugaard, Lotte Prescott and the staff of the dialysis department for the support. A special acknowledgement goes to Ole Hyrd-Hansen for several favours and oral glucose tolerance tests. Helene Meden showed her great expertise by analyzing the patients' energy intake.

During the training periods Louise Norman-Kristiansen, Christian Køppen and Pernille Faurschou assisted as co-trainers. I thank them all for their professionalism and motivational work with the patients.

From Bispebjerg Hospital I thank Michael Kjær many times for obtaining the muscle biopsies. I also thank him for his patience, when the patients once in a while forgot to show up as arranged, and for his very positive attitude toward the patients and also to the study.

Of course I thank the patients, who volunteered for the project. They participated with great enthusiasm and optimism.

Finally, my greatest thanks will go to my children and my wife. My daughter Cornelia kept me motivated by making a beautiful embroidery with the letters PHD and by keeping check on my ECTS points. My son Marius supported me in discussions of strength training and with his great skills in mathematics. Line Maria was as always a great help in discussions of processes during the work and she supported me all the way through.

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## **1. Previous work related to the thesis**

1. Heaf J, **Molsted S**, Eidemak I, Eiken P, Harrison AP, Prescott L, Flatman J.D-vitamin and Muscle Function in Patients with Chronic Kidney Disease. *Nephron* 2010; 115: c244-c250
2. **Molsted S**, Eidemak I, Sorensen HT, Harrison A, Kristensen JH, Andersen JL. Muscle Fibre Distribution and Size in Chronic Hemodialysis Patients *Scandinavian Journal of Urology and Nephrology* 2007; 41: 539-45
3. **Molsted S**, Prescott L, Heaf J, Eidemak I. Assessment and Clinical Aspects of Self-rated Health in Chronic Renal Failure Patients *Nephron* 2007; 106: c24-c33
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5. **Molsted S**, Heaf J, Prescott L, Eidemak I. Reliability Testing of the Danish Version of the Kidney Disease Quality of Life Short Form™ *Scandinavian Journal of Urology and Nephrology* 2005; 39: 498-502
6. **Molsted S**, Eidemak I, Sorensen HT, Kristensen JH. Five Months' Physical Exercise in Hemodialysis Patients: Effects on Aerobic Capacity, Physical Function and Self-rated Health *Nephron* 2004; 96: 76-81
7. **Molsted S**, Aadahl M, Schou L, Eidemak I. Self-rated Health and Employment Status in Hemodialysis Patients *Scandinavian Journal of Urology and Nephrology* 2004; 38: 174-78

## 2. Abbreviations

AA	amino acids
AUC	area under the curve
BG	blood glucose
BMI	body mass index
CSA	cross-sectional area
ESRD	end-stage renal disease
F	fasting
GFR	glomerular filtration rate
HbA1c	glycosylated haemoglobin
HD	haemodialysis
HOMA	homeostatic model assessment
HRQOL	health-related quality of life
IGT	impaired glucose tolerance
IR	insulin resistance
ISI	insulin sensitivity index
MHC	myosin heavy chain
NGT	normal glucose tolerance
NO	non-protein
OGTT	oral glucose tolerance test
P	plasma
PD	peritoneal dialysis
RFD	rate of force development
PR	protein
REPS	repetitions
RM	repetition maximum
sEMG	surface electromyography
SF-36	Short Form 36

### **3. Summary**

Muscle strength, which is reduced in patients undergoing dialysis, is also associated with a loss of muscle mass. One reason for this loss of muscle mass is a disturbed balance between protein synthesis and protein breakdown, which occurs in relation to renal disease and dialysis treatment. Low muscle strength has implications in terms of impaired physical function related to daily living.

Hence the aim of this study was to investigate the effects of strength training in patients undergoing dialysis and furthermore to investigate if potential effects could be improved by protein supplementation in relation to training sessions.

Patients undergoing dialysis at Frederiksberg Hospital, Rigshospitalet and Hillerød Hospital were invited to participate in the study. To be included in the study the patients had to be older than 18 years of age, to have been undergoing dialysis for a minimum of three months and to be able to complete the training programme. Patients were not included if they had severe neuropathy, amputation of a leg, diabetic retinopathy, dementia or were unable to speak Danish.

The patients were tested before and after 16 weeks control period and again after 16 weeks of strength training three times a week. Before the training period the patients were randomly assigned to one of two groups, one ingesting a protein drink the other a non-protein drink in relation to every training session.

The training programme consisted of five minutes of warm-up followed by three high-load exercises; leg press, knee extension and knee flexion.

The outcome measurements were health-related quality of life, physical performance, muscle strength, neuromuscular function, insulin sensitivity and size as well as type composition of muscle fibres.

Forty-one patients were included in the study. During the control period eight patients dropped out and during the training period four patients dropped out. None of the dropouts were related to complications associated with the training programme.

During the control period the measured variables remained unchanged. After the training programme health-related quality of life, physical performance, muscle strength, neuromuscular function and insulin sensitivity were found to be improved. The size of muscle fibre types 1 and 2 were unchanged, but within type 2 fibres the subtype 2X increased. The effects of training were not associated with the source of energy supply.

Strength training improved muscle strength even though only a modest muscle hypertrophy was noticed. The effect on muscle strength may therefore mainly be related to an improved neuromuscular function. The positive effect on insulin sensitivity may be important in the prevention and treatment of type 2 diabetes. The reason for the lack of effects of combining protein intake with training may be due to an insufficient amount of protein in relation to the disease-related chronic protein breakdown.

In conclusion, strength training in patients undergoing dialysis is associated with improvements, which have relevant implications for physical function and a better utilization of insulin.

#### **4. Dansk resume (Danish summary)**

Patienter med kronisk nyresvigt behandlet med dialyse har forringet muskelstyrke, som blandt andet skyldes tab af muskelmasse. En årsag til tabet af muskelmasse er manglende balance mellem proteinsyntese og proteinnedbrydning, som er påvirket af en række sygdomsrelaterede faktorer. Med reduceret muskelmasse og muskelstyrke følger en forringet fysisk funktion relateret til hverdagsaktiviteter.

Formålet med projektet, der ligger til grund for denne afhandling, var at undersøge effekten af styrketræning hos patienter i dialysebehandling og at undersøge om en eventuel effekt blev forøget ved at indtage protein i forbindelse med træningen.

Patienter i dialysebehandling på Frederiksberg Hospital, Rigshospitalet og Hillerød Hospital blev inviteret til at deltage i projektet. Inklusionskriterier var over 18 år i alder, dialysebehandling i minimum tre måneder og en tilstand der gjorde det muligt at følge træningsprogrammet. Eksklusionskriterierne var svær polyneuropati, amputation på en underekstremitet, diabetisk retinopati, demens eller manglende forståelse af dansk sprog.

Patienterne blev testet før og efter 16 ugers kontrolperiode samt før og efter 16 uger med styrketræning tre gange om ugen. Inden træningsperioden blev patienterne randomiseret til indtag af en proteindrik med 9,4 g protein eller en energidrik uden protein i forbindelse med træningen.

Træningsprogrammet bestod af fem minutters opvarmning efterfulgt af tre øvelser med høj belastning: Benpres, knæ-ekstension og knæ-fleksion. Træningsintensiteten blev gradvist øget i træningsperioden.

Før og efter kontrol- og træningsperioden fik patienterne målt selv-vurderet helbred, fysisk funktion, muskelstyrke, neuromuskulær funktion, insulinsensitivitet og størrelse samt fordeling af muskelfibre.

Enogfyrre patienter blev inkluderet i projektet, otte udgik i kontrolperioden og fire udgik i træningsperioden. Ingen patienter udgik på grund af komplikationer relateret til træningen. I kontrolperioden var der ingen ændring af testresultaterne. Efter træningsperioden var der forbedring af patienternes selv-vurderede helbred, fysiske funktion, muskelstyrke, neuromuskulær funktion og insulinsensitivitet. Andelen af type 2X muskelfibre blev reduceret og størrelsen på type 1- og type 2-muskelfibre var uændret. Træningseffekten var ikke afhængig af, om patienterne i forbindelse med træningen fik proteindrik eller drik uden protein.

Styrketræningen forbedrede muskelstyrke på trods af en begrænset muskelhypertrofi. Effekten på muskelstyrke var derfor primært relateret til forbedringer i det neuromuskulære system. Den positive effekt af insulinsensitiviteten er vigtig i forebyggelse og behandling af type 2-diabetes. Årsagen til at der ikke var nogen effekt af at supplere træningen med protein kan skyldes for lille mængde indtaget protein og at den øgede proteinopbygning ikke oversteg den grundlæggende sygdomsbetingede proteinnedbrydning.

Konklusionen på undersøgelsen er, at styrketræning til patienter i dialysebehandling er forbundet med forbedringer, der er relevante for fysiske funktion og for bedre udnyttelse af insulin.

## **5. Introduction**

During the last decade the number of patients with end-stage renal disease (ESRD) undergoing active renal replacement therapy in Denmark has increased by 38% from 3406 in 2000 to 4708 in 2010 (Dansk Nefrologisk Selskab 2011). The underlying reason for ESRD is most often unknown (26%). The predominant individual disease leading to ESRD however is type 2 diabetes followed by hypertensive/vascular nephropathy, glomerulonephritis and others (Dansk Nefrologisk Selskab 2011). The patients are treated with maintenance dialysis or kidney transplantation. Due to a lack of organs for transplantation and the patients' impaired health status the majority of the patients do not receive an organ transplant and therefore undergo chronic dialysis. In 2010, a total of 2011 patients were treated with haemodialysis (HD) and 544 with peritoneal dialysis (PD) in Denmark.

Patients undergoing HD or PD are suffering from a long list of comorbidities and experience limitations in their daily life due to the chronic disease and the ongoing treatment (Molsted et al. 2007). Some of the limitations are impaired physical performance, dependence on medical staff, reduced ability to travel, restriction of fluid intake as well as limitations of the type of food consumed so as to be in accordance with a diet containing reduced phosphate and potassium. The patients' health-related quality of life (HRQOL) is significantly impaired compared to the general population (Molsted et al. 2004a). Furthermore, the dialysis treatment as well as treatment of comorbidities is expensive, putting a pressure on the health care system (Sundhedsstyrelsen 2006). In addition to that, patients undergoing dialysis have a high mortality rate of 23.0 per 100 person years and 15.5 per 100 person years for HD and PD, respectively (Dansk Nefrologisk Selskab 2011).

If this was not enough, these patients suffer from fatigue, which together with reductions in mental health (Molsted et al. 2004a) may further reduce the motivation to be physically active. Therefore it is not surprising that low levels of physical activity among these patients have been documented (Brodin et al. 2001, Johansen et al. 2000). The low levels of physical activity in these patients may lead to a downward spiral with a further reduction of physical capacity. It is therefore of great importance to develop and improve training programmes suitable in the treatment of patients undergoing dialysis.

## **6. Background**

The primary task of skeletal muscle is to generate force and ultimately facilitate movement of the body. In addition, the skeletal muscles have important metabolic and storage functions and may therefore be regarded as an organ. Skeletal muscle is highly influenced by genetic markers, but it is also a dynamic tissue in which both size and structure are very adaptable to usage (Kraemer et al. 1995, Putman et al. 2004) and nutritional status (Paddon-Jones et al. 2006b). Many pathological stages are associated with muscular changes affecting muscle size and structure, and ultimately muscle function and metabolism (Andersen et al. 2000, Campbell & Leidy 2007, Pedersen 2011).



## 6.1 Skeletal muscle size and type

Skeletal muscles consist of different types of muscle cells also known as muscle fibres. The fibres are organized in bundles and the individual fibres are structured in bundles of myofibrils (Bottinelli & Reggiani 2000). The myofibrils contain a large number of structural and contractile proteins, the most abundant being actin and myosin. Most often various human skeletal muscle fibre types are defined by their content of the myosin heavy chain (MHC) isoform, since the content of a specific MHC isoform is closely related to the contractile properties of an individual fibre (Schiaffino & Reggiani 2011, Staron 1997). The human skeletal muscles contain three different MHC isoforms: The slow MHC 1 and the fast MHC 2A and MHC 2X. Since the three MHC isoforms in the individual fibres can exist either alone or be co-expressed two at a time, muscle fibre types can be divided into two, three, five or even more different fibre types depending on the degree of detail applied (Staron 1997). If stratified into three fibre types, type 1 fibres have the lowest maximum contraction velocity; type 2A fibres are three to five times faster and type 2X fibres are up to 10 times faster than the type 1 fibres (Harridge et al. 1996). Certain metabolic characteristics are related to these different fibre types; type 1 fibres have mainly an aerobic profile, type 2A fibres are both aerobic and anaerobic, whereas type 2X fibres are mainly anaerobic (Table 1).

**Table 1.** Important characteristics in the muscle fibre types.

<i>Characteristic</i>	<i>Type 1 fibre</i>	<i>Type 2A fibre</i>	<i>Type 2X fibre</i>
Contraction velocity	Slow	Fast	Very fast
Capillary density	High	High/medium	Medium/low
Mitochondria density	High	High/Medium	Medium/low
Aerobic enzymes	High	High/medium	Low
Anaerobic enzymes	Low	Medium/high	High

Muscle fibre types have different characteristics and the type distribution in the skeletal muscle is associated with physiological capacities. The Table is based on previous publications (Bottinelli & Reggiani 2000, Harridge et al. 1996).

Muscle fibre composition differs between the different skeletal muscles in the body. The soleus muscle has 75-80% type 1 fibres and 20-25% type 2A fibres and often no type 2X fibres making it relatively slow but of high endurance. On the other hand, the triceps brachii has only 30% type 1 fibres (personal communication Andersen, JL) making it a fast muscle but with less endurance. Many studies have investigated the human vastus lateralis muscle, and data shows an average muscle fibre composition of around 50% type 1 fibres and 50% type 2 fibres, but with considerable variations in-between individuals (Lexell et al. 1983). The relative amount of type 1 fibres is stable at least on a short term basis, whereas the composition of subtypes, type 2A and type 2X are more dynamic and largely depend on muscle usage. It is generally accepted that the relative number of type 2X fibres is down-regulated by physical activity and up-regulated by physical inactivity with a corresponding up-regulation and down-regulation of type 2A fibres (Andersen & Aagaard 2000).

The size of individual muscle fibres is determined by the cross-sectional area (CSA). Like fibre type distribution this varies in-between individuals. In addition, muscle fibre size is also affected by usage. Thus, strength training will result in muscle hypertrophy whilst inactivity will result in muscle atrophy (Paddon-Jones et al. 2006a, Fry 2004). Fibre size also changes through out life, increasing from birth until the age of 20-30 years. From here on and until around the age of 60 years only minor age related decreases in fibre size are seen. In the last phase of life the muscle fibre size is affected severely by an accelerated atrophy. The general age related muscle fibre atrophy, often referred to as sarcopenia, can not be avoided as such, but can be counteracted by strength training activity (Andersen 2003). Muscle fibre size also depends on gender and during adolescence fibre size in males increases more than in females, leading to males having a larger muscle mass than females in adulthood. Finally, various pathology stages are associated with muscle atrophy (Kim et al. 2008, Lunde et al. 2001, Molsted et al. 2007), atrophy that in some diseases may be counteracted by muscle usage.

Not only is the general fibre CSA affected during life, the ratio between slow type 1 and fast type 2 fibres' size is also affected. At a younger age type 2 fibres as a general rule have a slightly larger CSA than the type 1 fibres, which is more pronounced in males than in females. With increasing age there is a progression towards a shift in this ratio, consequently in older muscle a changed relation between type 1 and type 2 fibres area is seen, resulting in the type 1 fibres having a significantly larger CSA than the type 2 fibres (Andersen 2003). Combined with a gradually accelerating loss of muscle fibres in general in old age, this leads not only to a weaker but also a slower muscle (Volpi et al. 2004). A somewhat similar phenomenon is also evident in some pathological conditions and may be further accelerated by muscle disuse (Lunde et al. 2001).

## **6.2 Muscle strength**

Muscle strength is highly correlated with muscle mass (Fry 2004) and since muscle mass changes through life so will muscle strength decline with aging. This progressive decline towards an age-related loss of muscle mass and strength can not be avoided as such, but can be counteracted by strength training. Muscle strength can also be affected by pathological stages (Kim et al. 2008, Lunde et al. 2001), and a decrease in muscle strength is associated with a loss of physical performance. Low physical performance may lead to a further decrease in physical activity followed by not only increased risk of falling, but also an increased risk of metabolic diseases. Combined, those can lead to a downward spiral including an increased need for public health care, e.g. personal assistance in daily living.

Muscle strength is also dependent on neuromuscular function. Neuromuscular function is most often estimated by surface electromyography (EMG). An EMG recording during a muscle contraction will comprise the sum of action potentials of the activated muscle fibres. Thus, such data represents a complex outcome of motor unit recruitment and firing rate. Indeed EMG signal amplitude has been shown to be increased in parallel with increased muscle strength (Suetta et al. 2004, Aagaard 2003). Besides an increase

in EMG amplitude, muscle strength may also be associated with improved synchronized motor unit recruitment (Harrison et al. 2012). Thus, one can expect that an increase in muscle fibre area, in particular fast fibres, and an improved neuromuscular function will result in a more rapid rate of muscle contraction, also known as the rate of force development (RFD) (Aagaard 2003). Higher RFD may have important implications for physical performance, for example when a person is about to stumble, a rapid rate of muscle contraction in the relevant muscles may counteract an actual fall.

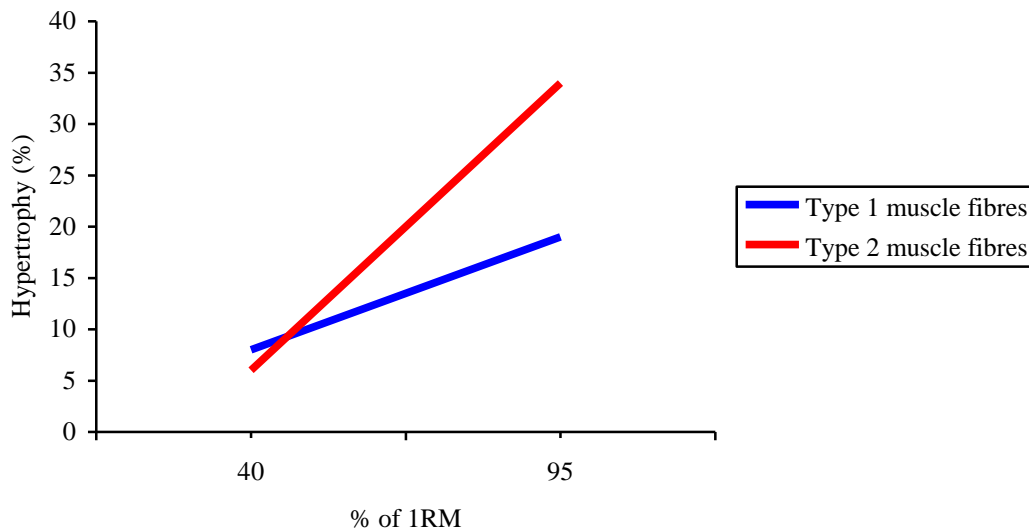
Muscle strength can be tested in several ways. In clinical practice, one repetition maximum (1RM) may be preferred since it can be tested using the same equipment as that used to conduct the exercise (Dong et al. 2010). More sensitive tests may be carried out using specific equipment to test speed and quantity of concentric, eccentric or isometric muscle strength (Highgenboten et al. 1988).

Even though physical performance may be associated with muscle strength, physical performance tests give a more adequate assessment of functions related to daily activities than muscle strength tests (Elliott et al. 2011). Several performance tests have been developed for persons in different age groups or in relation to different diseases (Avelar et al. 2011). Whilst objective assessments of physical performance are important the individuals' subjective experience is also relevant and self-rated physical function has become a natural part of outcome measurements in clinical trials (Elliott et al. 2011).

### **6.3 Strength training**

Strength training is the preferred training regimen to increase muscle strength (Fry 2004, Andersen & Aagaard 2010). An increase in muscle strength after strength training is associated with both muscle hypertrophy and improved neuromuscular function (Hakkinen et al. 1985). Strength training can be conducted with various loads, contraction speeds, number of sets, rest periods between sets and range of movements in the joints, all parameters potentially affecting the outcome of the training (Andersen & Aagaard 2010). Muscle hypertrophy is in general most pronounced after high-load strength training, involving loads not greater than 12RM (Fry 2004). In general, there is a linear correlation between the load in terms of percentage of 1RM and relative muscle hypertrophy (Fry 2004). This is true both for type 1 and for type 2 fibres, but the relative hypertrophy correlated with percentage of 1RM is greater in type 2 fibres compared with type 1 fibres (Figure 1). A hypertrophy of the type 2 fibres of 25-30% (somewhat less for type 1 fibres) within a three month period is not abnormal in young untrained healthy subjects after strength training programmes including high load exercise (Andersen et al. 2000).

**Figure 1.** Muscle fibre hypertrophy correlated to strength training loads.



Simplified correlation between percentage of 1RM in strength training programmes and hypertrophy in type 1 and type 2 muscle fibres. The greatest relative hypertrophy is noticed in type 2 fibres. Inspired by Fry AC (2004).

An increase in muscle strength as is typically seen during the early phase of a strength training programme is mainly due to improved neuromuscular function (Aagaard 2003), whereas a detectable increase in muscle fibre CSA is evident during the later stages.

Muscle hypertrophy induced by strength training involves an increased protein synthesis (see section 6.4). Even though strength training also induces protein breakdown, protein synthesis exceeds the protein breakdown leading to an accumulation of muscle tissue. Strength training-induced increases in muscle protein synthesis seem to be most pronounced in the first four hours post training, where protein synthesis may show a three to four fold increase (Burd et al. 2010, MacDougall et al. 1995, Kumar et al. 2009). Moreover, it has been proposed to still be doubled 24 hours after a strength training session and retain above pre training values for up to 48 hours after a strength training session (Kumar et al. 2009).

A major part of the studies on post training muscle protein synthesis have been conducted on healthy young men, but other experiments have shown that protein synthesis is affected by age and gender (Kumar et al. 2009). Thus, myofibrillar post training muscle protein synthesis is reduced with aging, and in addition it has been proposed that there may be a minor reduction in protein synthesis in young females compared to young men (Kumar et al. 2009). Furthermore, the influence of gender may increase in older age showing a further muscle protein synthesis rate decrease in older females compared to older men (Kumar et al. 2009).

Muscle protein synthesis is associated with the intensity of the strength training, but it seems to reach a plateau at 60% of 1RM (Kumar et al. 2009). Thus, the correlation between strength training load and muscle

hypertrophy may be associated with a prolonged synthesis period when the training is conducted with loads above 60% of 1RM.

Improved neuromuscular function after strength training consists of an increased motor neuronal output that may involve increased firing rates, increased motor neuron excitability, decreased pre synaptic inhibition and a down-regulation of inhibitory pathways (Henneman et al. 1965). The neuromuscular adaptations after strength training can be estimated using EMG since the EMG signals reflect spatial and temporal summation (Harrison et al. 2012). Indeed a previous review documents increased EMG amplitude after strength training (Aagaard 2003). Moreover, amplitude represents the in-phase activated motor units. Thus, amplitude changes after strength training may be due to increased motor unit recruitment or improved synchronization of existing active motor units. Finally, increased EMG amplitude after strength training has been noticed in parallel with an improved RFD, which has important implications for daily physical function.

#### **6.4 Amino acid metabolism**

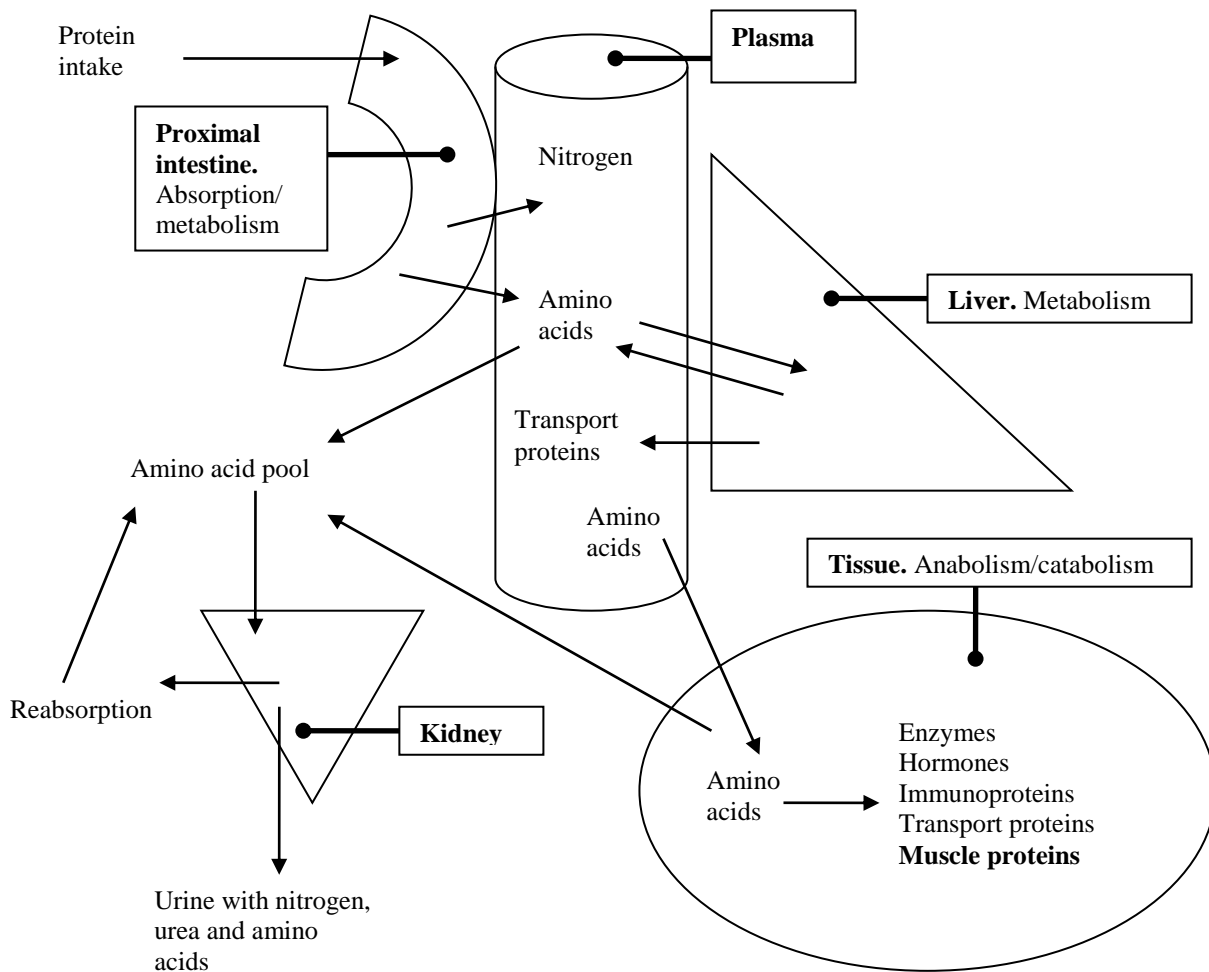
Strength training induces muscle protein synthesis leading to increased muscle mass. For muscle protein synthesis, essential amino acids (AA) are crucial and they are usually absorbed from ingested meat, milk, eggs, beans and pulses (Walker et al. 2011, Gropper et al. 2005).

The majority of the AA's absorbed into the systemic circulation are transported to the liver, which is the primary site for AA metabolism. From the liver the AA's are distributed in the body, where they are used in protein synthesis, in catabolization or in the recirculating free AA pool. The AA's are needed in the construction of several proteins, determined at the cellular level according to various functions (enzymes, peptide hormones, transport proteins, immune proteins, connective tissue and contractile proteins) (Gropper et al. 2005). A great amount of the AA's leaving the liver do so as branched-chain AA's which are needed for the contractile protein construction (Gropper et al. 2005).

The protein intake recommendation to the general population is 0.8 g per kilo body weight per day and in a nutritionally balanced situation only a small proportion of the AA's are used as an energy source. Proteins are constantly being broken down in the body and resynthesized so called protein turn-over. Thus, there exists a far greater body pool of proteins besides the circulating AA's and proteins.

During protein turn-over not all AA's are reused and some are excreted as urea primarily in the urine via the kidney, which has a crucial role in urea disposal. The glomeruli in the kidney act as a filter to the plasma, removing plasma urea to the filtrate. Some albumin passes the glomeruli and enters the filtrate but those are typically reabsorbed in the proximal tubular cells. Whilst the tubular cells are permeable to albumin this is not the case for urea, which is not reabsorbed (see Figure 2).

**Figure 2.** General overview of amino acid metabolism (anabolism/catabolism) in the body.



Ingested proteins are absorbed as proteins, peptides or AA's across the intestine and into circulation. The liver is the primary site for AA metabolism. The AA's are transported to where they are needed in anabolism or catabolism. The kidneys produce urine in which AA's may be excreted. The figure is based on Gropper and colleagues (2005).

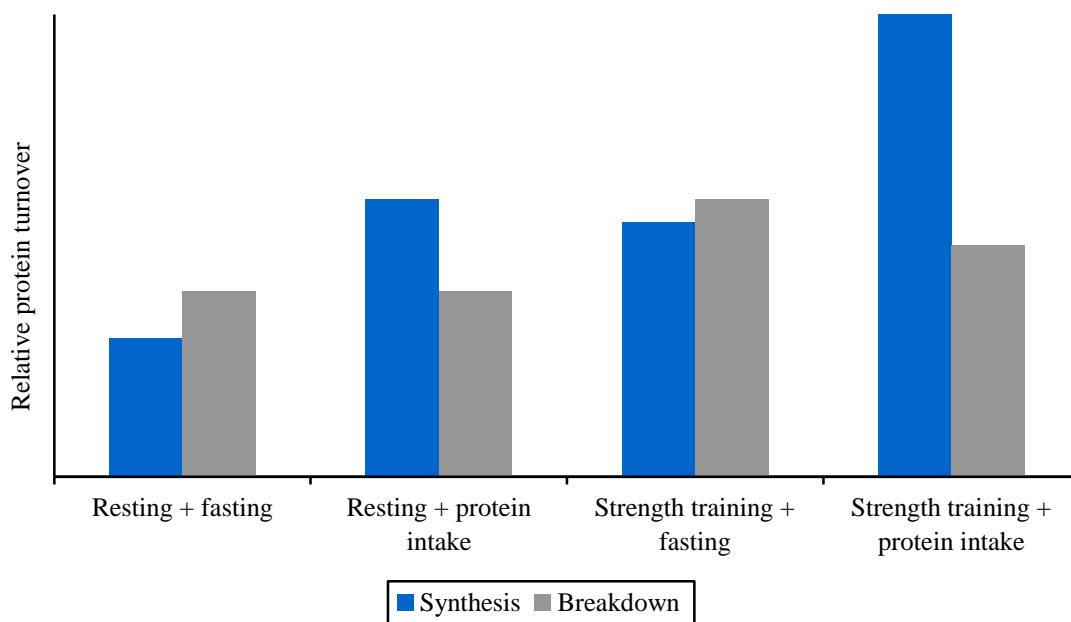
A daily protein intake of 0.8 g per kilo body weight may be enough to increase muscle strength and induce muscle hypertrophy after strength training. In order to achieve such an effect the total energy intake must comply with energy expenditure. If the energy uptake is insufficient circulating AA's or stored proteins are used as an energy source and this diminishes the potential for muscle hypertrophy. Besides building muscle mass proteins are also essential for hormone synthesis, many of which also play a role in training adaptations (Gropper et al. 2005).

## **6.5 Strength training and protein supply**

Whilst strength training increases muscle protein synthesis the same is also true for protein intake (Atherton & Smith 2012). Once AA's have been absorbed there follows a period when increased protein synthesis is induced. In general, 30 min after protein intake muscle protein synthesis is increased three-fold and it peaks after 90 min. Two hours after ingestion of food muscle protein synthesis returns to baseline, despite an elevated AA concentration (Atherton & Smith 2012). Amino acid absorption depends on the intake source of various proteins, and AA's from whey are as an example more rapidly absorbed compared to those from casein (Tipton et al. 2004). Amino acids from casein may on the other hand give a longer period of increased protein synthesis compared to that observed after whey intake, when whey or casein protein is ingested by healthy persons (Tipton et al. 2004).

During the last decade there has been an increased focus on the effects of supplementing proteins alongside strength training (Campbell & Leidy 2007, Phillips et al. 2005, Tang & Phillips 2009). The idea is to utilize a synergistic situation where protein synthesis is stimulated by strength training and AA uptake. At rest protein breakdown is rather stable, whilst protein synthesis is high after food intake and low in a fasting state (Campbell 2007). During a period with a moderate level of physical activity and sufficient energy intake there is a net balance between protein synthesis and protein breakdown (Campbell & Leidy 2007, Campbell 2007). When a strength training session is conducted, protein synthesis and also protein breakdown increases and if the strength training is conducted in a fasting situation, depending on the fasting time frame, protein breakdown exceeds protein synthesis (Campbell 2007). If the strength training is combined with energy intake, protein synthesis exceeds protein breakdown, evidently leading to muscle hypertrophy (Phillips et al. 2005, Tang & Phillips 2009, Campbell 2007). Energy intake increases the insulin level leading to a decreased protein breakdown and thereby further improves the training induced net protein balance (Atherton & Smith 2012). Protein synthesis induced by training is most pronounced in the hours after training and it has been proposed that protein synthesis reaches a higher level if training is conducted at the same time as a protein intake resulting in a synergistic effect (Esmarck et al. 2001, Levenhagen et al. 2001). Even though the timing of additional protein to strength training seems to be important, the synergistic effect of strength training and protein supply on muscle protein synthesis still exists 24 to 48 hours after a training session (Atherton & Smith 2012), which may explain why muscle hypertrophy occurs after strength training programs without protein intake immediately after the training session.

**Figure 3.** Protein turnover during protein intake and physical activity.



When a resting person goes from fasting to ingesting proteins then protein synthesis increases, whilst protein breakdown is rather stable. When a fasting person goes from resting to conducting strength training both protein synthesis and also protein breakdown increase. In addition the training induced protein synthesis lasts longer than the training induced breakdown. Finally, when strength training is combined with protein intake the effect on protein synthesis is synergistic whereas only a small change is seen in protein breakdown. The figure is based on Campbell & Leidy (2007) and Atherton & Smith (2012).

Studies on healthy persons have shown positive effects on muscle strength and muscle hypertrophy when combining strength training and a timed protein intake (Esmarck et al. 2001, Levenhagen et al. 2001, Andersen et al. 2005). One study showed 25% hypertrophy in type 2 fibres when a relatively low amount of proteins (10 g) was given in relation to strength training three times per week for 12 weeks (Esmarck et al. 2001). In another study 50 g of protein in relation to strength training three times weekly over 14 weeks was associated with a 26% increase in type 2 fibre CSA (Andersen et al. 2005). In addition to this, it has been confirmed that the timing of the protein intake in relation to exercise is important (Esmarck et al. 2001, Levenhagen et al. 2001).

Even though one study showed positive effects of combining protein intake with strength training in older subjects (Esmarck et al. 2001), the evidence for this effect especially among older persons has been debated (Campbell & Leidy 2007). Thus, it is difficult to draw conclusions about dose-response effects of timed protein intake in relation to strength training. However, it is important to note that relatively small amounts of proteins have been associated with increased muscle protein synthesis and that a signal effect from protein intake seems to be more important than the protein dose, which may explain why sufficient daily protein



intake during a strength training programme is associated with increased muscle strength and muscle hypertrophy (Atherton & Smith 2012).

## **6.6 Muscle glucose uptake**

Skeletal muscle plays an important metabolic role in the regulation of blood glucose homeostasis in terms of glucose disposal and it is the primary site of post prandial glucose uptake (DeFronzo & Tripathy 2009). Besides the skeletal muscle, fat tissue and the liver will uptake redundant blood glucose, which may be transformed to fat tissue when the glucose amount exceeds a certain level (DeFronzo & Tripathy 2009). In muscle, glucose is synthesized and stored as glycogen, which is used as glucose in aerobic and anaerobic processes.

Blood glucose homeostasis is regulated by insulin and glucagon. Elevated blood glucose triggers insulin release, which stimulates blood glucose uptake into the muscle. Muscular glucose uptake is also stimulated by muscle contractions *per se* and the glucose transporter 4 (GLUT4) is the primary glucose transporter in the cell membrane. In addition, glucose uptake capacity in muscle depends on capillary density, mitochondrial function, muscle fibre type composition and a complex system of intracellular pathways (Dela & Kjaer 2006).

If the increased blood glucose is not normalized by the insulin response hyperglycaemia is induced due to impaired sensitivity to insulin. This impaired insulin function is known as insulin resistance (IR). If the fasting and/or the postprandial blood glucose increase to certain levels the condition is categorized as type 2 diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003). Insulin resistance may be associated with genetic markers but it is also induced by physical inactivity and obesity especially in terms of visceral adiposity and liver fat deposition (DeFronzo & Tripathy 2009). Furthermore, systemic inflammation and dyslipidaemia are also key players in the development of or at least strongly associated with IR (Pedersen 2011, Colberg et al. 2010).

The muscular glucose uptake capacity is usually estimated by the euglycaemic hyperinsulinaemic clamp technique or the oral glucose tolerance test. In the clamp technique, glucose is infused to maintain a plasma glucose concentration in response to a fixed insulin level. This technique reflects primarily the skeletal muscle glucose uptake and is considered to be the gold standard for measuring insulin action *in vivo*. From the oral glucose tolerance test the Matsuda insulin sensitivity index (ISI) (Matsuda & DeFronzo 1999) and the homeostatic model assessment (HOMA) insulin resistance (Levy et al. 1998, Matthews et al. 1985) can be derived. Whilst the Matsuda ISI includes fasting and also post prandial blood glucose and insulin values, the HOMA model is based solely on fasting blood glucose and insulin values. Glycemic control is measured by fasting blood glucose or more precisely by glycosylated haemoglobin (HbA1c), which reflects glucose bound to haemoglobin. Glycosylated haemoglobin reflects the recent 8-12 weeks blood glucose levels and in

patients with type 2 diabetes it is associated with the relative risk for complications related to diabetes (Stratton et al. 2000).

Insulin resistance and type 2 diabetes are associated with an increased relative risk for medical complications including mortality (Stratton et al. 2000). The complications are related to micro- and macroangiopathic damage including cardiovascular disease. Whilst IR is induced by physical inactivity, physical activity is a recognised intervention in the prevention against and in the treatment of IR. Aerobic training increases insulin sensitivity and has traditionally been recommended as a force of treatment due to primary activation of type 1 muscle fibres, which are more insulin sensitive than type 2X fibres (Olsson et al. 2011). Furthermore, aerobic training increases muscle capillary density which is associated with better insulin sensitivity. In the last decade there has been a growing interest in the effects of various types of strength training, which has been shown to be effective on IR and glycemic control in patients with type 2 diabetes (Colberg et al. 2010, Gordon et al. 2009, Umpierre et al. 2011, Eves & Plotnikoff 2006). One study showed that even two strength training sessions weekly during a 16 week period improved insulin sensitivity in older men with type 2 diabetes (Ibanez et al. 2005) and achieved effects may at least be maintained by two strength training sessions weekly (Gordon et al. 2009). Whilst the effects of aerobic training on insulin sensitivity are associated with increased capillarity, this may not be the case after strength training in which a mass effect due to muscle hypertrophy has been suggested (Colberg et al. 2010). In addition a symphony of intracellular activities is improved after endurance training and also by strength training (Dela & Kjaer 2006). Thus, physical training is associated with more physiological improvements in patients with IR or type 2 diabetes. As with most other causative events the effects of physical training only exist for shorter periods when it has been conducted and the training should be conducted regularly to maintain achieved effects on insulin sensitivity.

## **6.7 Skeletal muscle size and type in patients undergoing dialysis**

In patients undergoing dialysis loss of lean body mass is common and it has negative implications in terms of impaired muscle strength and reduced metabolic capacity. Muscle atrophy may be established even before the need for renal replacement therapy in patients with chronic kidney disease (CKD) (Price et al. 2010). Thus, the uraemia *per se* and comorbidities related to CKD may be involved in muscle atrophy. Muscle mass loss may also have implications in terms of increased mortality risk and it has been proposed that in patients undergoing PD, lean body mass wasting was associated with mortality (Dong et al. 2008). Muscle atrophy prevention is therefore an important target in clinical practice (Price et al. 2010).

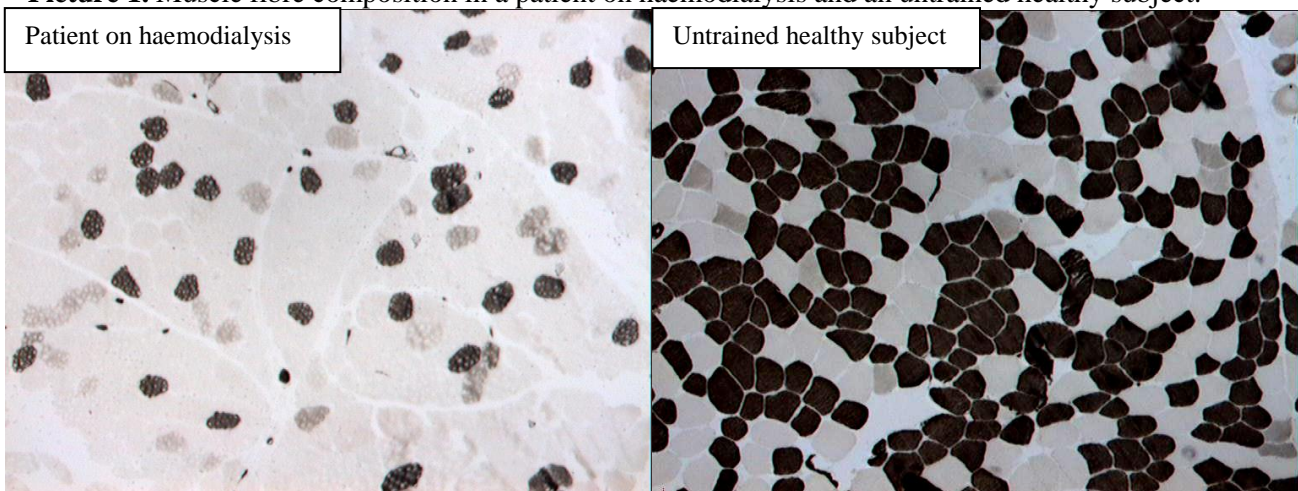
Only a few studies have analyzed muscle fibre composition and muscle fibre CSA in muscle tissue from biopsies obtained in patients undergoing dialysis. Three studies which determined fibre type composition from patients undergoing dialysis, either by ATP<sup>ase</sup> histochemistry or by analysis of MHC isoforms from muscle biopsies obtained in the vastus lateralis muscle can be found in the literature (Molsted et al. 2007,

Fahal et al. 1997, Kouidi et al. 1998). One study showed a normal mean proportion of type 1 fibres of 55% in seven patients undergoing HD (Kouidi et al. 1998). In two other studies both including 12 patients undergoing HD remarkably low proportions of type 1 fibres (35% (Fahal et al. 1997) and 31% (Molsted et al. 2007)) were reported. In one of these studies, 25% of the patients expressed less than 15% type 1 fibres as determined by MHC isoform content (Molsted et al. 2007). This remarkably low relative number of type 1 fibres seems to be supported by rodent data. Thus, in a study on subtotaly nephrectomised uraemic rats compared to healthy sham operated rats, a significantly lower proportion of type 1 fibres was found determined by MHC analyses (Taes et al. 2004).

In two of the afore mentioned studies on patients undergoing dialysis, a relatively large proportion of type 2X fibres (~ 30%) were observed (Molsted et al. 2007, Fahal et al. 1997). This relatively high number of type 2X fibres in patients undergoing dialysis fits well with the patients' low level of physical activity (Brodin et al. 2001, Johansen et al. 2000). However, even when taking the inactivity of these patients into account, the relatively low proportion of type 1 fibres could very well be influenced by the pathological condition imposed on the skeletal muscle. Thus, the observed changes may not only reflect disuse.

In the three studies of muscle fibre size in the vastus lateralis muscle in patients undergoing dialysis, there has been reported a reduced CSA of type 1 as well as type 2 fibres (Molsted et al. 2007, Fahal et al. 1997, Kouidi et al. 1998) (Picture 1). This reduction in muscle fibre size may be a reflection of the low levels of physical activity, but catabolism related to CKD and HD may also be an important factor.

**Picture 1.** Muscle fibre composition in a patient on haemodialysis and an untrained healthy subject.



Dark fibres, type 1 fibres; White fibres, type 2 fibres. Notice the fewer type 1 fibres and their reduction in size in the muscle of the patient on haemodialysis vs. the healthy subject (Molsted et al. 2007).

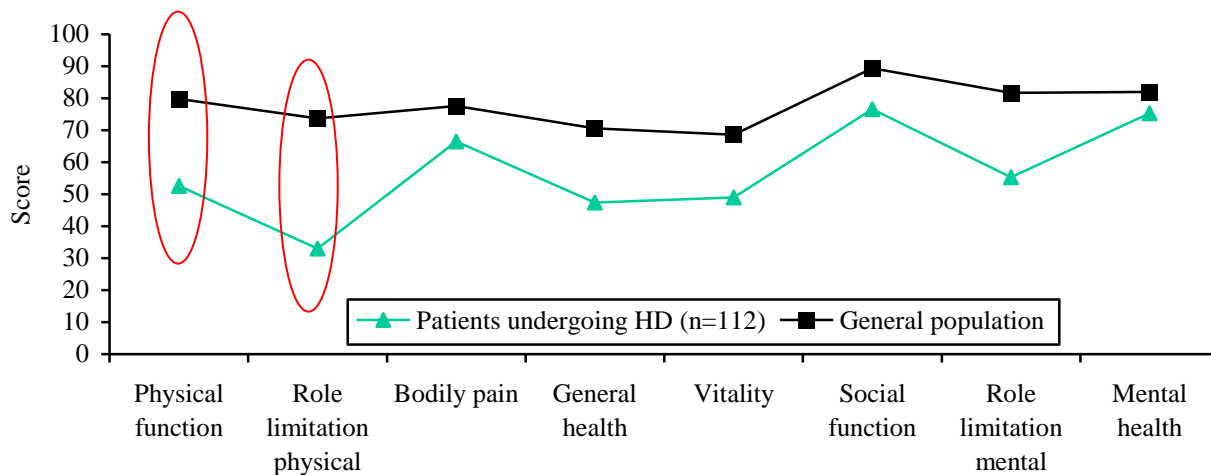
Previous studies have investigated muscle fibre size and architecture in patients with CKD without the need for renal replacement therapy. In a study of 22 patients with CKD, who were about to start chronic dialysis therapy (glomerular filtration rate (GFR) not reported), biopsies were obtained from the abdominal muscle (Sakkas et al. 2003). The aim of the study was to investigate abnormalities in skeletal muscle caused by

uremia. To avoid effects of physical inactivity the analyses were conducted on a non-locomotor muscle in the participants. The study did not show different muscle fibre composition between the patients and that of a control group, but the size of type 2 fibres was reduced in the patients with CKD compared to the controls. A similar tendency was shown in muscle biopsies obtained from the vastus lateralis muscle in men with CKD with a mean GFR of 14 ml/min\*1.73 m<sup>2</sup> (Clyne et al. 1993), whilst another study did not show differences in muscle fibre composition and size in elderly patients with CKD with a mean GFR of 17 ml/min\*1.73 m<sup>2</sup> compared to healthy controls (Heiwe et al. 2005). Thus, CKD may lead to a shift in the muscle size ratio between type 1 and 2 type fibres due to type 2 fibre atrophy, but previous data are not consistent.

### 6.8 Muscle strength in patients undergoing dialysis

Patients undergoing dialysis are known to have reduced muscle strength compared to healthy controls (Fahal et al. 1997, Bohannon et al. 1994, Johansen et al. 2003). Moreover, this lack of muscle strength severely affects the patients' physical performance and HRQOL (Molsted et al. 2007, Molsted et al. 2004a) (Figure 4).

**Figure 4.** Health-related quality of life in patients undergoing HD and in the general population.



Health-related quality of life assessed by using the questionnaire Short Form-36. A higher score indicates better health. Notice the significant difference between patients undergoing HD and the age and gender matched general population (Bjørner et al. 1997) in the scales *Physical function* and *Role limitation physical* (Molsted et al. 2004a).

### ***Protein synthesis and protein breakdown in patients undergoing dialysis***

The reduction in muscle strength in patients undergoing dialysis can potentially be caused by different factors. It has been proposed that a disturbed balance between protein synthesis and protein breakdown is associated with loss of muscle mass in patients undergoing dialysis (Garibotto 1999). During HD and PD there is an inappropriate removal of proteins. Indeed HD has been proposed to be catabolic *per se* (Lim et al.

2005) and the loss of proteins during a typical treatment regimen of three HD sessions per week has been proposed to correspond to a daily loss of seven g of proteins (Eidemak et al. 2011). The dialysis modality PD is not associated with the same level of protein breakdown as HD, but with PD the loss of proteins in the dialysis fluid is greater (Lim et al. 2005). Other potential reasons for a disturbance between protein synthesis and protein breakdown in patients undergoing dialysis are IR (Pupim et al. 2005, Lee et al. 2007, Siew et al. 2007), low levels of and resistance to anabolic hormones (Kopple et al. 2006, Carrero et al. 2011), acidosis (May et al. 1986), low grade inflammation (Zhang et al. 2009), and physical inactivity (Garibotto 1999).

Furthermore, an insufficient energy and protein energy intake may also be followed by a loss of protein stores. Malnutrition is common in patients undergoing dialysis and diet counselling is usually offered in dialysis centres. The nutritional status related to protein energy waste in the patients is estimated by protein concentrations and protein stores. The protein concentration is most often assessed using analyses of serum albumin and the protein stores are estimated via serum creatinine and lean body mass assessments.

Energy uptake after regular food intake may in some patients be impaired and such a condition may lead to reduced serum albumin concentration. Previous studies have investigated the protein uptake efficiency after oral energy supply in patients undergoing HD. The results show that plasma albumin values increase after energy supply as seen in healthy controls (Caglar et al. 2002, Eustace et al. 2000) and digestion problems may not be the primary explanation to low albumin concentrations, which more likely may be explained by other pathological issues or insufficient energy or protein-energy intake.

Whilst inter- or intra-dialytic protein supplementation improve protein synthesis and protein breakdown balance in patients undergoing HD (Veeneman et al. 2004), the nutritional recommendation according to protein intake to patients undergoing dialysis is still debated. It has been proposed that a daily protein intake of 1.2 g per kilo body weight in patients undergoing HD is sufficient (National Kidney Foundation). Due to the greater protein loss related to the PD treatment, these patients are recommended a level of protein intake of 1.2 to 1.3 g per kilo body weight (National Kidney Foundation).

### ***Neuromuscular function in patients undergoing dialysis***

The reduced muscle strength in patients undergoing dialysis may also be explained by impaired neuromuscular function. Neuropathy is a well known problem in patients undergoing dialysis and it may affect the motor nerves and thereby affect muscle strength. In a study of the lower limb motor nerve excitability properties of the tibialis anterior and extensor digitorum brevis muscles of 14 patients undergoing HD, the neuromuscular function was found to be negatively altered before a HD session (Krishnan et al. 2005). After the HD session the majority of nerve excitability parameters were normalized. These results are supported by data from another study that also investigated the neuromuscular function before and after a HD session (Harrison et al. 2006). The neuromuscular function estimated by sEMG of contractions of the 2<sup>nd</sup> dorsal interosseous muscle was improved after a HD session, whereas the EMG

signals were unchanged in the vastus lateralis muscle. The changed EMG signal was compared with the levels of minerals removed by HD and the authors suggested the impaired sEMG signal before HD to be correlated to high levels of phosphate.

In another study the sEMG signals from 21 patients undergoing PD and 21 patients with CKD were compared with signals from healthy controls (Heaf et al. 2010). Furthermore, data on physical performance were collected. Whilst there was no difference in sEMG signals between patients undergoing PD and patients with CKD, the sEMG signals were found to be impaired in the patients, and in addition, the impaired EMG signal was associated with reduced physical performance (Heaf et al. 2010). The lack of difference in the EMG signals between patients with CKD and patients undergoing PD may emphasize that neuromuscular function is negatively affected by uraemia in the stage before renal replacement therapy.

Using sEMG to test effects of training on neuromuscular function is not without problems, and the interpretation of the collected data should be made with caution. Intramuscular temperature, sub cutis thickness, intramuscular pH and electronic recording equipment quality may confound the results (Harrison et al. 2012). A great advantage in the sEMG method however, is that data can be collected in relation with other tests for example muscle strength testing, which gives the opportunity to correlate these different data. When sEMG is recorded during a 1RM test before and after an intervention it brings useful information on changes in neuromuscular activity together with muscle strength observations (Harrison et al. 2012).

Thus, the impaired muscle strength in patients undergoing dialysis has been shown to be associated with muscle atrophy as well as changed neuromuscular function. Disuse as well as pathology remain important determinants of reduced muscle strength in patients undergoing dialysis.

## **6.9 Rehabilitation in patients with chronic kidney disease**

In the rehabilitation of patients undergoing dialysis, there has traditionally been focus on aerobic type of training, with the purpose of significantly increasing aerobic capacity (Kouidi et al. 1998, Deligiannis et al. 1999a, Deligiannis et al. 1999b, Goldberg et al. 1983, Molsted et al. 2004b, Mustata et al. 2004, Sakkas et al. 2003). Other outcome measurements have also been used in studies with aerobic training and there has been shown positive effects on physical function and HRQOL (Molsted et al. 2004b, Kouidi et al. 1997, Painter et al. 2002). Physical training may with advantage be implemented in the stages before the need for renal replacement therapy in patients with CKD and studies have shown positive results after training in such patient samples (Boyce et al. 1997, Clyne et al. 1991, Heiwe et al. 2001).

Many of the patients undergoing dialysis are suffering from chronic heart disease. Thus, aerobic training with high intensities should in patients with ischaemic heart disease and in particular in patients with chronic heart failure be performed with caution. Strength training is not however associated with cardiac stress as is aerobic training, and may therefore apply to more patients undergoing dialysis than aerobic training. In addition, even though almost all of the patients are treated with erythropoietin (EPO) many of them have at

least in periods anaemia, which may make aerobic training at a useful level harder to overcome compared to strength training. In a previous study on patients undergoing HD the central factors haemoglobin concentrations and peak lactate concentration were not associated with aerobic capacity (Diesel et al. 1990). In contrast the authors found that muscle strength was associated with aerobic capacity and suggested the importance of including strength training in the rehabilitation to patients undergoing dialysis, since the peripheral muscular capacity may limit the aerobic capacity.

#### **6.10 Strength training and protein supply in patients undergoing dialysis**

Strength training is in contrast to aerobic training, associated with increased muscle mass and increased muscle strength and may thereby be a more potent form of training when trying to improve physical performance. Strength training with high loads has been tested in studies on patients undergoing HD outside but also during HD (Dong et al. 2010, Johansen et al. 2006, Cheema et al. 2007, Headley et al. 2002, Kopple et al. 2007). The results are inconsistent and may likely be related to differences in the training programmes especially in terms of their intensity. Strength training during dialysis definitely has relevance in a time saving aspect, but it might limit not only the exercise intensity but also the choice of strength training exercises.

With the knowledge of a disturbed balance between protein synthesis and protein breakdown in patients undergoing dialysis, there is an increased interest in the effects of combining physical training and protein supply in these patients.

In a recent study on patients undergoing HD the acute effect of exercise and protein intake during dialysis was investigated (Majchrzak et al. 2008). Eight patients went through two settings: In the first setting the patients ingested 33 g of protein during a HD session and in the second setting they performed a single exercise session containing three times 12 repetitions leg press at 75% of 1RM together with 33 g of protein intake during a HD session. The authors proposed that muscle protein synthesis was significantly higher when the exercise was added to the protein intake, suggesting that such an intervention on a long term basis could improve muscle volume and muscle strength (Majchrzak et al. 2008). Another study investigated the possible effect of combining strength training with an oral protein supplement (Dong et al. 2010). The patients were randomized to protein supply with strength training, or protein supply alone. The protein supply was 40 g of protein and 22 patients completed the 6 month intervention. The study showed that the nutritional intervention alone increased muscle strength, which suggests how seriously muscle strength is affected by a habitual nutritional intake in these patients. Surprisingly, the study did not show any further benefits on muscle strength when the exercise was added. The study had some important limitations which were also noted by the authors, e.g. the exercise programme consisted of only three sets of leg press, and the progression of the exercise did not seem adequate.

### **6.11 Muscle glucose uptake in patients undergoing dialysis**

Studies of patients undergoing dialysis have shown incidences of IR from 32% to 60% of the patients (Sakkas et al. 2008, Sit et al. 2005, Takenaka et al. 2007). Insulin resistance in patients undergoing dialysis is, besides acidosis, caused by the same underlying conditions as in the general population. However, there may be other contributing factors in these patients due to the complex nature of kidney diseases and related comorbidities.

Besides being associated with morbidity and mortality IR also seems to play a significant role in the loss of muscle protein in patients undergoing dialysis by disturbing the balance between protein synthesis and protein breakdown (Pupim et al. 2005, Lee et al. 2007, Siew et al. 2007). In non-diabetic patients undergoing dialysis, it was shown that IR estimated by HOMA-IR was inversely associated with muscle protein estimated by DEXA scanning (Lee et al. 2007). Another study showed that in non-diabetic patients undergoing HD, HOMA-IR was associated with protein breakdown estimated by stable isotope methodology (Siew et al. 2007). Finally, a further increase of muscle protein breakdown when patients undergoing HD have diabetes has been suggested (Pupim et al. 2005).

Two studies have investigated the effects of physical exercise on IR in patients undergoing dialysis. A randomized trial showed a significant decrease in fasting insulin after aerobic training three times a week for nine months in eight patients undergoing HD compared to a control group with five patients undergoing HD (Goldberg et al. 1983), whereas in a non-controlled study on patients undergoing HD no change was found in the HOMA-IR after three months of aerobic training twice weekly (Mustata et al. 2004). The difference between these results may likely be due to a greater training dose in the study of Goldberg and colleagues (1983) compared to the study of Mustata and colleagues (2004).



## **6.12 Aim and hypotheses**

With the experience of previous studies (Molsted et al. 2007, Molsted et al. 2004a, Molsted et al. 2007, Harrison et al. 2006, Molsted et al. 2004b), I decided to investigate the effects of strength training in patients undergoing dialysis. In order to achieve the most pronounced effect the training was conducted outside of dialysis. The training programme needed to be simple and not time consuming for the participants, so it may be replicated in clinical practice afterwards and hopefully be found attractive by the patients. With the possible effects of combining strength training and timed protein intake, I designed a study that in addition to a control period, incorporated a randomization of the participants to timed protein intake or no protein intake in connection to strength training in patients undergoing dialysis.

**The aim** of the present study was to adopt a controlled design, to investigate the effects of a high-load progressive strength training programme on health-related quality of life, physical performance, muscle strength and power, electromyography, muscle fibre composition and size, and insulin sensitivity in patients undergoing dialysis. In addition this study has compared changes between patients randomly assigned to exercise with a timed protein supplement or to exercise with a timed isocaloric non-protein energy supplement.

### **Hypotheses**

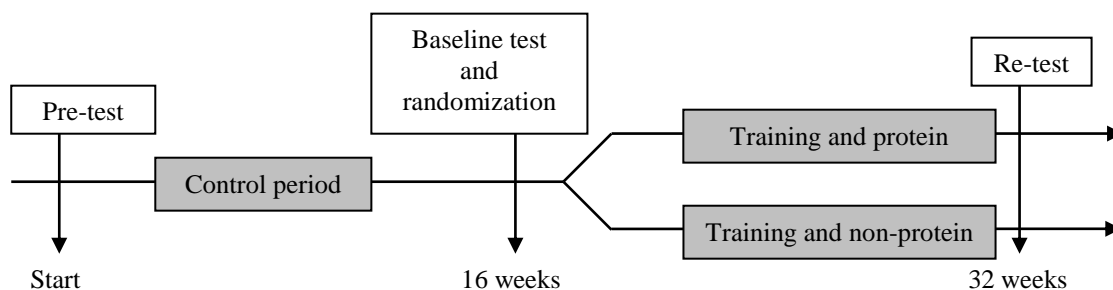
1. Strength training increases muscle strength and physical performance in patients undergoing dialysis.
2. Strength training induces a change in the muscles fibre composition of patients undergoing dialysis.
3. Strength training induces hypertrophy at the muscle fibre level in patients undergoing dialysis.
4. Strength training increases insulin sensitivity in patients undergoing dialysis.
5. Strength training benefits are more effective when undertaken in combination with a timed intake of proteins supplied to patients undergoing dialysis.

## 7. Material and methods

### 7.1 Study design

The patients were invited to participate in a control period comprising 16 weeks, without any intervention, followed by a period of 16 weeks with a strength training intervention. Thus, the participants served as their own controls. Prior to the training period, the patients were randomized with the aid of a computer programme ([www.randomization.com](http://www.randomization.com)) into two groups: Strength training with an intake of protein (PR) or strength training with an intake of an isocaloric non-protein product (NO). The design of the study is presented in Figure 5.

**Figure 5.** Design of the study.



### 7.2 Participants

Patients from three Danish dialysis centres (Rigshospitalet, Frederiksberg Hospital and Hillerød Hospital) were recruited to the study.

To be included the participants had to meet the following criteria:

- Age above 18 years
- HD or PD treatment for more than three months
- Capable of participating in the training programme

Patients were excluded if they met any of the following criteria:

- Severe diabetic retinopathy
- Amputation of a lower limb
- Severe peripheral polyneuropathy
- Dementia
- Unable to speak Danish
- Participation in other conflicting trials
- Undergoing anti-coagulation treatment with Fondaparinux (Arixtra®).

After having provided patients with both written and oral information about the study, informed consent was obtained from all patients. The protocol was approved by the local ethical committee (H-D-2008-124) and registered on [www.controlled-trials.com](http://www.controlled-trials.com) (ISRCTN72099857).

### **7.3 Blinding**

The investigators who performed the tests of sEMG and OGTT were blinded to what group the patients were randomized. Moreover, the analyses of the plasma from the OGTT and the determination of muscle fibre size and fibre composition from the muscle biopsies were performed by individuals who were blinded to both the time of the tests and to the results of the randomization.

### **7.4 Training programme**

The training was conducted during three time frames with up to 14 patients at a time. The training consisted of progressive high load strength exercises three times a week for 16 weeks. Physiotherapists and exercise instructors supervised the patients during the training. Training was made available to the patients 5 days a week (Monday to Friday). The three training days each week were chosen by the patients themselves, who were advised to spread out the sessions over the week, but exercise on two consecutive days was permitted. The exercise began with 5 minutes of warm-up on a stationary ergometer. Three obligatory exercises were performed at each exercise session: leg press, leg extension, and leg curl (Pictures 2-4).

**Picture 2.** Leg press.



**Picture 3.** Leg extension.



**Picture 4.** Leg curl.



In the training programme the number of exercises were limited to three to make the programme simple and in an attempt to avoid it being time consuming to the patients. The three exercises were primary chosen in order to improve physical function related to the lower extremities.

The rest period between each set was 60-90 seconds (the time between repetitions was not regulated). During the training period the load was increased and the number of repetitions decreased correspondingly from 15 to 6 repetitions as shown in Table 2. In the first phase of the training programme a relative high number of repetitions and low number of sets were conducted. This was done in order to minimize delayed onset muscle soreness and to ensure that the patients were able to complete the training as well as adopting the correct technique. The progression during the training period was adjusted according to changes in 1RM or 6RM, which was tested six times during the training period. The relative high load of resistance in the training programme was chosen in an attempt to ensure improvement in muscle strength and muscle mass.

**Table 2.** Protocol of 48 exercise sessions covering the 16 weeks of the training.

<i>Exercise</i>	<i>Session 1-6</i>	<i>Session 7-12</i>	<i>Session 13-24</i>	<i>Session 25-36</i>	<i>Session 37-42</i>	<i>Session 43-48</i>
Leg press (sets • reps)	4 • 15	5 • 15	5 • 12	5 • 8-10	5 • 6-8	4 • 6-8
Knee extension (sets • reps)	3 • 15	3 • 15	4 • 12	5 • 8-10	5 • 8	4 • 8
Knee flexion (sets • reps)	3 • 15	3 • 15	4 • 12	4 • 10	4 • 8	4 • 8

Abbreviation: reps = repetitions.

*Protein and non-protein supplements.* Isocaloric supplements were ingested immediately after each exercise session: The PR group ingested a protein supply (Renilon 7.5, Nutricia) of 125 ml containing 9.4 g of protein from whey with 14.3% leucine, 25 g carbohydrate and 12.5 g lipid, 1050 Kj; the NO group ingested an energy supplement (Calogen, Nutricia) of 54.5 ml containing 2.4 g of carbohydrate and 27.3 g lipid, 1050 Kj. The protein source contained all essential AA's. Both supplements contained a low amount of potassium and phosphate as recommended to patients undergoing dialysis. Such change in oral energy supplies does not give the product an optimal taste and with respect to the patients' appetite, which in some cases is limited, the dose was chosen to ensure that all patients were able to ingest it after the training sessions. The patients were instructed not to ingest meals at least two hours on either side of the training sessions.

## **7.5 Comorbidity, physical activity, food intake and blood tests**

Data on renal disease and morbidity were obtained from the patients' case records. The level of comorbidity was assessed using The Index of Coexistent Disease (Miskulin et al. 2001) by one person (S. Molsted). Physical activity outside the training was assessed using a single question from a previous study (Saltin & Grimby 1968) included in the validated questionnaire The Danish Health and Morbidity Survey 2005 Questionnaire (National Institute of Public Health). Food intake, besides that provided as a supplement to the training, was assessed using a modified 7-day version of the Inter99 food frequency questionnaire (Toft et al. 2008) and the energy intake was determined by a trained dietician with the aid of Master Diätist Data software (Anova A/S).

Standard blood parameters were analyzed in the laboratories of the hospitals comprising those servicing the Capital Region.

#### **7.6 Health-related quality of life**

To investigate HRQOL the Danish version of the questionnaire Short Form 36 (SF-36) was used (Bjørner et al. 1997). The questionnaire comprises eight dimensions covering physical, mental and social aspects of health. For each dimension a score from 0 to 100 was calculated according to standard procedures. Scores were also transformed into a physical component scale and a mental component scale. Higher scores indicate better health. Participants' data were compared with data from an age and gender matched general population sample (Bjørner et al. 1997).

#### **7.7 Muscle power and strength measurements**

Leg extensor power was measured using the Nottingham Leg Extensor Power Rig. The patients were asked to sit in an upright position with their arms folded across their chest, and their active leg towards the push-pedal, whilst their free leg rested on the floor. The push-pedal was located in front of the seat, which makes the direction of movement almost horizontal. First, the leg corresponding to the same side as their dominant hand was measured; thereafter their non-dominant leg was measured. The subject was instructed to push the pedal as hard and as fast as possible. The measurement was repeated at least five times and until no further improvement could be recorded on two consecutive occasions. The inter-trial rest period was 30 seconds. For each subject, the highest value attained was used as result. The data were recorded, computed, and expressed in watts (W) using the Leg Rig software package (PC214E; University of Nottingham, Medical Faculty Workshops, Queen's Medical Centre, Nottingham, UK).

**Picture 5.** Nottingham Leg Extensor Power Rig



Maximal voluntary isometric knee extension was tested in an adjustable dynamometer chair (Good Strength, Metitur Ltd., Jyväskylä, Finland). The measurements were done at a knee angle of  $60^\circ$  from full extension with arms folded across the chest. First, the leg corresponding to the same side as their dominant hand was measured; thereafter their non-dominant leg was measured. The patients were instructed to rapidly produce as much force as possible and hold it for 5 seconds. A minimum of three tests separated by 60 seconds of rest was conducted. For each subject the best performance with the highest value followed by a lower value was accepted as the result. The maximal voluntary contractions were measured in Newtons (N). All data were recorded and stored on a computer using the Good Strength software package (version 3.11. Metitur Ltd., Jyväskylä, Finland).

**Picture 6.** Good Strength, Metitur Ltd.



### **7.8 Physical performance**

Physical performance was measured using the Chair Stand Test from the Senior Fitness Test (Rikli & Jones 2004, Rikli & Jones 2001). The test required the patients to rise to a full standing position and return to a seated position as frequently as possible within a 30 second time frame, whilst maintaining their arms folded across their chest at all times. The time taken to complete the first 5 rises to a standing position was also recorded.

### **7.9 sEMG Recordings**

The sEMG recordings were performed before and after the exercise period in conjunction with the knee extension equipment (see Picture 6) in the gym where the training took place. To avoid exercise initiated delayed onset muscle soreness, and to ensure that the patients were familiar with the training equipment, the baseline sEMG tests were carried out one week after the first exercise session.



The recordings were performed on the mid thigh region, and specifically on the vastus lateralis muscle, while patients were sitting in an upraised position. A dynamic test was done during a 1RM test of complete knee extension from a 90-degree flexion. An isometric test was done with a 20 seconds full extended knee with 50% of the result of the 1RM test. Each participant made the tests with the same machine position at baseline and at re-test.

The study used a double differential electrode configuration, with electrodes (N-00-S & R-00-S; Blue Sensor R, Medicotest A/S, Ølstykke, Denmark) configured as described previously (Harrison et al. 2006). sEMG recordings were taken *via* an ML 131 amplifier connected to a PowerLab 4/25T A/D converter (AD Instruments, Chalgrove, Oxfordshire, UK) with a further connection to a Mac PowerBook Air with Chart v. 5.5.6 Software. Input impedance was 200 M $\Omega$  differential, and a high- and a low-pass filter of 3 Hz and 500 Hz, respectively, were used. Sampling speed was set to 40,000 per second.

Recordings, which were taken following the guidelines laid out in the *European Recommendations for Surface ElectroMyoGraphy* as detailed by the SENIAM project (Hermens et al. 1999). Information regarding any expected/anticipated electromyography performance results was not divulged, and patients were not allowed to follow their sEMG recordings on the computer screen in real time. The recorded sEMG signal was assessed as described previously (Harrison et al. 2006) in terms of signal frequency (Hz) and peak-to-peak amplitude (V), using Chart analysis software and Peak Parameters, and Spike Histogram extensions (AD Instruments, Chalgrove, Oxfordshire, UK).

### **7.10 Muscle biopsies**

Muscle biopsies were obtained from the mid region of the vastus lateralis muscle on a non-HD day. The vastus lateralis muscle is often preferred for research studies due to its large size, making the biopsy obtaining relatively uncomplicated. In addition, studies have shown that complications after biopsies in the vastus lateralis are very seldom and uncomplicated. Furthermore, the vastus lateralis fibre composition is rather consistent, making this muscle relevant for use when more biopsies from the same muscle in the same individual are needed (Lexell et al. 1983). Furthermore, the exercises performed in the present study all target the vastus lateralis muscle directly or indirectly.

Anticoagulants were paused for three days leading up to the biopsy and the international normalized ratio was tested before the biopsy was obtained. Local anaesthesia of the skin, sub cutis and fascia was induced using 1% lidocain. Muscle biopsy tissue was obtained through a five-millimetre incision with the aid of a Bergström biopsy needle using suction. Muscle biopsies were mounted with Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands) and immediately frozen in isopentane cooled in liquid nitrogen and stored at -80 °C until analysis.

### 7.11 Analyses of muscle biopsies

*ATPase histochemistry.* Serial sections (10 µm) of the muscle biopsy samples were cut using a cryostat maintained at -20 °C. Myofibrillar ATPase histochemistry was performed at pH 9.40 after pre-incubation at pH; 4.37, 4.60, and 10.30 (Brooke & Kaiser 1970). Computer image analysis was performed using an image analysis system (TEMA, Scan Beam ApS, Hadsund, Denmark). Fibres were subsequently classified as type 1, type 1/2A, type 2A, type 2AX, and type 2X (Andersen & Aagaard 2000). The number of the minor fibre types (1/2A and 2AX), were so few in some biopsies, that a statistically reliable determination of their size was not meaningful. Therefore, calculations of fibre type CSA were only performed on the three major fibre types (1, 2A, and 2X).

Staining of capillaries was performed using the double staining method (Qu et al. 1997). The double staining method is an immunohistochemical method that uses both anti-UEA-I and anti-collagen type IV antibodies (double staining) to stain both capillaries and muscle fibre borders on the same section. Only truly horizontally cut fibres were analyzed. The day after obtaining the biopsy HD was performed with heparin levels according to post operation guidelines.

### 7.12 Insulin sensitivity

For the investigation of insulin sensitivity, patients undergoing treatment with insulin were excluded.

A 2-hour OGTT with 75 g of glucose was performed after an over-night fast and within a minimum of 18 hours of training. Patients with type 2 diabetes paused their oral antidiabetic medication 72 hours before the test. Samples of plasma glucose (PG), plasma insulin (PI), and plasma C-peptide were drawn at -30, -15, 0, 30, 60, 90, and 120 minute intervals. Fasting values were determined as the mean of the samples from -30, -15 and 0 minutes. The patients were categorized according to diagnoses (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003):

- Normal glucose tolerance (NGT): Fasting PG <6.1 mmol/l and 2-hour PG <7.8 mmol/l
- Impaired fasting glucose (IFG): Fasting PG 6.1-6.9 mmol/l and 2-hour PG <7.8 mmol/l
- Impaired glucose tolerance (IGT): Fasting PG <6.1 mmol/l and 2-hour PG between 7.8-11.0 mmol/l
- IFG and IGT: Fasting PG 6.1-6.9 mmol/l and 2-hour PG between 7.8-11.0 mmol/l
- Type 2 diabetes: Fasting PG ≥7.0 mmol/l and/or 2-hour PG ≥11.1 mmol/l

Besides levels of PG and PI, insulin sensitivity was estimated in terms of total and incremental insulin as well as the PG area under the curve (AUC) taken from the OGTT. The Matsuda Insulin Sensitivity Index (ISI) was calculated according to the following formula;

$10,000 / ((\text{fasting PG} \cdot \text{fasting PI}) \cdot (\text{mean OGTT PG} \cdot \text{mean OGTT PI}))^{0.5}$  (Matsuda & DeFronzo 1999) and the HOMA2 was calculated by the programme HOMA Calculator (The Oxford Centre for Diabetes).

Blood samples were taken from the antecubital vein or the HD arteriovenous fistula. The same procedure was performed for each test. Plasma glucose concentrations were analyzed using the glucose-hexokinase

method (Glucose-quant®, Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Plasma insulin and proinsulin C-peptide were measured using a Sandwich Elektrochemiluminescens-immunoassay (ECLIA) kit (Elecsys, Roche Diagnostics GmbH, D-68298 Mannheim, Germany). All tests were performed on a Roche Modular SWA analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany).

The OGTT was performed identically with regard to the dialysis-procedure for each individual, before and after the control and training period. Patients undergoing PD met for the OGTT without PD fluid and the recently used fluid before the OGTT contained the same glucose concentration before and after the control and training period.

### **7.13 Statistical methods**

Statistical analyses were carried out using PASW® Statistics 18 under the guidance and consultation of a statistician (LIFE, Copenhagen University).

The distribution of the data was tested using Q-Q plots. Since these results showed the data not to be normally distributed, all subsequent statistical analyses were performed using non-parametric tests.

The Mann-Whitney test (Exact significance) was used to compare the PR and the NO groups at pre-test. The Wilcoxon Signed Ranks Test was used to test for differences between pre-test and baseline and between baseline and re-test, as well as to compare the differences during the control period with any difference during the training period.

The second test (baseline test) is used in the analyses of the control period ( $\Delta$  control period) and also the training period ( $\Delta$  training period). In order to perform a summery measure, The One Sample Wilcoxon signed Rank Test was used to test the difference between the two periods ( $\Delta$  control period -  $\Delta$  training period).

Bivariate correlations were tested using Spearman correlation test.

Data are presented as the mean  $\pm$  standard deviation (SD), as the mean  $\pm$  standard error of the mean (SEM), as the range, or as counts and percentages. All tests were two-tailed and significance at the level of  $p \leq 0.05$ .

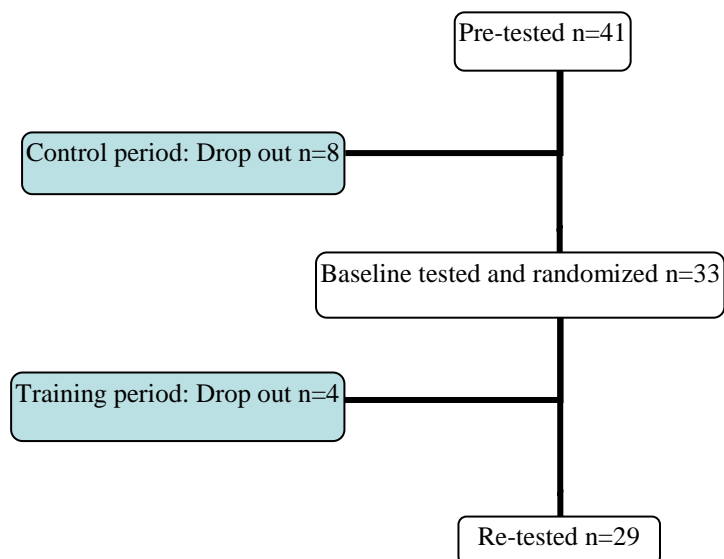
## 8. Results

### 8.1 Patient characteristics

A total of 41 patients were included in the study. The participation flow through the study is presented in the flow chart in Figure 6. Dropout during the control period was due to lack of time (n=1), medical complications not related to the study (n=5 (erysipelas n=1; cancer n=2; neuropathy n=1; heart disease n=1)), discomfort related to muscle biopsy collection (n=1), and moving to another dialysis centre (n=1). During the training period the dropout was due to medical complications not related to the training (n=3 (arteriovenous fistula operation n=2; pneumonia n=1)) and death not related to the training (n=1).

Characteristics of the 29 patients who completed the study are presented in Table 3. The study sample comprised more men than women, reflecting the gender distribution in Danish patients undergoing dialysis. The patients' mean age of 55 years covering a range from 29 to 74 years. When physical activity outside the training was assessed by the patients using a validated question, only three patients were characterized as being inactive. There were no differences between any of the characteristics between the PR and NO groups.

**Figure 6.** Inclusion and dropout during the study



**Table 3.** Characteristics of the participants.

<i>Characteristic</i>	<i>All participants (n=29)</i>	<i>Protein group (n=16)</i>	<i>Non-protein group (n=13)</i>
Gender (F/M)	13/16	7/9	6/7
Age (years)	55 ± 14	54 ± 15	56 ± 14
Dialysis modality (HD/PD)	24/5	13/3	11/2
Duration of renal disease (years)	7.9 ± 6.8	6.5 ± 5.0	9.4 ± 8.3
Duration of chronic dialysis (years)	5.0 ± 5.7	5.1 ± 4.5	4.9 ± 7.2
Comorbidities (score 0-3)	2.3 ± 0.8	2.5 ± 0.7	2.1 ± 0.8
<i>Primary disease</i>			
Type 1 diabetes	2	1	1
Type 2 diabetes	1	1	0
Hypertension	1	1	0
Polycystic	7	2	5
Glomerulonephritis	10	6	4
Nephrosclerosis	2	2	1
Other/unknown	5	3	2
<i>Physical activity</i>			
High active	3	2	1
Medium active	6	2	4
Moderate active	16	9	7
Inactive	3	3	0
Unknown	1	0	1

Data are presented as mean ± SD or count. There were no difference between the protein and the non-protein groups' characteristics.

F = female; M = male; HD = haemodialysis; PD = peritoneal dialysis.

## 8.2 Patient compliance

The patients participated in  $42.5 \pm 0.8$  (range 33-51) exercise sessions during  $16.2 \pm 0.1$  (range 14-17) weeks. The mean number of exercise sessions/week was  $2.63 \pm 0.04$  (range 2.12-3.00), corresponding to  $88 \pm 1\%$  (range 71-100%) of the planned exercise sessions. There was no difference in the compliance between the PR and NO groups.

## 8.3 Side effects

Four patients experienced muscle pain related to the training. The pain lasted one to two weeks and in none of the cases was the reported muscle pain of a degree that needed suspension from training. No other side effects affecting the training protocol were reported.

## 8.4 Clinical variables related to dialysis

In Table 4 selected standard parameters from clinical practice are presented. During the control period there was a decrease in p-albumin. There was no difference in p-albumin between the PR and NO groups at pre-test, baseline and re-test. No other changes in the listed variables were noticed during the control or training period and there was no difference of changes in the variables between the PR and NO groups.

**Table 4.** Control parameters from clinical practice during the study.

<i>Parameter</i>	<i>Pre-test</i>	<i>Baseline</i>	<i>Re-test</i>
Haemoglobin (mmol/l)	7.5 ± 0.2	7.6 ± 0.2	7.4 ± 0.1
Albumin (g/l)	41.2 ± 0.6	40.3 ± 0.7 *	40.8 ± 0.7
C-reactive protein (mg/l)	6.6 ± 1.2	6.1 ± 1.7	7.6 ± 1.8

Data are presented as mean ± SEM. \*  $p < 0.05$  between pre-test and baseline.

Body weight was unchanged during the control period (from  $75.8 \pm 3.7$  kg to  $75.5 \pm 3.6$  kg) and increased (from  $75.5 \pm 3.6$  kg to  $76.6 \pm 3.7$  kg,  $p < 0.05$ ) during the training period. This was also true for the BMI (data not shown). The patients demonstrated a wide range in BMI on inclusion to the study. In clinical nephrology a BMI below  $23 \text{ kg/m}^2$  has been considered to be low. In 38% of the patients BMI values were below this limit. According to general BMI interpretations, 24% of the patients had a BMI below  $20 \text{ kg/m}^2$  and 13% had a BMI above  $30 \text{ kg/m}^2$ . After the training period the distribution of the BMI showed significant changes and now only 14% of the patients had a BMI below  $20 \text{ kg/m}^2$  and 10% had a BMI above  $30 \text{ kg/m}^2$ . During the training period, the changes of body weight and BMI were not different in-between the PR and NO groups.

The energy intake did not change during the study (pre-test  $9418 \pm 453 \text{ Kj} \cdot \text{day}^{-1}$ ,  $130 \text{ Kj} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ,  $1.3 \pm 0.1 \text{ g protein} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ; baseline  $9836 \pm 519 \text{ Kj} \cdot \text{day}^{-1}$ ,  $136 \pm 9 \text{ Kj} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ;  $1.4 \pm 0.1 \text{ g protein} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ; re-test  $9567 \pm 423 \text{ Kj} \cdot \text{day}^{-1}$ ,  $129 \pm 8 \text{ Kj} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ;  $1.3 \pm 0.1 \text{ g protein} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ) and there were no differences in-between the groups during the training period (PR group  $-609 \pm 387 \text{ Kj} \cdot \text{day}^{-1}$ ,  $-12 \pm 6 \text{ Kj} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ,  $-0.15 \pm 0.10 \text{ g protein} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ; NO group  $207 \pm 675 \text{ Kj} \cdot \text{day}^{-1}$ ,  $1 \pm 9 \text{ Kj} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ,  $-0.03 \pm 0.11 \text{ g protein} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ ).

## 8.5 Health-related quality of life

Health-related quality of life measured as the scores of the questionnaire SF-36 is presented in Table 5. While there were no changes in the scores in the control period, several scales increased in the score after the training period. Scores of *Physical Function*, *Bodily Pain*, *Role limitation – Emotional*, *Mental Health* and the summary scale *Physical Component Scale* were improved after the training period. Surprisingly the scores of the scale *Role limitation – Physical* was unchanged after the training. During the training period there were no differences between the PR and NO groups' scores.

At baseline *Physical Function*, *General Health* and *Vitality* were impaired compared to the age and gender matched general population sample. After the training period *Physical Function* and *Vitality* were without statistically significant differences from the general population. *General Health* remained impaired, and maybe a little surprising so did *Role limitation – Emotional* although an increase in numeric scores.

**Table 5.** Patients' health-related quality of life measured by using SF-36 during the study. Higher score indicates better health.

<i>Scale</i>	<i>Pre-test</i>	<i>Baseline</i>	<i>Re-test</i>	<i>P control</i>	<i>P training</i>	<i>P periods</i>	<i>P one-sample</i>
Physical Function	68 ± 3	66 ± 4	74 ± 4	NS	< 0.05	NS	NS
Role limitation – Physical	46 ± 6	60 ± 8	59 ± 8	NS	NS	NS	NS
Bodily Pain	75 ± 5	71 ± 4	77 ± 4	NS	< 0.05	< 0.01	< 0.01
General Health	50 ± 4	49 ± 4	54 ± 4	NS	NS	NS	NS
Vitality	53 ± 5	56 ± 3	61 ± 4	NS	NS	NS	NS
Social Function	82 ± 4	82 ± 3	85 ± 3	NS	NS	NS	NS
Role limitation – Emotional	76 ± 4	70 ± 7	75 ± 8	NS	< 0.05	NS	< 0.05
Mental Health	79 ± 3	79 ± 3	83 ± 3	NS	< 0.05	NS	NS
Physical Component Scale	40 ± 2	40 ± 2	43 ± 2	NS	< 0.01	NS	NS
Mental Component Scale	53 ± 2	53 ± 2	54 ± 2	NS	NS	NS	NS

Data are presented as mean ± SEM. NS = not significant

Significant changes are given for the control period, the training period, the difference between the control period and the training period, and as a one-sample test of the difference between the  $\Delta$  control period and the  $\Delta$  training period.

## 8.6 Muscle power and muscle strength

To adjust the exercise intensity 1RM was performed six times during the training programme. Knee extension 1RM increased from  $56 \pm 5$  to  $82 \pm 8$  kg,  $p < 0.001$ , and leg press 1RM from  $94 \pm 9$  to  $212 \pm 17$  kg,  $p < 0.001$ . There was no difference in the increase between the PR and NO groups.

During the control period the strength of leg extension and knee extension remained unchanged (Table 6). These tests increased significantly during the training period and there were significant differences between the control and training periods. The increase in the muscle power measured as leg extension remained significant even after adjusting for body weight. The knee flexion strength increased during the control period and was unchanged after the training period (Table 6).

## 8.7 Physical performance

Physical performance was not changed during the control period but it was improved after the training period. This was true for the number of rises the patients could perform within the 30 seconds as well the time used for the first 5 rises in the Chair stand test (Table 6). In both tests the change in the training period was significantly different from the control period.

**Table 6.** Muscle power, muscle strength, and physical performance during the control and training period.

<i>Parameter</i>	<i>Pre-test</i>	<i>Baseline</i>	<i>Re-test</i>	<i>P</i> <i>control</i>	<i>P</i> <i>trainin</i> <i>g</i>	<i>P</i> <i>period</i> <i>s</i>	<i>P one-</i> <i>sample</i>
<i>Muscle power</i>							
Leg extension, right (W)	145 ± 12	142 ± 13	183 ± 16	NS	< 0.001	< 0.01	< 0.005
Leg extension, left (W)	141 ± 12	138 ± 12	172 ± 16	NS	< 0.001	< 0.01	< 0.005
Leg extension/body weight, right (W/kg)	1.95 ± 0.14	1.91 ± 0.16	2.38 ± 0.18	NS	< 0.001	< 0.01	< 0.005
Leg extension/body weight, left (W/kg)	1.88 ± 0.14	1.84 ± 0.17	2.28 ± 0.18	NS	< 0.001	< 0.01	0.001
<i>Muscle strength</i>							
Knee extension, right (N)	338 ± 19	343 ± 20	413 ± 25	NS	< 0.001	< 0.01	0.001
Knee extension, left (N)	324 ± 22	318 ± 20	376 ± 22	NS	< 0.001	< 0.01	0.001
Knee flexion, right (N)	134 ± 13	157 ± 13	174 ± 14	< 0.05	NS	NS	NS
Knee flexion, left (N)	140 ± 12	162 ± 13	179 ± 15	< 0.05	NS	NS	NS
<i>Physical performance</i>							
Chair stand test, 30 sec. (no.)	14 ± 1	14 ± 1	17 ± 1	NS	< 0.001	< 0.001	< 0.001
Chair stand test, 5 reps. (sec.)	10.4 ± 0.6	10.7 ± 0.8	8.6 ± 0.6	NS	< 0.001	< 0.01	< 0.005

Data are presented as mean ± SEM. W = watts; N = Newton; NS = not significant

Significant changes are given for the control period, the training period, the difference between the control period and the training period, and as a one-sample test of the difference between the  $\Delta$  control period and the  $\Delta$  training period.

## 8.8 sEMG recordings

In the test of neuromuscular function during maximum concentric contraction, the sEMG frequency was unchanged (Table 7). The amplitude of the sEMG increased after the training period. When the amplitude was adjusted for the resistance (weight lifted (kg) from the 1RM) the amplitude remained unchanged after the training period.

In the isometric hold of 50% of the 1RM, there were no changes in the sEMG frequency AUC or the amplitude AUC during the 20-second sEMG recording. Data on the 20-second sEMG recording are presented in Figures 7 and 8.

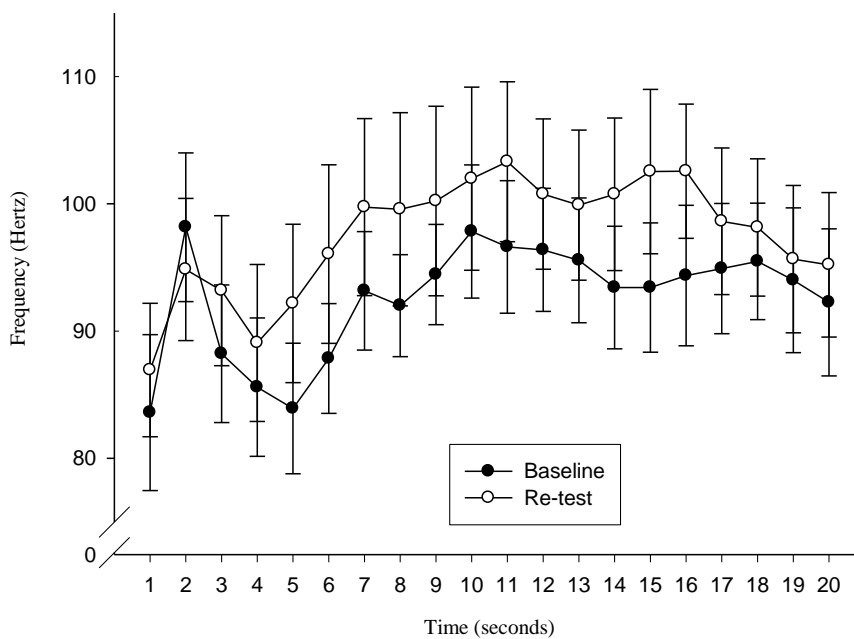


**Table 7.** Surface EMG of maximum dynamic contractions and 20 seconds of isometric holds before and after the training period.

<i>Parameter</i>	<i>Baseline</i>	<i>Re-test</i>
<i>Dynamic 1RM 1. Sec</i>		
Frequency (Hz)	77 ± 8	79 ± 4
Amplitude (mV)	0.322 ± 33	0.483 ± 54*
<i>Dynamic 1RM 1. sec/kilo</i>		
Amplitude/kilo (mV/kg)	0.0079 ± 0.0011	0.0078 ± 0.0009
<i>Isometric 50% of 1RM 20 sec</i>		
Frequency AUC	1801 ± 100	1879 ± 117
Amplitude AUC	3.75 ± 0.58	5.42 ± 0.76
<i>Isometric 50% of 1RM 20 sec/kilo</i>		
Amplitude/kilo AUC	0.163 ± 0.023	0.202 ± 0.027

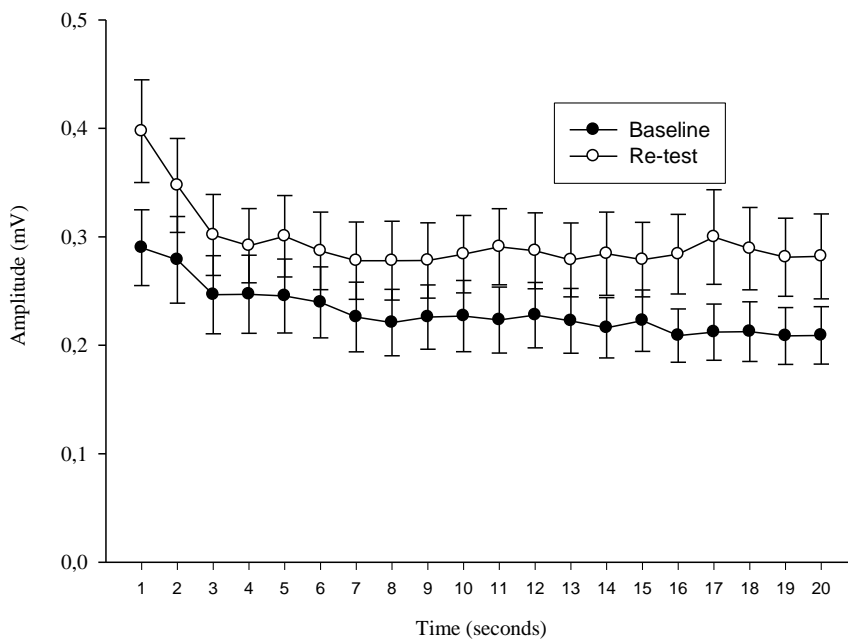
Data are presented as mean ± SEM. \*  $p < 0.05$  between baseline and re-test. The dynamic test is presented as the first second during the knee extension. The isometric knee extension was conducted with 50% of the 1RM resistance at baseline and 1RM at re-test.

**Figure 7.** Surface EMG frequency during 20 seconds of isometric full knee extension (n=25).



Data presented as mean (SEM). There was no difference between the baseline and the re-test area under the curve.

**Figure 8.** Surface EMG amplitude during 20 seconds of isometric full knee extension (n=25).



Data presented as mean (SEM). There was no difference between the baseline and the re-test area under the curve.

## 8.9 Muscle fibre analyses

Muscle fibre type, size and capillary density are presented in Table 8. Muscle fibre composition did not change during the control period. During the training period the relative number of type 2X fibres was reduced. There was no difference on the effect of training on muscle fibre composition in-between the PR and NO groups.

The size of the two major fibre types (type 1 and type 2) remained unchanged during the control and training period. Within type 2 fibres, type 2X fibres increased after the training period while the CSA of type 2A fibres were unchanged (Table 8).

Somewhat surprisingly, in 18 out of 22 (82%) subjects the size of type 1 fibres exceeded the size of the type 2 fibres at baseline. The ratio of the fibre sizes (type 1( $\mu\text{m}^2$ )/type2( $\mu\text{m}^2$ )) was  $1.45 \pm 0.08$  before training and  $1.30 \pm 0.09$  after training.

The capillary density remained unchanged during the study with the exception of a decrease in capillaries per fibre in type 1 fibres during the control period (Table 8).

There were no differences in changes of relative muscle fibre composition, size or capillary density in-between the PR and NO groups (Table 9).

**Table 8.** Composition of muscle fibre types, muscle fibre size and muscle fibre capillary density during the study.

<i>Parameter</i>	<i>Pre-test</i>	<i>Baseline</i>	<i>Re-test</i>	<i>P</i> <i>control</i>	<i>P</i> <i>training</i>	<i>P</i> <i>periods</i>	<i>P one-sample</i>
<i>Fibre type, n=25</i>							
Fibre (n)	173 ± 12	173 ± 11	173 ± 13	NS	NS	NS	NS
Type 1 (%)	46 ± 3	44 ± 3	48 ± 3	NS	NS	NS	NS
Type 2A (%)	38 ± 3	36 ± 3	40 ± 2	NS	NS	NS	NS
Type 2X (%)	17 ± 3	19 ± 3	12 ± 3	NS	0.001	< 0.01	< 0.05
<i>Fibre size, n=22</i>							
Fibre (n)	170 ± 14	168 ± 14	163 ± 15	NS	NS	NS	NS
Type 1 (µm <sup>2</sup> )	4605 ± 303	4440 ± 290	4478 ± 370	NS	NS	NS	NS
Type 2 (µm <sup>2</sup> )	3281 ± 242	3190 ± 219	3575 ± 274	NS	NS	NS	NS
Type 2A (µm <sup>2</sup> )	3682 ± 296	3510 ± 246	3773 ± 296	NS	NS	NS	NS
Type 2X (µm <sup>2</sup> )	2607 ± 216	2552 ± 182	2950 ± 258	NS	< 0.05	NS	NS
<i>Capillary density, n=22</i>							
Fibre (n)	170 ± 14	168 ± 14	163 ± 15	NS	NS	NS	NS
Type 1 (capillary/fibre)	3.0 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	< 0.05	NS	NS	NS
Type 2A (capillary/fibre)	2.4 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	NS	NS	NS	NS
Type 2X (capillary/fibre)	1.7 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	NS	NS	NS	< 0.005
All fibre types (capillary/fibre)	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	NS	NS	NS	NS
All fibre types (capillary/mm <sup>2</sup> )	299 ± 11	283 ± 13	276 ± 14	NS	NS	NS	NS

Data are presented as mean ± SEM. NS = not significant

Significant changes are given for the control period, the training period, the difference between the control period and the training period, and as a one-sample test of the difference between the Δ control period and the Δ training period.

**Table 9.** Change of muscle fibre composition, size and capillary density in the protein group and the non-protein group during the training.

<i>Variable</i>	<i>Protein group</i>	<i>Non-protein group</i>
$\Delta$ Fibre type (%)	(n=16)	(n=9)
Type 1	5 $\pm$ 4	2 $\pm$ 6
Type 2A	1 $\pm$ 3	7 $\pm$ 4
Type 2X	-6 $\pm$ 3	-11 $\pm$ 6
$\Delta$ Fibre size ( $\mu\text{m}^2$ )	(n=15)	(n=7)
Type 1	62 $\pm$ 223	-14 $\pm$ 639
Type 2	267 $\pm$ 343	638 $\pm$ 310
Type 2A	170 $\pm$ 328	464 $\pm$ 247
Type 2X	437 $\pm$ 252	314 $\pm$ 372
$\Delta$ Capillary per fibre (capillary/fibre)	(n=15)	(n=7)
Type 1	0.02 $\pm$ 0.21	-0.17 $\pm$ 0.17
Type 2A	0.08 $\pm$ 0.16	0.03 $\pm$ 0.29
Type 2X	0.16 $\pm$ 0.17	0.26 $\pm$ 0.35
All fibre types	0.03 $\pm$ 0.09	<0.01 $\pm$ 0.07
$\Delta$ Capillary per area (capillary/mm <sup>2</sup> )	(n=15)	(n=7)
All fibre types	-2.5 $\pm$ 21.5	-16.7 $\pm$ 32.3

Data are presented as mean  $\pm$  SEM. There were differences between the groups.

The individual changes of muscle fibre size during the training period in all patients were not significantly associated with the daily energy intake per kg at baseline or the daily protein intake per kg at baseline (data not shown). In the PR group, the individual changes of muscle fibre size during the training period were not associated with the protein supplement (9.4 g) per daily protein intake per kg at baseline (data not shown).

### 8.10 Insulin sensitivity

Five of the patients were not included in the OGTT due to insulin treatment for diabetes. Four of the patients, who completed the study, were not re-tested in terms of glucose tolerance due to illness, change of dialysis modality, or problems arising during the OGTT. Thus, data on 23 patients are presented. The characteristics of this subgroup of patients are presented in Table 10. There were no differences between the PR and NO groups with the exception of a significant higher score of comorbidity in the PR group. In bivariate correlations there was no significant association between comorbidity and the training period changes of blood glucose, insulin, Matsuda ISI, HOMA2, relative muscle fibre composition, muscle fibre size or any of the analyses of capillary density (data not shown). Physical activity outside the training remained unchanged during the study. During the study the clinical control parameters haemoglobin, albumin and C-reactive protein remained unchanged. There was no difference in changes in-between the PR and the NO groups in any of the two periods. Body weight remained unchanged during the control period and increased during the training period (72.8  $\pm$  3.7 to 73.8  $\pm$  3.8,  $p < 0.05$ ).

**Table 10.** Characteristics of the participants from the analysis of insulin sensitivity.

<i>Parameter</i>	<i>n=23</i>	<i>Protein n=14</i>	<i>Non-protein n=9</i>
Age (years)	52 ± 15	53 ± 15	51 ± 14
Gender (f/m)	10/13	6/8	4/5
Dialysis modality (HD/PD)	19/4	11/3	8/1
Duration of chronic dialysis (years)	4.3 ± 4.3	5.1 ± 4.8	3.1 ± 3.3
Duration of renal disease (years)	6.3 ± 4.5	6.7 ± 5.1	5.7 ± 3.8
Body mass index (kg/m <sup>2</sup> )	23.8 ± 4.4	22.9 ± 4.4	25.3 ± 4.2
Albumin (g/l)	41.7 ± 3.3	42.7 ± 3.4	40.1 ± 2.7
Haemoglobin (mmol/l)	7.4 ± 1.0	7.6 ± 1.1	7.2 ± 0.7
C-reactive protein (mg/l)	5.7 ± 6.5	5.6 ± 5.9	6.0 ± 8.0
Comorbidities (score 0-3)	2.2 ± 0.8	2.5 ± 0.8	1.8 ± 0.7 <sup>a</sup>
<i>Primary disease</i>			
Type 2 diabetes	1	1	0
Hypertension	1	1	0
Polycystic	6	2	4
Glomerulonephritis	7	5	2
Nephrosclerosis	3	2	1
Other/unknown	5	3	2
<i>Physical activity</i>			
High active	2	2	0
Medium active	6	3	3
Moderate active	11	6	5
Inactive	4	3	1
<i>Glucose tolerance diagnose</i>			
Normal glucose tolerance	11	7	4
Impaired glucose tolerance	9	6	3
Type 2 diabetes	3	1	2

Data are presented as mean ± SEM or count. <sup>a</sup>  $p < 0.05$  between groups.

F = female; M = male; HD = haemodialysis; PD = peritoneal dialysis

Data for the patients during the study are presented in Table 11. Fasting as well as 2-hour plasma (p) glucose was unchanged during the study. At pre-test only one patient showed a fasting p-glucose value above the normal range (< 6.1 mmol/l). The fasting as well as the 2-hour p-insulin was not changed during the control period but were significantly reduced after the training period.

Plasma glucose and p-insulin AUC of the OGTT were analyzed by using the mean values from the OGTT. All incremental as well as total AUC's were unchanged during the control period. In the training period, the total p-insulin AUC decreased.

The Matsuda ISI, which includes all p-glucose and p-insulin measurements from the OGTT, did not increase as expected after the training period. The  $p$ -value was 0.089 and if one outlier from the sample was removed, the outcome increased significantly.

The source of energy supply and dialysis modality was not associated with any of the significant or non-significant changes of fasting p-insulin, 2-hour p-insulin, fasting p-glucose, 2-hour p-glucose, AUC's, Matsuda ISI or HOMA2 (data not shown).

The decrease in fasting p-insulin during the training period was found to be associated with the baseline fasting p-insulin ( $r = 0.654, p = 0.001$ ), showing that the effect of training on fasting p-insulin was dependent on the baseline level of fasting p-insulin.

One patient was treated with a stable dose of sulfonylurea during the study period.

**Table 11.** Blood glucose, insulin and other measures of insulin sensitivity in the patients during the study.

<i>Variable</i>	<i>Pre-test</i>	<i>Baseline</i>	<i>Re-test</i>	<i>P</i> <i>control</i>	<i>P</i> <i>training</i>	<i>P</i> <i>periods</i>	<i>P one-</i> <i>sample</i>
Fasting glucose (mmol/l)	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.2	NS	NS	NS	NS
2-hour glucose (mmol/l)	8.4 ± 0.8	8.9 ± 0.6	8.5 ± 0.7	NS	NS	NS	NS
Fasting insulin (pmol/l) #	65 ± 10	65 ± 8	53 ± 7	NS	< 0.01	NS	NS
2-hour insulin (pmol/l)	326 ± 52	423 ± 70	326 ± 52	NS	< 0.05	NS	NS
Fasting C-peptide (pmol/l)	3220 ± 362	3234 ± 418	2732 ± 352	NS	< 0.05	NS	NS
2-hour C-peptide (pmol/l)	7562 ± 454	8282 ± 777	7699 ± 614	NS	NS	NS	NS
Glucose AUC total	36 ± 2	36 ± 2	38 ± 2	NS	NS	NS	NS
Glucose AUC incremental	15 ± 2	15 ± 1	16 ± 1	NS	NS	NS	NS
Insulin AUC total	1529 ± 197	1708 ± 219	1518 ± 219	NS	< 0.05	NS	NS
Insulin AUC incremental	1262 ± 173	1448 ± 198	1307 ± 208	NS	NS	NS	NS
Matsuda ISI	4.8 ± 0.6	4.6 ± 0.5	5.1 ± 0.5	NS	NS	NS	NS
HOMA2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	NS	NS	NS	NS
<i>Glucose tolerance</i>							
Normal	11	9	9	NS	NS	NS	NS
Impaired	9	11	12				
Type 2 diabetes	3	3	2				

Data are presented as mean ± SEM. #Normal range 10-125 pmol/l. NS = not significant.

Significant changes are given for the control period, the training period, the difference between the control period and the training period, and as a one-sample test of the difference between the  $\Delta$  control period and the  $\Delta$  training period.

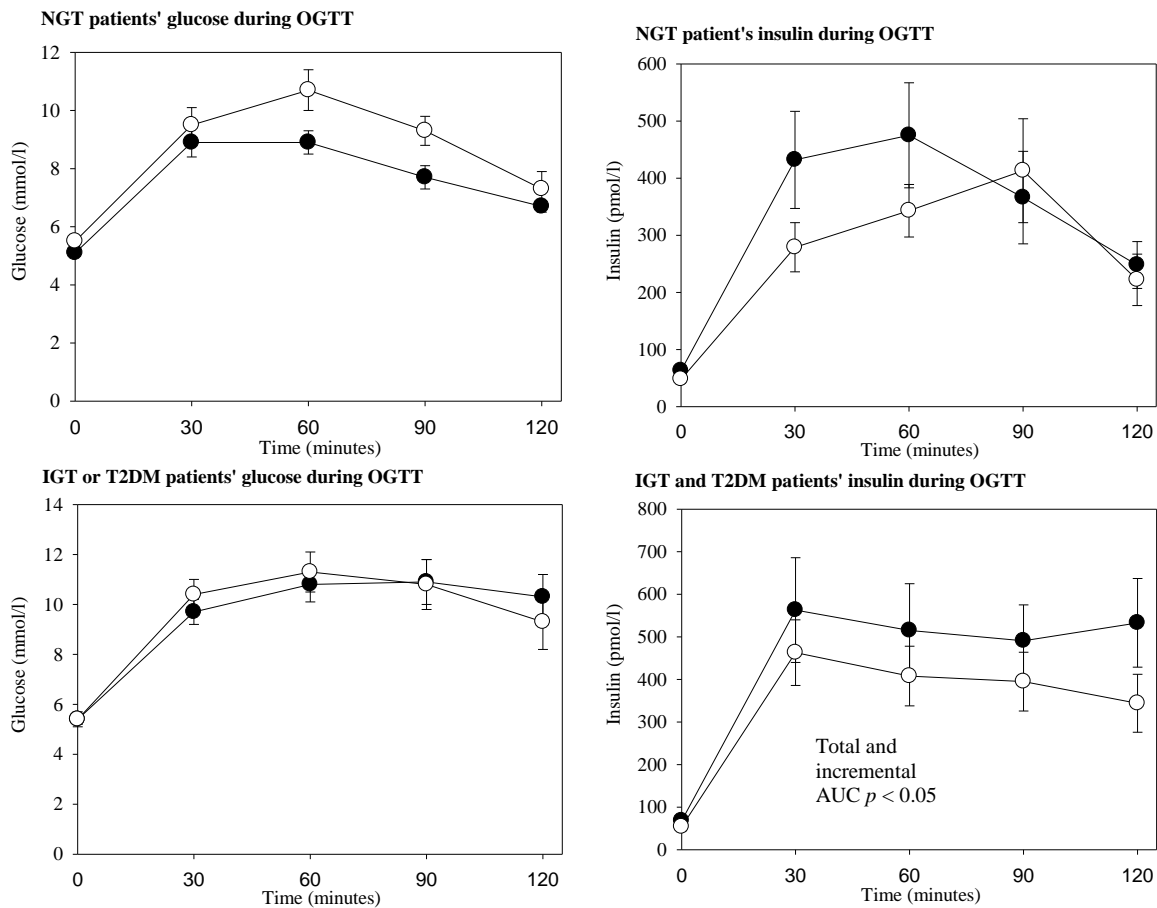
Patients were stratified according to glucose tolerance diagnosis at baseline (NGT vs. IGT and type 2 diabetes) and the response to the training in the strata are presented in Table 12. None of the p-glucose and p-insulin measures in the NGT patients were significantly changed by training. In the IGT and type 2 diabetes patients there were significant effects on fasting p-insulin, 2-hour p-insulin, total p-insulin AUC and incremental p-insulin AUC. The AUC for p-glucose and p-insulin in NGT and IGT or type 2 diabetes patients are presented in Figure 9.

**Table 12.** Blood glucose, insulin and other measures of insulin sensitivity in patients stratified according to glucose tolerance before the training period.

Variable	Baseline	Re-test	P
<b>Normal glucose tolerance (n=9)</b>			
Fasting plasma glucose (mmol/l)	5.1 ± 0.1	5.5 ± 0.1	NS
2-hour plasma glucose (mmol/l)	6.7 ± 0.2	7.3 ± 0.6	NS
Fasting plasma insulin (pmol/l) #	60 ± 8	52 ± 7	NS
2-hour plasma insulin (pmol/l)	248 ± 41	222 ± 45	NS
<b>Impaired glucose tolerance or type 2 diabetes (n=14)</b>			
Fasting plasma glucose (mmol/l)	5.4 ± 0.2	5.4 ± 0.3	NS
2-hour plasma glucose (mmol/l)	10.3 ± 0.9	9.3 ± 1.1	NS
Fasting plasma insulin (pmol/l) #	68 ± 12	54 ± 10	< 0.05
2-hour plasma insulin (pmol/l)	533 ± 104	344 ± 68	< 0.05

Data are presented as mean ± SEM. AUC = area under the curve. # Normal range 10-125 pmol/l. AUC = area under the curve

**Figure 9.** Glucose and insulin during oral glucose tolerance test



Glucose and insulin during oral glucose tolerance test before ● and after ○ 16 weeks of strength training in patients with normal glucose tolerance (NGT) (n=9) and impaired glucose tolerance (IGT) or type 2 diabetes (T2DM) (n=14).





## 9 Discussion

Strength training used as an intervention in patients undergoing dialysis had in the present study several positive effects and was well tolerated by the patients. Self-rated physical function, physical performance, muscle power, muscle strength and insulin sensitivity were all improved. Likewise, the strength training increased neuromuscular function and induced moderate changes in muscle fibre type composition, which could be interpreted as first signs of fundamental reconstruction of the skeletal muscle, favourable in combating the obvious or incipient metabolic disorder observed in this patient group. Somewhat surprisingly, and in spite of the vigorous strength training, it was not possible to establish any significant hypertrophy at the muscle fibre level. In addition, no effects of giving a timed protein intake in relation to strength training sessions were observed.

The participation compliance of  $88 \pm 1\%$  of all training sessions during the strength training programme was high taking into consideration this very ill patient group. Moreover, the drop out rate was acceptable compared to data reported in other studies on patients undergoing dialysis (Bohm et al. 2010). The training programme was well tolerated, with no adverse effects preventing the patients from training. As expected there was delayed onset muscle soreness in the early training phase, but this did not interrupt the training programme.

The clinical relevance of the positive effects of training on muscle power and muscle strength are obvious, since both physical performance as well as self-rated physical function was improved. The patients' score for the SF-36 scale *Physical Function* was significantly improved during the training period with no change during the control period, but with no difference in-between changes during the periods. When the patients' baseline *Physical Function* score was compared to the score from the age- and gender-matched general population it was significantly lower. After being improved by the training the *Physical Function* score was still below the general population score but without being significantly different. The patients reported less bodily pain after the training period by increased scores of the scale *Bodily Pain*. The data do not refer to specific bodily pain, but the improvement is interesting since similar results have been shown in our previous study of exercise in patients undergoing HD (Molsted et al. 2004b). In general, the SF-36 scale scores were not as impaired, compared to the age- and gender-matched general population, as we have shown previously (Molsted et al. 2007, Molsted et al. 2004a). An explanation for this could be the result of improved HRQOL during the last decade in patients undergoing dialysis. In a recent study HRQOL data from 1995 and 2006 in patients undergoing HD were compared with data from the general population (Mazairac et al. 2011). Data from the study of Mazairac and colleagues (2011) seem to indicate that during the last decade HRQOL was improved more in the patients than was noticed in the general population. The authors proposed that the improvement of HRQOL was associated with increased levels of haemoglobin and decreased phosphate levels in the blood. On the other hand, the relatively high SF-36 scores from the present study could also be a result of inclusion of a selected group of patients.

There might have been positive self-reported effects (e.g. less tiredness, less cellulite) which are not captured in the included outcome measurements. Some of these effects might have been noticed if HRQOL had been measured using the disease specific questionnaire KDQOL-SF (Molsted et al. 2005, Hays et al. 1994). The KDQOL-SF was not used due to its length and due to the planned focus on physical function of the generic questionnaire SF-36.

Physical performance in patients undergoing dialysis has been shown to be impaired in several studies and is a treatment target in order to improve the ability to be physically active and thereby delay the need for personal physical assistance. The improvements in physical performance and HRQOL in the present study may be important parameters for the patients to stay motivated towards being physically active, which may have benefits for the patients' health. Physical activity is associated with decreased relative mortality risk in patients undergoing HD (Tentori et al. 2010) as well as in the general population. Thus, beside effects on physical performance and HRQOL the strength training may be associated with decreased mortality risk.

The results of the studies from Cheema and colleagues (2007) and Johansen and colleagues (2006) differ from the results in the present study by reporting unchanged physical performance and only partly improved muscle strength after strength training in patients undergoing HD. One could speculate if this was due to the training dose in the studies where the training was conducted during HD. Exercise during HD may not only limit the choice of exercise but also the intensity. With respect to this, strength training during HD has been shown to have other positive effects on self-rated physical function and it is time saving for the patients, who may therefore find it attractive.

In a previous randomized study on patients undergoing HD we showed that aerobic capacity could be improved by only two endurance training session per week for five months (Molsted et al. 2004b). In the same study we found an improved physical performance in the training group measured by a squat test but without a significant difference between the training and the non-training control groups. Even though the patients from our previous study conducted training only two times weekly their training dose may be comparable to the present study due to 60 min of training per session. With a modest effect on physical performance in the previous study encompassing endurance training the present study of strength training showed a more pronounced effect on physical performance with a significant difference between data in the control and the training periods.

The significant improvement of muscle strength and muscle power was expected due to the dose, intensity and progression in the training programme. The most pronounced effects were noticed in the dynamic muscle power test showing mean improvements of 25% and 29% in the left and right legs, respectively. The muscle strength gain in the leg press and the knee extension occurred in spite of a modest muscle hypertrophy at the fibre level and may therefore mainly be achieved as a result of improved neuromuscular function including motor unit synchronization. The improvement in muscle strength in the present study was achieved in parallel with an increase in the amplitude from the sEMG signal, which was an expected result of

the strength training (Aagaard 2003). When the amplitude data were adjusted for the increased resistance (lifted weight), the amplitude remained unchanged. The reason for this finding is uncertain, but could be the result of enhanced spatial summation (Henneman et al. 1965). If so, this could lead to the suggestion that an improved synchronization of the activated motor units was the main contributor to the improved muscle strength. The sEMG baseline recording was made three training sessions after the training programme had started. The untrained patients might well have improved their neuromuscular function in the very early phase, which could then of course not be detected due to the nature of the testing protocol. Thus, using a true baseline test may have been associated with minor but even further improvements of neuromuscular function. It has to be noted though, that this type of signal analysis reveals a finding that is consistent with what is known to occur during the initial period following commencement of exercise training (Aagaard 2003). Typically one observes an improvement in neuromuscular recruitment and activation of “coordination” that may precede fibre hypertrophy in these patients.

Previous data have suggested that neuromuscular function is affected by uraemia (Krishnan et al. 2005, Harrison et al. 2006) although data concerning potential neurotoxins was beyond the scope of the present study. Besides which the strength of this study is that sEMG data were collected with the same relation to dialysis before and after the training period. Thus, the major changes in neurotoxins noticed after dialysis should not affect the sEMG data. Of course recordings of sEMG from muscles of this type of patient are not without problems, and the interpretation of data should not be made without careful considerations. The results may be affected by several parameters, such as the configuration of the recording electrodes (size, distance apart etc.), skin thickness and subcutaneous tissue composition and depth, blood flow, muscle fibre diameter and orientation as well as the number of active motor units within the muscle (De Luca 1997). In addition to these parameters are intermediate factors including the choice of electrode filter, the risk of cross-talk from adjacent muscles or underlying tissue, the occurrence of spatial filtering as a consequence of fibre depth, the volume of the muscle that is detectable, and the conduction velocity of muscle fibres (Harrison et al. 2012). However, most of these parameters were carefully controlled, making the data important in the interpretation of the increased muscle strength.

Previous studies have shown relatively few type 1 fibres in patients undergoing HD compared to healthy controls (Molsted et al. 2007, Fahal et al. 1997). In our previous study we did not find an association of low type 1 fibre proportions and pathology or physical inactivity, but there might be a connection between these conditions since the same pattern has been noticed in spinal cord injury subjects, which are physically inactive and do not have neurological stimulation to their muscles (Andersen et al. 1996). However, the previous low proportions of type 1 fibres (Molsted et al. 2007) were not evident in the present study, in which the type 1 proportion was closer to what can be expected in the general population.

Before the training was conducted a relatively high proportion of type 2X fibres was found and strength training generated a decrease in the relative number of this fibre type. This was an expected finding since this

outcome of high-load strength training has been shown to be a robust response in healthy subjects (Putman et al. 2004, Andersen & Aagaard 2000). A more pronounced transformation from 2X to 2A fibres was expected if compared to a study on healthy subjects, who underwent a similar type and volume of strength training with high loads and a structured progression (Andersen & Aagaard 2000). The reasons for the limited transformation observed could be several, but may be found in difficulties of recruiting the large type 2X motor units during voluntary contractions, a scenario that could exist especially in infrequently used muscles. Furthermore, this result may be influenced by the heterogeneous sample of patients with a wide range of comorbidities and age (Andersen 2003).

It was expected that the training dose used in the present study would increase muscle mass. Even though body weight increased in the present study the hypertrophy was very modest, since significant hypertrophy was only observed in the type 2X fibre pool. It could be suggested that the expected hypertrophy may have been suppressed by the patients' disturbance in terms of protein balance. Thus, the achieved post exercise protein synthesis may have been exceeded by protein breakdown caused by uraemia (Bailey et al. 2006), the dialysis treatment (Lim et al. 2005), acidosis (May et al. 1986), low grade inflammation (Zhang et al. 2009), IR (Pupim et al. 2005, Lee et al. 2007, Siew et al. 2007) or a low level of anabolic hormones (Kopple et al. 2006, Carrero et al. 2011). In addition, increasing age diminishes the protein synthesis rate (Kumar et al. 2009). Even when the protein supply and its potential subsequent protein synthesis were added to the potential training-induced protein synthesis, no further hypertrophy was noticed. Although speculative, the training period may not have been long enough to induce hypertrophy in this sample of chronic diseased patients. The result is partly supported by earlier studies. Two previous studies have investigated the effect of strength training on muscle CSA in patients undergoing HD. Johansen and colleagues (2006) found a limited hypertrophy of 2.5% in CSA compared to a 6.8% atrophy in a control group and thereby a positive net effect of 9.3% of the quadriceps muscle measured by magnetic resonance imaging, whereas Cheema and colleagues (2007) found no significant hypertrophy when using computed tomography scanning. Compared to the 6.8% atrophy during a three month control period reported by Johansen and colleagues (2006), the non-significant atrophy in the control period in the present study was only 4.5% and 2.8% in the type 1 and type 2 fibres, respectively, in spite of an enhanced time frame of 16 weeks (3.7 months) compared to the period in the study of Johansen and colleagues (2006). When the changes during the control period and the training period in the present study are combined, the type 2 fibre CSA positive net effect was 14.9%, but this was not significant.

In regard to the lack of effects on muscle fibre size the result may be affected by a type 2 error risk. Even though power calculations on collected data should be interpreted with caution, it may be performed to suggest sufficient numbers of patients in coming studies. Whilst the mean CSA in type 2 fibres increased by  $385 \mu\text{m}^2$ , the power based on the present data including 22 patients was only 29%. If the power should reach

80%, then 76 patients should be included assuming an equal data distribution and variance. Thus such a calculation suggests a high risk of a type 2 error in the present data.

When the patients were stratified to those achieving hypertrophy and those not achieving hypertrophy in the type 2 muscle fibres after strength training, the only noticed difference was a weight gain in the hypertrophy group and a weight loss in the atrophy group. Thus, in the collected data in the present patient sample it was not possible to suggest other variables associated with change in muscle fibre size after training.

At pre-test, the ratio between the size of type 1 and type 2 fibres showed somewhat unusual values. In general, and in healthy subjects the size of type 2 fibres should exceed or at least equal the size of type 1 fibres. In the majority of the patients from this study the opposite was observed. The finding of this study is supported by data from a previous study (Sakkas et al. 2003) in which the CSA of type 1 fibres were larger than that noticed for type 2 fibres in patients undergoing dialysis. A phenomenon also shown in uraemic men with CKD (Clyne et al. 1993). Why this is so is difficult to say and we have no good reason to suggest if the affect is due to the disease itself or the limited use of muscles in patients undergoing dialysis.

At pre-test IR was observed in 56% of the included patients, thereby representing an important treatment target. As expected there were patients with undiagnosed type 2 diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003). This was found in 11% of the patients and emphasizes the need for attention towards this comorbidity in clinical practice.

The strength training improved insulin sensitivity in all patients, but when patients were stratified according to their glucose tolerance diagnosis, the effects were present only in patients with IGT or type 2 diabetes. The effects were consistently shown in the AUC of total and incremental insulin as well as in the fasting and 2-hour p-insulin.

Fasting and 2-hour p-insulin represent two aspects of insulin sensitivity in skeletal muscle. The post prandial 2-hour p-insulin may provide a more pronounced representation of peripheral muscle insulin sensitivity compared to fasting p-insulin, which to a greater extent is based on a closed action between liver glucose and the beta cell function to maintain homeostasis (Faerch et al. 2010). This is in accordance with the results from the present study where the greatest effect was noticed in the 2-hour p-insulin after training.

The effect on insulin sensitivity was achieved after a relatively limited weekly duration of training which was up to three times 30 min. In relation to that it is important to note that the conducted exercise activated the large human skeletal muscles vastus lateralis, gluteus maximus and the hamstring muscles. Thus, even though the weekly training duration may not seem long, metabolism may be changed through activation of a large amount of muscle mass.

In the present study blood glucose data remained unchanged. The fasting values were in most cases within a normal range, whereas 2-hour p-glucose values exceeded the normal range in several patients even after the training programme. It could be speculated that a prolonged period of training is necessary to decrease the 2-hour p-glucose in these patients' with their relatively inactive muscles.

Besides changes in intracellular pathways, aerobic training reduces IR through an increased capillary density, increased mitochondrial density and a change in fibre type composition, whereas strength training besides changing fibre type composition may be effective due to increased muscle mass. It is not surprising that capillary density remained unchanged and muscle fibre type composition was changed by a relative decrease of the number of type 2X fibres in parallel with an increase in insulin sensitivity. Type 2X fibres have the lowest glucose uptake capacity (Olsson et al. 2011) and high proportions of type 2X fibres have been associated with the relative risk of IR (Nyholm et al. 1997). The change in muscle fibre composition in the present study fits well with the achieved effect on insulin sensitivity, but other improvements in the metabolic capacity, e.g. GLUT-4 transporters, insulin receptors, glycogen synthase or glycogen levels may also be involved.

A decrease in abdominal or other fat tissue is associated with improved insulin sensitivity (Bacchi et al. 2012). In patients with type 2 diabetes strength training may be associated with reduced fat mass whilst body weight has been suggested to be stable (Gordon et al. 2009). Body composition was not measured in the present study and it is therefore unknown as to whether the improvement in insulin sensitivity was associated with a reduction in abdominal or other body fat. This is however unlikely since the body weight increased in spite of the modest muscle hypertrophy.

There were no effects on insulin sensitivity of combining protein with training compared to the non-protein supply. The results of the present study are supported by a recent study on obese patients with type 2 diabetes in which a high protein diet and strength training were not followed by a decrease in fasting p-insulin more than that shown after strength training and a normal diet (Wycherley et al. 2010).

In regard to the lack of effects on the 2-hour p-glucose this result may also be affected by a risk of a type 2 error. In a power calculation on the present data to suggest sufficient numbers of patients in future studies p-glucose decreased by 0.99 mmol/l in 14 patients with IGT. When  $p = 0.05$  the result was associated with a power of 25% and if the power should reach 80% then 54 patients should be included. This calculation suggests another high risk of a type 2 error in the present study.

The present study did not include a control group receiving high load strength training without timed energy supply. It is therefore unknown if the energy supply *per se* had any additional effect on the outcome. It cannot be ignored that even a non-protein energy supply on the exercise days could have had a minor effect on the outcome. On the other hand, protein synthesis is induced by protein intake and a study on healthy subjects showed that protein homeostasis remained unchanged after a post exercise intake of non-protein energy (Levenhagen et al. 2002) and there are no data to suggest that this should be different for patients undergoing dialysis. Furthermore, the patients' basic nutritional status may be important with regard to achieving effects with a nutritional supplement. With a low energy intake a nutritional supplement may be used as energy rather than to improve protein synthesis and induce muscle hypertrophy, but there was no association between baseline energy intake or baseline protein intake and effects on muscle fibre size or

muscle strength. When the supplemented protein of 9.4 g was adjusted for daily protein intake per kg body weight in the PR group, there was no correlation to the relative protein supplement or change in muscle fibre size after the training period.

The source of the protein supplement was based on leucine from whey, which has been recommended as a relevant supply in relation to strength training (Kim et al. 2010). While the source of the protein seems adequate, the quantity of protein supplied may not have been enough to achieve an additional effect in these patients. Even though a study in older healthy persons showed effects of adding 10 g of protein after each exercise session (Esmarck et al. 2001), this might not be enough to stimulate muscle protein synthesis, which exceeds protein breakdown in patients marked by disease-related catabolism. The present 9.4 g of protein in relation to three exercise sessions per week is a relatively low amount compared to the patients' daily mean protein intake per kg body weight. In relation to this it is important to note that not only the amount but also the timing of the additional protein intake is important, but whilst studies proposing positive effects of adding protein intake to strength training do exist, evidence of an effect in elderly persons is still debated (Campbell 2007). Further studies of such interventions in patients with chronic diseases are now needed if we are to draw more definitive conclusions about such a relation and potential benefits. The protein dose in the present study was chosen to design an intervention that was possible to replicate in clinical practise. Low appetite is common in patients undergoing dialysis and by reducing the phosphate and potassium in the protein drink as in the present study the taste does not improve. Thus, ingesting a higher protein dose immediately after training than that used in this study may be possible in a selected patient sample but may not be relevant for broader samples. The question remains as to whether an additional protein intake besides what was ingested in this study could augment the effect. Lim VS et al (2005) suggested that an intradialytic nutritional supplement may blunt the catabolic effect of HD, which may exceed the protein synthesis achieved by the training and the post-training protein intake. The data are supported by an intervention study in which patients improved their muscle strength and body weight after six months of protein supply during dialysis (Dong et al. 2010). Thus, in order to decrease protein breakdown and thereby improve the balance between protein synthesis and protein breakdown, protein supply during dialysis may have the same relevance as a protein supply in relation to training in patients undergoing dialysis.

The data on energy and protein intake in the present study was based on the previous seven days of food intake. The method was chosen to simplify the collection of these data even though more specific methods may be more valid. Such data may be associated with recall bias and over- or under-estimations by the patients and thereby represent a limitation in the present study.

The effects of the tested strength training may have important clinical implications. It was safe and could be conducted within a 30 min time frame. Even though high loads are needed to achieve the effects presented in this study, the exercises are simple and participants do not need special skills to be able to perform them. As in other clinical trials this study included a sample of patients who fulfilled specific criteria, but there should

be no reasons why a broader part of dialysis centre populations should not be able to participate in training programmes like the present.

The relatively high percentage of patients with IGT and type 2 diabetes underscores the relevance of a focus on IR. By using the fasting and two hour data of the OGTT to estimate patients' glucose tolerance, it will be possible to identify patients who may benefit from strength training with regard to insulin sensitivity.

## **10 Limitations in the study**

In the previous paragraphs limitations related to specific areas of the study have been discussed. As a general limitation the lack of a control group receiving training only should be noted. This was not included due to the limited capacity in the training intervention and the trial period and would with the included number of patients have minimized the number of patients allocated to each group. In addition, this study is limited by the relatively low number of participants.

Several outcome measurements could have been included and would with no doubt have improved the study. The amount of outcome measurements was limited by the physical capacity of the chronic diseased participants. The measurement of glucose tolerance could have been replaced by the gold standard euglycaemic hyperinsulinaemic clamp technique and the measurement of muscle size at the fibre level would have been provided by a whole muscle scan, facilitating the interpretation of improved insulin sensitivity.

## **11 Conclusions**

In conclusion, high-load strength training was well tolerated by patients undergoing this form of treatment and the compliance to the training programme was high. Strength training was associated with clinically relevant improvements in HRQOL, physical performance, muscle power and muscle strength. The magnitude of the increase in muscle strength could not be explained by muscle hypertrophy and may more likely be related to neuromuscular adaptations. Furthermore, strength training induced a decrease in the relative number of fast type 2X fibres and in parallel increased insulin sensitivity.

The data from the present study did not reveal any indications of additional benefit of combining the training programme with a timed protein intake, which may be due to insufficient protein synthesis compared to the increased protein breakdown in these patients and/or an inadequate amount of ingested proteins. Given the multiple positive effects, high-load strength training should be recommended to patients undergoing dialysis, whereas effects of combined timed protein intake as a means of further improving strength training was not evident in the present study.



## **12 Perspectives**

A strength training programme as used in the present study or similar programmes including heavy loads can be used in clinical practice and also conducted by a broader sample of patients undergoing dialysis. The costs of the training programme have not been calculated, but this kind of rehabilitation is generally relatively cheap, especially compared to the costs of dialysis and the costs associated with comorbidities that such a programme may reduce. As a minimum the training programme should be recommended to the patients, who can choose to follow the programme outside of their dialysis centre if it is not implemented in the centre itself. Thus, strength training may not only be effective in the treatment of dialysis patients but may also be used more widely to combat comorbidities in other chronic diseases, or as a preventive measure to hinder or slow down the development of comorbidities associated with chronic disease.

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