CHAPTER 1

GENERAL INTRODUCTION
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The life expectancy within the western population is increasing, resulting in a tremendous rise in older people. In the Netherlands, the population of people above the age of 65 years will increase by more than 50% within the next 20 years [22]. In this increasing elderly population, the prevalence of age-related diseases will augment, resulting in high morbidity as well as a rise in health care costs. Therefore, maintaining a healthy and independent lifestyle is of major personal as well as economical interest. Two age-related changes of the musculo-skeletal system, eventually resulting in higher morbidity are 1) osteoporosis, characterised by a low bone mineral density (BMD), and 2) sarcopenia (age-related decrease in muscle mass and quality).

In older people, not only the muscle force generating capacity is reduced due to sarcopenia, but also the oxidative capacity of the muscle is reduced, resulting in a lower exercise tolerance [27]. Furthermore, the maximal velocity of muscle contraction also decreases and muscles become slower [130]. Taken together, determinants of muscle contractile characteristics change considerably which may complicate the execution of daily activities and may result in walking limitations. For instance, the recovery after tripping is impaired in older people compared to younger individuals, resulting in increased fall risk [136,137]. The low BMD in combination with the high number of falls among older people results in a high fracture risk [80]. Since fractures in older people are associated with higher morbidity and mortality [79], prevention of loss in muscle performance (muscle force generating capacity and oxidative capacity) as well as loss in bone mass is highly indicated.

There is growing evidence that the amount of daily physical activity is inversely related to fracture risk in older people [126] and physical exercise training may therefore help to maintain an independent lifestyle. Since muscle performance and bone mass are reduced with increasing age, exercise training should aim to increase and/or preserve muscle force generating capacity, oxidative capacity and bone mass. To our knowledge, as yet, it is not known how these three traits can be improved within one training program.

The aim of this thesis was to investigate: 1) whether in older people, parameters of physical functioning are associated with bone quality and fracture risk and 2) whether it is possible to increase muscle performance (maximal muscle force and oxidative capacity) as well as bone mass by combining peak power and endurance training. The
knowledge acquired during the training study is requisite for development of training programs to improve and maintain musculo-skeletal health in older people and contribute to an independent lifestyle. The results of the study addressing the first aim may contribute to the selection of older people at high fracture risk for whom a training program is strongly indicated. In addition, these tests for physical functioning may be used to evaluate training programs that aim to increase muscle performance and bone mass in order to reduce fracture risk.

In order to develop a training program aiming to increase muscle force generating capacity, oxidative capacity and bone mass, insight in the physiology of muscle and bone tissue is required. In the following sections, muscle characteristics which contribute to the force generating capacity and oxidative capacity of the muscle will be discussed, as well as characteristics of bone tissue and bone remodelling. In addition, age-related changes on muscle and bone tissue will be discussed as well as how these two tissues adapt to different types of training.

Muscle physiology
The main function of skeletal muscles is to maintain posture and to induce limb movements. Skeletal muscles are composed of muscle fibres which are embedded in a network of connective tissue and insert via aponeuroses and tendons into ligaments or bones. Muscle fibres are multinucleated cells and consist of myofibrils, which are built by sarcomeres arranged in series. Actin and myosin filaments are two major filaments of the sarcomeres and involved in muscle contractions. Muscles fibres are innervated by motor neurons. A motor neuron together with the muscle fibres it innervates forms a motor unit. The number of motor units within a muscle as well as the number of muscle fibres within a motor unit varies considerably between muscles and depends on the function of the muscle. Muscles used for fine motor control usually contain small motor units whereas muscles used to generate high forces contain larger motor units.

In response to an action potential, calcium ions are released from the sarcoplasmic reticulum (SR) and bind to troponin-C. This results in exposure of myosin binding sites on actin filaments which allows the formation of cross-bridges between myosin heads and actin filaments. Muscle contraction occurs when the myosin heads pull the actin filaments toward the centre of the sarcomere. Uptake of calcium ions by the SR
results in its removal from the troponin-C. Consequently, myosin binding sites on the actin filaments are blocked again by tropomyosin, leading to relaxation of the muscle. Adenosine triphosphate (ATP) is required for the detachment of cross-bridges as well as for the transport of calcium ions back into the SR. The contraction velocity as well as the relaxation rate of muscle fibres are dependent on the ATPase activity associated with the different myosin heavy chain (MyHC) isoforms expressed in skeletal muscle fibres.

Muscle fibre types
Skeletal muscles express four different MyHC isoforms: type I, type IIA, type IIX and type IIB (the latter extremely rare in human skeletal muscles) of which a muscle fibre is composed (also combinations of the different MyHC isoforms). Muscle fibres predominantly consisting of MyHC type I will further be referred to as type I fibres, muscle fibres predominantly consisting of MyHC type IIA as type IIA fibres etc. Type I muscle fibres have the slowest contraction velocity and type IIB the fastest. The type I and IIA muscle fibres have generally a small fibre cross-sectional area (FCSA), are fatigue resistant (high mitochondrial density and myoglobin content) and are particularly responsible for postural adjustments and/or movements which are performed for a prolonged period of time at a low power output. The faster (type IIX and IIB) muscle fibres have a large FCSA, are less fatigue resistant (lower mitochondrial density and myoglobin content) and are used for powerful movements. Motor units consisting of slow muscle fibres are generally small (consisting of a relatively low number of muscle fibres), whereas motor units of fast muscle fibres consist of relatively more fibres having also a large FCSA and are predominantly used during powerful contractions. Therefore, the muscle fibre type composition in combination with the mean FCSA are major determinants of the force generating capacity of a muscle.

Mitochondria
The oxidative capacity of the muscle is largely determined by the number of mitochondria as well as by their efficiency. Mitochondria are the organelles within a muscle fibre which are best known for their ATP production via oxidative phosphorylation. Within the mitochondria, two systems contribute to the aerobic production of ATP: the tricarboxylic acid (TCA) cycle, also known as citric acid cycle or Krebs cycle, and
the electron transport chain (ETC). In the cytosol, glycogen is converted into pyruvate via glycolysis which can then enter the mitochondria. Within the mitochondria, pyruvate, fatty acids or amino acids are converted into acetyl-CoA to enter the TCA cycle. Several biochemical reactions occur in the TCA cycle, during which nicotinamide adenine dinucleotide (NAD\(^+\)) and flavin adenine dinucleotide (FAD) are reduced forming NADH and FADH\(_2\), respectively. Electrons and protons (H\(^+\)) are separated from NADH and FADH\(_2\) and transferred to the ETC at the inner membrane of the mitochondria. The ETC uses the energy obtained from the transfer of electrons to pump H\(^+\) ions across the inner membrane into the intermembrane space of the mitochondria. The high proton concentration in the intermembrane space creates an electrochemical proton gradient across the mitochondrial inner membrane, which allows the flow of H\(^+\) through the ATP synthase back into the mitochondrial matrix, generating ATP. The transport of the electrons through the ETC is only possible in the presence oxygen which acts as a final electron acceptor.

Succinate dehydrogenase (SDH) is an enzyme which is involved in the TCA cycle as well as the ETC and its activity is a representative measure for mitochondrial activity [188]. Maximal oxygen consumption of a single muscle fibre has been shown to be linearly related to the SDH activity [188]. Therefore, by analysing SDH activity using calibrated histochemistry, the \(\text{VO}_{2\text{max}}\) of a muscle cell can be determined. This measure is useful to obtain an indication of the oxidative capacity of a muscle.

**Bone physiology and bone remodelling**

Important functions of the skeleton are to provide mechanical support to the body, protect vital organs and enable limb movements with the attached muscles. In addition, bone serves as an important storage for calcium and phosphate. The human skeleton consists of approximately 80% compact bone, also known as cortical bone and of 20% spongy bone, also known as cancellous or trabecular bone. Cortical bone is composed of osteons (also known as Haversian system) which are made up of lamellae surrounding a central canal, the Haversian canal [169]. The shaft of long bones is made up of cortical bone. Trabecular bone, typically located within the proximal and distal ends of long bones and in vertebrae and pelvis, consists of rods and plates, which are aligned in the directions of the loading [169]. Trabecular bone consists of trabecular bone packets, which are the equivalent of osteons in cortical bone [106].
Bone remodelling

In the adult skeleton, bone is constantly in a process of remodelling. After a signal (i.e. altered mechanical loading, damage or paracrine or endocrine factors), bone remodelling starts with bone resorption by osteoclasts, followed by the formation of new bone by osteoblasts. By secretion of H⁺ ions and protease cathepsin K, osteoclasts dissolve minerals and degrade the surrounding bone matrix. Following this, osteoblasts synthesise type 1 collagen which is the major constituent of the new bone matrix to be formed around the osteoblast (approximately 90% of all bone matrix proteins), as well as non-collagenous bone matrix proteins (i.e. osteocalcin and osteopontin) [29]. The initial collagen matrix deposited by osteoblasts is not yet mineralised and is referred to as osteoid. The osteoid will, by the deposition of calcium and phosphate, become mineralised [29].

After this process, osteoblasts either become bone lining cells on the bone surface, or osteocytes when they are incorporated in the mineralised bone matrix or undergo apoptosis [116]. Osteocytes are located in lacunae of the bone matrix and connected to each other via their processes that lie within very thin canals (canaliculi) within the bone matrix. This network enables communication between different types of bone cells [11]. Osteocytes are presumed to orchestrate the mechanical loading-induced bone adaptation [94].

Age-related changes in muscle and bone tissue

Muscle

In older people, muscle force generating capacity is reduced [130]. The reduction in muscle force generating capacity may be due to one or more of the following mechanisms: a reduction in the number of muscle fibres (mainly type II fibres), muscle fibre atrophy and/or a reduction in specific force of muscle fibres [130]. This age-related loss of muscle mass and quality is referred to as sarcopenia (Greek meaning loss of flesh). Factors that may contribute to sarcopenia are reduced physical activity, hormonal and nutritional changes as well as an increased prevalence of chronic diseases [130].

Besides muscle force generating capacity, also the oxidative capacity of human skeletal muscle is reduced with aging [27]. In older people, both number of mitochondria and activity of oxidative enzymes are reduced, which results in reduced maximal rate of ATP production [66,81]. A possible explanation for this reduced mitochondrial
function is the age-related increase in mitochondrial DNA (mtDNA) abnormalities [66]. Although, the majority of the mitochondrial enzymes are encoded in nuclear DNA, also mtDNA encodes several proteins which are essential for the ETC. With increasing age, the incidence of mutations of mtDNA is increasing in humans, resulting in less functional mitochondrial proteins [66]. This mitochondrial dysfunction is presumed to enhance the production of reactive oxygen species (ROS) which again can induce damage on mtDNA, resulting in a vicious circle.

Despite the fact that mitochondrial function and thereby oxidative capacity is reduced with aging, muscles of older people were shown to be more fatigue resistant compared to those of younger individuals [142]. This paradoxical observation may be explained by the fact that during aging in humans, there is a relative increase in slow type I muscle fibres [130]. Although aging muscles may be more fatigue resistant, older people may still experience difficulties in the execution of daily activities. As their force generating capacity is reduced [142], activities such as stair climbing occur at a higher percentage of their maximal force generating capacity. This may eventually still lead to a reduced exercise tolerance. In older people, low walking speed was shown to be associated with mortality [153]. In addition, oxidative capacity has been shown to be positively related to walking speed [25]. This suggests that although fatigue resistance may be increased in older people, the reduced oxidative capacity observed in aging muscles may still be an important target for training to maintain an independent lifestyle. Therefore, not only muscle force generating capacity should be improved in older people, but also the oxidative capacity of the muscle.

Bone
During childhood and adolescence, much more bone is deposited than withdrawn, so the skeleton grows in both size and density. The amount of bone mass can keep growing until the age of approximately 30 years [127]. At that point, bones have attained their maximum strength and density, known as peak bone mass. A variety of genetic (i.e. gender and race) and environmental (i.e. exercise habits and diet) factors influence peak bone mass. After the third decade, bone mass begins to decrease gradually and this decrease is accelerated in women during the first few years after menopause due to lack of oestrogen [116,127]. High peak bone mass is beneficial for a healthy bone mass at an older age, suggesting that people should aim at attaining a
high peak bone mass [12,127].

When bone resorption exceeds the rate of bone formation, the thickness of cortical bone as well as the thickness and number of trabeculae of trabecular bone will decrease [168]. The negative balance of bone resorption and bone formation results in an increased bone loss as observed in people with osteoporosis. Osteoporosis is characterised by a low BMD (>2.5 standard deviations below the mean value of peak bone mass of young healthy women between 20-30 years of age) and micro-architectural deterioration, leading to fractures after minimal to moderate trauma. Osteoporosis can have different causes and can be classified into primary and secondary osteoporosis. Primary osteoporosis is caused by the oestrogen- and/or age-related decline in bone mass eventually resulting in a low BMD. Secondary osteoporosis is bone loss due to specific conditions, such as chronic diseases, medication use, inactivity and hormonal changes [74].

**Adaptive responses of muscle and bone tissue to training**

*Improving muscle performance by training*

Skeletal muscles have the ability to undergo large adaptations in muscle fibre size and oxidative capacity depending on the physical demands [191]. Exercise training consisting of long duration exercise at low forces (further referred to as endurance training) increases the oxidative capacity by stimulating mitochondrial biosynthesis (i.e. mitochondrial content and activity of oxidative enzymes) [69], whereas training consisting of short contractions during which muscles generate high forces (further referred to as peak power training) increases muscle fibre size by increasing the muscle protein synthesis rate eventually resulting in higher maximal muscle force [113].

*Increasing muscle fibre size – Changes in muscle mass are the net effects of changes in the rate of protein synthesis and/or in the rate of protein degradation [46]. Two major growth factors involved in the regulation of muscle mass are: insulin-like growth factor 1 (IGF-1) and myostatin [46,99]. IGF-1 is considered a positive regulator of muscle fibre size as it activates the Akt/mammalian target of rapamycin (mTOR) signalling pathway and activation of this pathway has been shown to increase the rate of protein synthesis [37,46,47]. Akt increases the rate of protein synthesis but also negatively regulates the rate of protein degradation by the phosphorylation of forkhead box transcription factors O (FoxO) [46]. The phosphorylation of FoxO in-
hibits its nuclear translocation and thereby the transcription of the muscle specific E3 ubiquitin ligases, muscle ring finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx) [46]. Note however, that although IGF-1 is known for its role in muscle hypertrophy, it has recently been suggested that hypertrophy can also occur in an IGF-1 independent manner [59,172,173] and that activation of mTOR in the early phase in response to mechanical loading may be Akt independent [125].

Myostatin on the other hand has opposite effects to those of IGF-1 and is considered a negative regulator of muscle fibre size [99,120,122]. Myostatin was shown to affect protein synthesis by reducing Akt/mTOR signalling in rat muscle as well as in differentiated human skeletal muscle cells in vitro [2,183]. However, the effects of myostatin on protein degradation have been questioned [2,183]. Overexpression of myostatin in rat tibialis anterior muscle and in differentiated human skeletal muscle cells in vitro did not increase the expression levels of MuRF-1 and MAFbx [2,183]. In contrast to those findings, several muscle wasting diseases are associated with increased myostatin levels as well as increased levels of MuRF-1 and MAFbx [28,49]. In addition, overexpression of myostatin was shown to stimulate expression of MuRF-1 and MAFbx in mice muscle [120], suggesting that myostatin does not only attenuate protein synthesis but also stimulates protein degradation. Mechanical loading of rat muscle has been shown to increase IGF-1 and reduce myostatin mRNA expression [64]. Analysing the expression levels of IGF-1 and myostatin as well as down-stream targets may provide indications regarding the response of training on the regulation of adaptation of muscle fibre size.

*Increasing oxidative capacity* – The oxidative capacity of the muscle increases with a higher mitochondrial biosynthesis. Endurance exercise has been shown to stimulate mitochondrial biosynthesis and thereby increasing the oxidative capacity [70]. During endurance exercise, cellular energy decreases (increased adenosine monophosphate (AMP):ATP ratio), resulting in phosphorylation and thereby activation of AMP-activated protein kinase (AMPK) [70]. Following this, AMPK phosphorylates one of the major regulatory proteins for mitochondrial biosynthesis, peroxisome proliferator-activated receptor (PPAR) γ co-activator 1α (PGC-1α), which then translocates into the nucleus and serves as a co-activator for several transcription factors such as PPARs, nuclear respiratory factor and oestrogen-related receptor [104,109,165]. This results in the transcription of nuclear-encoded mitochondrial proteins as well as mitochondrial
transcription factor A (TFAM) [104,109,165]. Transcription of mitochondrial proteins encoded on mtDNA is initiated by TFAM after its translocation into the mitochondria [109]. Besides stimulating mitochondrial biosynthesis, AMPK has been suggested to regulate turnover of contractile proteins by inhibiting mTOR activity [16,50] and stimulating FoxO activity [162]. This suggests that endurance training has an effect on 1) oxidative capacity by stimulating mitochondrial biosynthesis and 2) muscle fibre size by attenuating the rate of protein synthesis as well as increasing the rate of protein degradation, resulting in relatively small muscle fibres with a high oxidative capacity.

Increasing muscle fibre size and oxidative capacity simultaneously – From the above is evident that peak power and endurance training activate distinct signalling pathways within muscle fibres, resulting in different muscle phenotypes [176]. The objective of training to improve both maximal force and oxidative capacity, does not only apply to older people, but also to sports men. In several sports, training strategies have to be developed to optimise the increase in maximal force and oxidative capacity simultaneously. Due to the fact that activation of AMPK, which stimulates mitochondrial biosynthesis, inhibits the activation of mTOR and may stimulate the expression of E3 ligases, and that Akt activates mTOR and inhibits protein degradation, concurrent adaptation of both muscle fibre traits within one muscle fibre seems to be limited [4,5,26,191]. This suggests that muscle fibre size and oxidative capacity may be tightly regulated within one muscle fibre. Comparison of size and oxidative capacity of different types of muscle fibres over a wide range of species has shown that muscle fibre size and the average mitochondrial enzyme activity per unit of volume are inversely related (Figure 1.1) [189]. This inverse relationship suggests that limitation of oxygen diffusion within a muscle fibre causes a size constraint, implying that training for oxidative capacity causes a reduction in size and vice versa [191]. This indicates that hypertrophy and an increase in oxidative capacity are likely mutually exclusive within one muscle fibre.

It seems that improving both muscle fibre traits simultaneously requires training strategies to get around the interference effect of peak power and endurance training. A possible strategy could be a combination of training consisting of exercises by which muscle fibres are trained fibre type specifically. This implies that during endurance training only the small and slow motor units should be trained to increase their oxidative capacity, while during peak power training the large and fast motor units
should be trained to increase their size. Using such muscle fibre type specific adaptation, the entire muscle may increase in size and oxidative capacity simultaneously.

Generally, during low intensity endurance exercise, high oxidative muscle fibres are mainly recruited, whereas during peak power training, larger low oxidative fibres are mostly responsible for the generated power [33]. Investigation of the muscle fibre type specific responses requires a model in which high and low oxidative muscle fibres can be analysed separately. The rat medial gastrocnemius muscle (GM) is a compartmentalised muscle and composed of a high and low oxidative compartment which are recruited according to specific tasks [32,33]. During low intensity activities, the high oxidative compartment is active, whereas only during high intensity activities also the muscle fibres of the low oxidative compartment consisting of type IIIX and IIB fibres are recruited [33]. Such task specific recruitment in rat GM allows investigation of fibre type specific effects of concurrent training within one muscle.

![Figure 1.1. Inverse relationship between fibre cross-sectional area and the maximal rate of oxygen uptake.](image)

Over a wide range of species, muscle fibre size and the maximal rate of oxygen consumption (calculated from cross-sections of fibres strained for succinate dehydrogenase, SDH) are inversely related. Images: cross-sections stained for SDH of (from left to right): right ventricular wall of normal rat myocardium, rat extensor digitorum longus muscle, human vastus lateralis muscle, iliofibularis muscle of Xenopus laevis; scale bar 100 μm. ratMCT: right ventricular cardiomyocytes of a monocrotaline-induced pulmonary hypertensive rat; humanCHF: vastus lateralis muscle of human chronic heart failure patient. Figure adapted from [8,189,191].
Increasing bone mass by training

During training, ground reaction forces as well as forces due to muscle contractile activity are exerted onto bone. These forces cause deformations in the stiff mineralised bone matrix. Deformation of the load-bearing matrix induces a flow of interstitial fluid within very thin canals within the matrix, the canaliculi. Osteocytes are connected to each other via their processes that lie within these canaliculi. The fluid flow induces a fluid shear stress, specifically onto the cell processes of the osteocytes. The mechano-sensitive osteocytes are presumed to transform the mechanical stimuli into a biochemical signal [11]. In addition to osteocytes, osteoblasts also respond to mechanical stimuli [96]. However, it is believed that the biochemical signals from osteocytes are responsible for the adaptation of bone mass and structure to mechanical loading, by regulating the differentiation as well as the activity of osteoblasts and osteoclasts [11,94]. The differentiation and activation of osteoblasts are affected by osteo-anabolic factors, such as nitric oxide, prostaglandin E2 (PGE2), bone morphogenic proteins (BMPs), wingless (Wnt) signalling proteins and growth factors [29,94]. For the differentiation and activation of osteoclasts, receptor activator of nuclear factor-κB ligand (RANKL) plays an important role which is expressed by osteoblasts [29]. By the binding of RANKL to its receptor RANK on the osteoclast, osteoclastogenesis is stimulated. Osteoblasts also produce osteoprotegerin (OPG) which can bind to RANKL and therefore inhibit osteoclastogenesis [29]. Mechanical loading may induce bone adaptation by increasing and/or decreasing the expression of the above mentioned factors.

Indeed, in response to mechanical loading, gene expression levels of several proteins involved in the regulation of osteoblast and osteoclast activity are altered [96,143,152,164]. Osteoblastic cells as well as murine stromal cells in vitro respond to mechanical loading by increasing the osteoprotegerin (OPG)/RANKL mRNA ratio. This increase is accomplished by an elevation in the expression of OPG mRNA and/or a reduction in the expression of RANKL mRNA [92,93,96,97]. One mechanism by which mechanical loading of bone may result in an increase in bone mass, is the inhibition of osteoclastogenesis due to an elevated OPG/RANKL ratio. Another mechanism is the increase in expression levels of osteo-anabolic factors, such as IGF-1. IGF-1 stimulates proliferation of pre-osteoblast and their differentiation into osteoblasts and as such increases the rate of synthesis of bone matrix [29,45]. This indicates that mechani-
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cal loading stimulates the activation of osteoblasts and/or suppresses that of osteoclasts, affecting bone turnover in favour of bone formation.

High forces on bone have been shown to be very effective to increase bone mass \([17,158]\). Total duration of the loading regime seems less important to increase the osteogenic response \([55,186,190]\), as bone cells have been shown to become desensitized to mechanical loading \([17]\). Five to ten jumps increased bone mass and strength of rat tibia and femur to a similar extent as 40 jumps \([186]\). Several short sessions of mechanical loading, with a rest period in between, were more effective to increase the osteogenic response compared to one long session \([148,149,150,151]\). A recovery period of 8 hours was reported to be sufficient to restore mechano-sensitivity of bone cells to loading \([149]\). This suggests that training aiming to increase the osteogenic response should consist of short bouts of high impact exercise with sufficient rest between the training sessions to restore mechano-sensitivity of bone cells.

High impact exercise is generally acknowledged to increase the osteogenic response. Since in osteoporotic older people, high-impact loading may increase the risk of (vertebral) fractures \([129]\), high-impact exercise may be more harmful rather than stimulatory for bone formation. In contrast, low-impact training would be a more suitable option to increase bone formation in older people. Endurance training aimed to increase muscle oxidative capacity is a low-impact training. In young growing rats, such low-impact endurance training on a treadmill, was shown to stimulate bone formation \([55,73,78,82,83]\). The question is whether such low-impact endurance exercise also stimulates bone formation in mature bone. This raises the question whether additional endurance training on top of peak power training enhances the osteogenic response and may be a useful training strategy to increase bone formation with a minimal amount of high-impact loading.

Possible interaction between muscle and bone tissue
Throughout human life span, muscle mass and bone mass are tightly related. During growth and/or increased mechanical loading, the increase in muscle mass is accompanied by an increase in bone mass, whereas during aging and/or unloading muscle atrophy is accompanied by bone loss \([86,146]\). Muscle activity plays an important role in the adaptation of bone tissue by enabling movements that induce high impact loading onto the bone as well as by pulling on bone. Besides the effects of mechani-
cal loading onto bone due to muscle contractile activity, muscle tissue may also alter bone turnover via paracrine and/or endocrine pathways. Recently, it was shown that in response to stretching, myotubes were shown to secrete factors which affect the osteoclastogenesis \textit{in vitro} (unpublished data, Juffer \textit{et al.}). Evidence for paracrine signalling between muscle and bone has been provided in a study, in which bone fractures were healing slower when the periosteum was separated from the muscle by a nitrocellulose membrane [89]. This suggests that muscle tissue has the ability to contribute to the adaptation process of bone tissue via paracrine or endocrine signalling pathways.

Mechanical loading of rat muscle has been shown to increase IGF-1 and reduce myostatin expression levels [64]. Since IGF-1 is considered an anabolic factor and stimulates bone formation [29], the muscle-derived increase in IGF-1 may eventually result in increased serum levels and may then also affect bone tissue. Myostatin, on the other hand, is an anti-anabolic factor. Myostatin-deficient mice were shown to have increased bone density, enhanced osteogenic differentiation of marrow-derived stem cells and a faster bone repair [39,60]. If paracrine and/or endocrine signalling between muscle occurs, then it is conceivable that reduced myostatin expression levels after mechanical loading may also have a positive effect on bone formation. In skeletal muscles, endurance exercise was shown to up-regulate the expression levels of interleukin 6 (IL-6) and IL-10, as well as to down-regulate the expression levels of pro-inflammatory cytokines such as tumour necrosis factor α and IL-1, inducing an anti-inflammatory response [107,135]. This suggests that peak power and endurance training may affect the adaptive response of bone tissue via paracrine and/or endocrine signalling. The combination of peak power and endurance training may even create a better or prolonged osteogenic environment for the osteoblasts as compared to one type of training alone. A combined peak power and endurance training may thereby have an additional effect on bone formation via paracrine and/or endocrine signalling.

\textbf{Outline of the thesis}

The aim of this thesis was to investigate: 1) whether in older people parameters of physical functioning are associated with bone quality and fracture risk and 2) whether it is possible to increase muscle performance (muscle force generating capacity and
oxidative capacity) as well as bone mass by a combined peak power and endurance training in rats. With the knowledge acquired by this training study we aim to obtain cues for improvement of training programs to maintain musculo-skeletal health.

To investigate how physical functioning is related to bone quality and fracture risk in older people, an epidemiological study within the Longitudinal Aging Study Amsterdam was performed and presented in Chapter 2. Physical functioning was divided into three measures: 1) maximal handgrip strength representing the overall muscle strength; 2) physical performance representing muscle strength as well as coordination and 3) physical activity which may not only give an indication on what would be possible with respect to muscle force, coordination and endurance, but what is actually done by the person. The results of this study may help to develop criteria for selecting individuals at high fracture risk for whom a training program is highly indicated. In addition, measures for physical functioning may be used to evaluate training programs that aim to increase muscle performance and bone mass in order to reduce fracture risk.

To investigate the effects of peak power, endurance and combined peak power and endurance training on muscle force generating capacity, oxidative capacity and osteogenic response, we performed a 6 weeks training study in rats. During the 6 weeks training study, rats were subjected to either peak power training, endurance training, concurrent peak power and endurance training or no-training (control). The training was performed on a treadmill for 5 days per week and the concurrent training group performed two training sessions on these 5 days with an 8 hour rest between the sessions. Since the increase in muscle fibre size and oxidative capacity seems mutually exclusive within one muscle fibre, we investigated whether fibre type specific adaptation during peak power and endurance training allows the increase of both traits within the whole muscle. To assess effects of training, we investigated the medial gastrocnemius muscle (GM), which is composed of a high and low oxidative compartment. In Chapter 3, we investigated whether by using task specific recruitment of GM, additional endurance training on top of peak power training does not attenuate the increase in maximal force in response to peak power training only. The effects of peak power, endurance and concurrent peak power and endurance training on the oxidative capacity of rat GM were studied in Chapter 4. In Chapter 5, we investigated whether concurrent peak power and endurance training enhances the osteogenic
response in rat tibia compared to either one type of training alone and whether this may be a useful training strategy to increase bone mass with a minimal amount of high-impact loading.

Finally in **Chapter 6**, we discuss the main findings of the different studies presented and provide suggestions for how a training program can be best developed to improve both muscle performance and bone mass. Furthermore, we provide suggestions for future research that is warranted to improve the understanding of the adaptive responses of muscle and bone tissue to different types of training and to further optimise training strategies aiming to increase muscle performance and bone mass.