CHAPTER 6

GENERAL DISCUSSION
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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 a combined peak power and endurance training in rats. We showed that there are gender-specific differences in the associations between physical functioning and bone quality or fracture risk (Chapter 2). In men, higher physical performance scores as well as higher handgrip strength were associated with reduced fracture risk. In women, a moderate to high level of physical activity was associated with reduced fracture risk. This suggests that these tests for physical functioning are useful to identify older people at high fracture risk, but in a gender-specific manner.

For the second aim, we showed that in compartmentalised medial gastrocnemius muscle, additional endurance training attenuated the increase in maximal force as observed after peak power training alone. In addition, concurrent peak power and endurance training did not increase the oxidative capacity but reduced the oxidative capacity of low oxidative fast type muscle fibres (Chapters 3 & 4). For bone, combined peak power and endurance training may be a more successful training strategy to enhance the osteogenic response compared to peak power training alone (Chapter 5). Although training did not induce changes in bone turnover in trabecular bone, the decrease in mRNA expression levels of insulin-like growth factor 1 (IGF-1), osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL) in cortical bone after peak power training was counteracted by additional endurance training. The results from our training study in rats suggest that the combination of peak power and endurance training cannot be used to increase muscle force generating capacity, oxidative capacity and bone mass.

As the concurrent training performed in our study did not increase muscle force generating capacity, oxidative capacity and bone mass simultaneously, it remains to be determined whether it is possible to increase these three traits with one type of training and how this could be achieved. In the following sections, mechanisms are discussed by which concurrent peak power and endurance training may have attenuated the training adaptation of skeletal muscle compared to peak power training or endurance training alone. Regarding the adaption of bone tissue, age-related differ-
ences in the adaptation of bone as well as methodological aspects of bone measures will be discussed. In addition, it will be discussed how training may have affected the osteogenic response by muscle-derived growth factors in a paracrine or endocrine manner. Finally, based on the observations of this thesis suggestions are made regarding the development of a training programs to increase force generating capacity, oxidative capacity and bone mass.

**Implication of the results for development of training strategies to increase maximal muscle force and oxidative capacity**

The results of our study suggest that despite the fibre type specific adaptation of high and low oxidative muscle fibres, additional endurance training on top of peak power training attenuated the increase in maximal force (Chapter 3). Our findings are in line with results from human studies, reporting an attenuated increase in maximal muscle force of thigh muscles (as measured by knee extension or leg press) after concurrent training [38,65,155]. However, there are also studies showing that concurrent training in humans increase maximal muscle force of the tight muscles and cross-sectional area (CSA) of the quadriceps (as measured by magnetic resonance imaging or fibre CSA in biopsies of vastus lateralis muscle) to a similar extent as peak power training alone [48,57,71,87,111]. It is evident that within the literature there are controversies regarding the interference effect between peak power and endurance training. In the following paragraphs, possible mechanisms underlying this discrepancy between findings and molecular mechanisms involved in the interference between peak power and endurance training will be discussed. Four issues will be addressed: 1) comparison of human and rat studies, 2) is the increase in maximal force dependent on the frequency or duration of the endurance training sessions?, 3) what is the role of AMPK in limiting the increase in muscle fibre size?, and 4) role of hypoxia in the regulation of muscle fibre size and oxidative capacity.

**Comparison of human and rat studies**

The above mentioned studies investigated effects of concurrent training in humans [38,48,57,65,71,87,111,155]. Here we used a rat model to study these effects. This model allows the investigation of fibre type specific adaptation. Based on knowledge regarding the molecular regulation of hypertrophy and mitochondrial biosynthesis, it
is expected that within a muscle fibre the increase in muscle fibre size and oxidative capacity are mutually exclusive [5,26,191]. However, this interference between the adaptive response to peak power and endurance training may not account for the whole muscle, since different muscle fibres are recruited during distinct tasks and may adapt in a fibre-type specific manner. The rat medial gastrocnemius muscle (GM) is 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 low oxidative compartment consisting of type IIX and IIB fibres is recruited [33]. Such task specific recruitment in rat GM allows investigation of fibre type specific effects of concurrent training within one muscle. The task specific recruitment of the compartmentalised rat GM may be compared to fibre recruitment of human skeletal muscles that occur according to the size principle. In addition, fibre type specific effects of concurrent training may also apply to the synergistic muscles. Possibly, endurance training may increase the oxidative capacity of high oxidative soleus muscle, whereas peak power training may increase fibre CSA of low oxidative muscle fibres of plantaris or gastrocnemius muscle. However, in humans, the fibre type specific adaptation within a muscle as well as between muscles is unclear.

The rat model allowed to investigate contractile characteristics, phenotype characterisation and changes in molecular markers for particular adaptive processes within the same muscle. In contrast to the isolated force measurements of rat GM, studies in humans are only able to assess the effects of training on the maximal force of the entire thigh muscle. In addition, biopsies taken from the vastus lateralis muscle may not be representative for the changes that occurred within the entire muscle. A limitation of studying rats is that the effects may not directly be translated to humans. In addition, it should be noted that in older people, muscles may respond differently to peak power and endurance training as compared to muscles of younger individuals. However, the possibilities to investigate the molecular processes in rats may give essential basic information directing further research in humans.

Is the increase in maximal force dependent on the frequency or duration of the endurance training sessions?
A difference between the studies reporting an attenuated increase in maximal force and
those that do not, is the frequency of the training sessions [38, 48, 57, 65, 71, 87, 111, 155]. By performing maximally 5 training sessions per week (2-3 sessions of peak power and 2-3 session of endurance training), the increase in maximal force of the thigh muscles after peak power training does not seem to be attenuated when combined with endurance training [48, 57, 71, 87, 111]. However, more frequent training sessions (up to 11 training sessions for the concurrent training group) attenuated the increase in maximal force and/or CSA of the thigh muscles [38, 65, 95, 155]. An attenuated increase in maximal force was also shown in our study in which the concurrent training group performed 10 training sessions per week (5 peak power and 5 endurance training sessions). Recently, a meta-analysis of concurrent training studies in humans analysed the pooled effect sizes of maximal force, hypertrophy and power observed after concurrent training compared to one type of training alone [195]. With increasing frequency and duration of the endurance training, the effect sizes of maximal force, hypertrophy and power were shown to decrease [195]. This indicates that frequency and duration of the endurance training are negatively related to the increase in maximal force, hypertrophy and power [195]. For an optimal increase in maximal force, performing endurance training at low frequencies (2-3 times/week) may be a successful modulation of the training strategy and may allow the increase in both muscle fibre size and oxidative capacity.

What is the role of AMPK in limiting the increase in muscle fibre size?

A possible explanation for how high frequency and/or duration of the endurance training may have attenuated the increase in muscle fibre size and thereby maximal force, is that the rate of protein degradation may be increased in response to endurance training. Protein degradation may be increased by the activation of AMP-activated protein kinase (AMPK) [191], attenuating muscle fibre hypertrophy and therefore maximal muscle force.

With increasing duration of endurance training as well as by training with low muscle glycogen levels which may occur when two training sessions are performed per day, cellular energy rich phosphate levels decrease (increasing AMP:ATP ratio) [199]. AMPK is an enzyme, sensitive to changes in AMP:ATP ratio, as the γ subunit of AMPK contains binding sites which mutually exclusive bind either AMP or ATP [61]. The binding of AMP to AMPK triggers conformational changes in the γ subunit of
AMPK which allows phosphorylation of AMPK by AMPK kinases [61]. Phosphorylated (active) AMPK is known to enhance the mitochondrial biosynthesis via phosphorylation of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), but may also increase protein degradation by increasing nuclear forkhead box transcription factors O3 (FoxO3) levels and thereby increasing the expression of E3 ligases muscle ring finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx) [162]. Although AMPK may increase protein degradation, PGC-1α which is increased in response to AMPK activation may protect muscles from atrophy [163]. Mouse muscles overexpressing PGC-1α were protected against denervation-induced muscle fibre atrophy [163]. PGC-1α was shown to reduce the FoxO3-dependent transcription of E3 ligases within the nucleus [163]. These findings seem contradictory and raise questions about the effects of endurance exercise on protein degradation. Possibly, the distinct effect of AMPK and PGC-1α on muscle degradation may be time-dependent or fibre type-dependent. As an acute effect of the decrease in energy rich phosphate levels, AMPK may increase protein degradation, whereas high PGC-1α content may, in the long-term, protect the muscle from muscle atrophy. Since PGC-1α protein content in rat GM is substantially higher within high oxidative muscle fibres as compared to low oxidative muscle fibres (Chapter 4), these high oxidative muscle fibres may be more protected from atrophy than low oxidative muscle fibres. This raises the question whether fibre size of high and low oxidative muscle fibres are affected differently by endurance training.

Performing endurance training at relatively low intensity, preserving high cellular energy rich phosphate levels may be a useful strategy to increase muscle fibre size and oxidative capacity. As mentioned above, this may reduce the AMPK-induced protein degradation. In addition, the recruitment of only high oxidative muscle fibre may also contribute to the maintenance of fibre size, as these high oxidative muscle fibres contain high levels of PGC-1α and may thereby be protect from atrophy. The increase in oxidative capacity can also occur in an AMPK-independent manner [179,197]. The repeatedly contracting muscles induce high intracellular calcium levels. These calcium levels stimulate the activity of calcineurin and calcium/calmodulin protein kinase (CaMK) which are known to increase the expression of PGC-1α and therefore stimulate the transcription of proteins involved in oxidative metabolism [103,197]. Therefore, during low intensity long duration endurance training, the higher calcium levels may increase mitochondrial biosynthesis without a large decrease in cellular en-
ergy rich phosphate levels. In conclusion, these findings suggest that the increase in muscle fibre size as well as oxidative capacity within a whole muscle may be possible when endurance training only recruits high oxidative muscle fibres and preserves the cellular energy rich phosphate levels of low oxidative muscle fibres that increase in fibre size by peak power training.

Role of hypoxia in the regulation of muscle fibre size and oxidative capacity

Muscle fibre size – Besides minimising AMPK-induced protein degradation, low intensity endurance training has another advantage on the preservation of muscle fibre size. Muscle fibre size may be preserved due to the fact that at a low intensity, muscle fibres become less hypoxic. Due to hypoxia, intracellular production of reactive oxygen species (ROS) may increase which was shown to enhance protein degradation by activating nuclear factor kappa-B (NF-κB) [140,141]. The substantial reduction in succinate dehydrogenase (SDH) activity in type IIB fibres reported in Chapter 4 suggests that during peak power training, the oxygen supply to type IIB fibres could not meet its demand and may have induced hypoxia in the core of the low oxidative type IIB muscle fibres. Although we observed an increase in maximal force after peak power training only, the limited increase in muscle fibre CSA may be due to a concomitantly increased protein degradation. This increased protein degradation may have been ROS-mediated.

Differences in ROS production as well as ROS scavenging capacity were observed between high and low oxidative muscle fibres [3]. ROS production (measured as hydrogen peroxide, H₂O₂) in high oxidative type I muscle fibres was substantially lower than that of low oxidative type II (in particular IIB) fibres as measured in situ in permeabilised fibre bundles of rats skeletal muscles [3]. Besides the higher ROS production in low oxidative muscle fibres, within these muscle fibres, ROS scavenging capacity was shown to be lower than within high oxidative muscle fibres (even after correcting for mitochondrial content) [3]. Possibly, in our study during the peak power training, type IIX and IIB fibres became hypoxic which may have resulted in an increased ROS production. As low oxidative muscle fibres have a reduced ROS scavenger capacity, the increased ROS concentrations in response to peak power training may have limited the hypertrophic response within these low oxidative muscle fibres.

In contrast to an increase in ROS-mediated protein degradation in response to hy-
poxia, positive effects of hypoxia on muscle fibre size have also been reported [167]. Recently, during hypertrophic exercise in humans, hypoxia induced by occlusion was proposed to be an effective strategy to enhance the hypertrophic response [167]. This raises questions about the effects of hypoxia on the regulation of muscle fibre size and whether hypoxia is a disadvantage or an advantage during training. A possible mechanism by which the potential negative effects of ROS could be minimised is by increasing ROS scavenging capacity. In response to 12 weeks of hypertrophy training in rats, production of antioxidative enzymes, such as superoxide dismutase were shown to increase which in turn may reduce the ROS induced damage [166]. An increased ROS scavenging capacity contributes to the protective system against ROS-induced cell damage. From the above it is clear that the adaptive response to hypoxia and the role of ROS and ROS scavengers in the regulation of muscle fibre size is still unclear. This is subject for further research in which fibre type specific differences in the adaptive responses should also be considered.

**Oxidative capacity** – Besides a possible negative effect of hypoxia on muscle fibre size, hypoxia may also negatively affect oxidative capacity. Transfection of rat extensor digitorum longus muscle (EDL) with hypoxia inducible factor 1α (HIF-1α) showed a substantial decrease in SDH activity of type IIB fibres [112]. This loss of mitochondrial enzyme activity could be due to an increase in mitochondrial degradation (mitophagy). In cultured mouse embryo fibroblasts and rat cardiac myocytes, hypoxia was shown to induce mitophagy [58,200]. Similar to the reduction in SDH activity in type IIB fibres reported for HIF-1α transfected EDL muscle fibres [112], we also observed lower SDH activity in type IIB fibres after peak power training (Chapter 4). Possibly, the reduced SDH activity after peak power training may be HIF-1α dependent. In response to brief trains of electrical stimulation of the EDL, designed to recruit type IIX and IIB fibres, HIF-1α protein levels were shown to increase in EDL muscle [112]. As such type of muscle activation is fairly similar to that during our peak power training, which induced a reduction in SDH activity within the fibres of the low oxidative compartment, it is conceivable that the peak power training caused an elevated mitochondrial degradation in type IIB fibres by increasing HIF-1α. These data suggest, that when performing training to increase the oxidative capacity of the muscle, hypoxia within the cores of low oxidative muscle fibres should be prevented.
In contrast to the effects of peak power training on oxidative capacity, recently two weeks of high intensity training (HIT, 8-12 x 60 s of all-out exercise with 75 s of recovery in between) in humans was shown to increase levels of regulatory proteins involved in the stimulation of mitochondrial biosynthesis [108]. This suggests that this type of training is beneficial to increase oxidative capacity despite the fact that the core of muscle fibre may have been hypoxic. However, the effect of HIT on regulatory proteins of mitochondrial biosynthesis was only analysed in homogenized protein extracts obtained from vastus lateralis biopsies [108]. This approach does not allow to investigate the muscle fibre type specific effects of training. As basal expression levels of genes involved in oxidative metabolism differ between high and low oxidative muscle fibres [103,171,191], and the adaptation to the training programs used in this study was shown to be fibre type specific (Chapters 4), the fibre type specific effects of HIT in humans are unclear. The discussed findings suggest that the role of hypoxia on the increase in mitochondrial biosynthesis of the different muscle fibres still remains to be determined. Future studies on the effectiveness of training strategies that possibly induce hypoxia, should investigate 1) the fibre type specific response to hypoxia and 2) synthesis as well as degradation of mitochondrial proteins.

**Bone quality and fracture risk: training and testing**

Although concurrent peak power and endurance training may not be optimal to increase maximal muscle force and oxidative capacity simultaneously, for bone the combination of these two training modalities had no negative effect on the osteogenic response (Chapter 5). We hypothesised that combined training enhanced the osteogenic response by stimulating bone formation in response to peak power training and attenuating bone resorption by endurance training. Additional endurance training on top of peak power training counteracted the reduction in mRNA expression levels of osteo-anabolic factors observed after peak power training only. Despite the fact that concurrent training did not increase mean mRNA expression levels of genes involved in bone formation or bone formation as measured by histomorphometry, the variation of mRNA expression levels was substantially higher than that of the other groups. This elevated variation in several genes involved in bone formation suggests that some rats responded to daily combined training for 6 weeks with a cumulative positive effect on mRNA levels of genes involved in bone formation.
This may in the long-term enhance the osteogenic response. These results suggest that the combined peak power and endurance training group consisted of responders and non-responders. In rats, individual differences in training-induced adaptation [184] as well as differences of running behaviour were shown previously [147]. Also in humans, such subject-to-subject variability in the adaptive response to training is well known [182], suggesting that in order to optimise the training response, the personal differences should be taken into account and training should be tailored individually.

To improve the understanding on the adaptive response of bone tissue to training and optimise future training studies, two issues will be discussed in the following paragraphs: 1) training-induced changes in bone quality and fracture risk, and 2) methodological considerations to test effects of training on bone quality.

Training-induced changes in bone quality and fracture risk

Low bone mineral density (BMD) is an important determinant for fracture risk. As mechanical loading of bone is essential for the stimulation of bone formation, increased physical activity and/or skeletal muscle performance may contribute to a high BMD. An epidemiological study on a population of older (> 65 years) women showed that increased physical activity was shown to be associated with a reduced hip fracture risk [51]. Explanations for this reduction may be 1) a reduction in fall incidents (due to improved muscle performance) and/or 2) an enhanced bone quality. Other parameters of physical functioning may also be related to a reduced fracture risk. In Chapter 2, we showed that different parameters for physical functioning are associated with fracture risk and that these associations differ among men and women. In men, increased handgrip strength and physical performance were associated with reduced fracture risk, whereas in women, moderate to high level of physical activity was related to a substantially reduced fracture risk (Chapter 2). Measuring physical functioning in older people may help selecting individuals at higher fracture risk for whom training intervention is strongly indicated. Possibly, these measures for physical functioning may also be useful to evaluate training programs that aim to reduce fracture risk. However, whether the increase in physical functioning in response to training is also associated with reduced fracture risk, remains to be determined.

Training programs aiming to reduce fracture risk should improve muscle characteristics that may contribute to a reduction in number of falls as well as increasing
bone mass. It is well known that bone mass can most effectively be increased when training is performed at a young age [52,85]. This suggests that bone mass should be increased at a young age, before peak bone mass has been reached. Although the loss of BMD due to aging cannot be prevented by training, the loss can be attenuated by regular running exercise [123]. In chapter 5, we assessed effects of peak power, endurance and combined peak power and endurance training on the osteogenic response of rat tibia. Similar physical training stimuli applied to bone in young growing rats for two months were shown to increase bone mass during growth [78,82]. However, in young adult rats, the training performed in our study was not able to increase bone mass (as measured in the secondary spongiosa by histomorphometry). This raises the question what type, intensity and duration of the training is necessary to induce an increase in mature trabecular bone mass. Although bone mass is a strong determinant of bone strength, bone strength itself was not measured in this study. It is however the ultimate goal of most training studies to avoid fractures and therefore improve bone strength. Possible measurements that may reveal training-induced changes on bone strength will be discussed in the following paragraphs.

Methodological considerations to test effects of training on bone quality
The results of our epidemiological study, reported in Chapter 2, show that handgrip strength and level of physical activity were not associated with bone quality. However, these parameters were both associated with reduced fracture risk. This suggests that higher handgrip strength and physical activity level may reflect muscle contractile characteristics resulting in reduced fall incidence rather than a higher bone quality. However, higher handgrip strength and physical activity may also be associated with bone characteristics that are not assessed by dual-energy X-ray absorptiometry (DXA) or quantitative ultrasound (QUS). In response to training, new bone is formed at the sites which are subjected to large deformations. This site-specific adaptation may not result in a great increase in BMD determined from a large area of the skeleton, as observed after pharmacological interventions. Rather, bone formation may only be increase at the sites where high mechanical stress is applied. This site-specific adaptation in response to training can be very effective in increasing bone strength locally. Since forces exerted onto weak bone induce a larger deformation of the bone matrix as compared to the same forces applied to stronger bone, sites where the
bone matrix is relatively weak will particularly increase in strength. These small local effects may therefore decrease fracture risk substantially.

In response to 16 weeks of axial loading of the rat ulna, areal BMD was only 5% higher compared to that of the contralateral unloaded tibia, whereas ultimate force and energy to failure (measured by mechanically loading of the tibia to failure) were increased by 64% and 94%, respectively [150,151]. This suggests that bone strength as measured by ultimate force and energy to failure may give a more valid indication of bone adaptation in response to training than BMD measurements. However, due to the invasive character of these measurements, it is ethically not possible to perform those measurements in clinical studies. In the studies previously mentioned on the effects of axial loading of the rat ulna on bone quality (i.e. BMD, ultimate force and energy to failure), peripheral quantitative computed tomography (pQCT) measurements were performed in addition to DXA measurements [150,151]. Similar to DXA, pQCT measurements can also be performed in humans [77]. Compared to DXA, pQCT is able to assess bone structural changes, which may be better indices of bone quality than BMD. In contrast to the approximately 3% increase in volumetric BMD of the midshaft of the ulna in response to loading, minimal second moment of inertia (I\textsubscript{min}, representing mediolateral thickening) and maximal second moment of inertia (I\textsubscript{max}, representing the craniocaudal plane) were 50% and 10% increased, respectively [151]. These measures reflect the structure’s resistance to bending (bending rigidity) by taking into account both CSA and material distribution. Besides I\textsubscript{min} and I\textsubscript{max}, also several structural changes such as trabecular and cortical bone density and thickness as well as trabecular number can be assessed using high resolution pQCT [121]. The ultimate goal of training-induced bone adaptation studies is to avoid new fractures. However, studies assessing fracture risk need to include a high number of individuals with a long follow-up period. Therefore, when studying effects of training on bone strength, not only BMD should be determined but also site-specific adaptation and structural changes reflecting bone strength should be assessed.

In Chapter 5, we presented data of training-induced changes on the regulation of bone turnover of cortical bone determined from differences in mRNA expression levels and bone turnover indices in trabecular bone as measured by histomorphometry. In trabecular bone, no changes in bone turnover were observed in response to training. Six weeks of such physical stimuli as applied during the training may not
induce changes in trabecular bone as measured by histomorphometry. Possibly, training performed in our study induced other structural changes in bone. Other parameters which may be important contributors to bone strength are collagen content and cross-linking. With increasing age, type I collagen content in iliac crest bone of individuals ranging from 20-90 years of age was shown to decrease while mineralisation increased [6], suggesting that this may induce more brittle bones and consequently increase fracture risk. Not only the collagen content, also the longitudinal orientation of collagen fibres in cortical bone, were shown to be predictive for tensile strength [117]. The more the collagen fibres were oriented in longitudinal direction, the stronger the bone [117]. This suggests that collagen content as well as its orientation may contribute to bone strength. Training may induce such structural changes and thereby increase bone strength.

The proportion of enzymatic (lysine hydroxylase and lysyl oxidase) and non-enzymatic (advanced glycation end products, AGE) cross-links may also contribute to bone strength [160]. In elderly, the higher amount of AGE cross-links compared to younger subjects was suggested to play a role in the age-related deterioration of bone quality [160]. In diabetic rats that had impaired enzymatic cross-links and increased non-enzymatic AGE cross-links compared to healthy rats, maximum load and energy absorption of the femur were lower compared to those of healthy rats with similar BMD and collagen content [159]. Therefore, analyses of collagen content as well as the type of cross-links are warranted as these will give more insight in training-induced changes in bone strength. Future research on age-related or training-induced adaptation of bone strength should include measurements of these parameters of bone structure.

Possible interaction between muscle and bone tissue
Bone adaptation is induced by the deformation of the bone matrix due to ground reaction forces and/or forces due to muscle contractile activity that are exerted onto bone. In addition, muscle contractile activity may also induce bone adaptation in a paracrine or endocrine manner by the release of growth factors and cytokines [86]. 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 osteo-
genic environment as compared to one type of training alone and may thereby have an additional effect on bone formation via paracrine and/or endocrine signalling. In the following paragraph, we will discuss how the different training modalities may have stimulated and/or inhibited the transcription of anabolic and catabolic factors in muscle and how muscle may have affected bone remodelling in a paracrine or endocrine manner. If the combination of peak power and endurance training enhanced the osteogenic environment as compared to peak power training alone, this provides valuable information for the development of training programs for osteoporotic elderly people for whom high impact loading should be minimised.

Mechanical loading of muscle and bone cells is known to increase the expression levels of anabolic factors. However, the peak power training performed in our study reduced the mRNA levels of IGF-1Ea in both rat GM and tibia (Chapter 3 and 5). In addition, after peak power training, myostatin mRNA expression levels were 2-fold higher in the low oxidative compartment of GM compared to those of controls (Chapter 3). IGF-1 is considered an anabolic factor as it is involved in the synthesis of bone matrix [45] and the proliferation of pre-osteoblast to osteoblasts [29]. Myostatin, on the other hand has opposite effects and is known to inhibit proliferation and differentiation of osteoprogenitor cells [39]. The increased myostatin levels after peak power training could have attenuated the osteoblastogenesis in a paracrine or endocrine manner. In addition, the lower levels of IGF-1 mRNA in tibia could also have contributed to a decrease in the osteoblastogenesis. Since peak power training was aimed to increase muscle fibre size and bone formation, these anti-anabolic effects of the peak power training performed in our study were unexpected. This type of peak power training may not be effective to enhance the osteogenic response directly by increasing expression levels of anabolic factors in bone or indirectly by increasing expression levels of anabolic factors in muscle. In both, bone and muscle tissue, additional endurance training on top of peak power training counteracted the decrease in anabolic factors as well as the increase in catabolic factors. This suggests that endurance training may have an osteogenic effect. However, we did not observe changes in mRNA expression levels of alkaline phosphatase, osteocalcine and collagen type 1 or bone turnover as measured by histomorphometry. This suggests that 6 weeks of combined peak power and endurance training did not increase bone formation and it is unclear whether in the long-term, the effects of combined training on muscle tissue may also
affect bone turnover in a paracrine or endocrine manner. In addition, it remains to be determined whether the combination of endurance training with another type of high-impact training induce muscle adaptation that may enhance the osteogenic response via paracrine or endocrine signalling and thereby may be effective to enhance bone formation with a minimal amount of high impact loading.

Clinical implications and future directions to improve the understanding of training-induced adaptation of muscle performance and bone mass

Clinical implications
With increasing age, muscle performance (force generating capacity as well as oxidative capacity) and bone mass decline. In this thesis, we investigated whether it was possible to increase maximal force generating capacity, oxidative capacity and bone mass during a 6 week training period when combining peak power and endurance training. Our result suggest that combined peak power and endurance training as performed in our studies is not appropriate to increase all three traits. Despite the lack of a concomitant positive effect on muscle fibre size, oxidative capacity and bone mass, the studies of this thesis provide indications of how a training program should be designed to optimise the adaptive training response of muscle and bone.

Muscle – We suggest that when aiming to improve muscle force generating capacity and oxidative capacity simultaneously, the endurance training should be performed approximately 2-3 times per week at low intensities. To minimise protein and possibly mitochondrial degradation, hypoxia within fast muscle fibres should be prevented by performing the training at low intensities and/or with sufficient rest between sessions. More insight is needed into the intensity level and duration of the training at which the muscles become hypoxic. To increase maximal muscle force, short bouts of peak power exercise should be performed with sufficient rest between the repetitions for recovery.

Bone – In osteoporotic older people, high-impact loading is dissuaded. A major reason for this is that high-impact loading increases the risk of (vertebral) fractures [129]. Training with a relatively high amount of low-impact exercises may therefore be more appropriate to increase bone formation in older people. We suggest that for bone, endurance training in addition to short periods of high-impact loading may be an effective strategy to enhance the osteogenic response. These training sessions for
peak power and endurance should be separated by a rest period, as bone cells become desensitised to mechanical loading. Whether 8 hours is also optimal to restore mechano-sensitivity of human bone cells remains to be determined.

Suggestions for future research

Assessing fracture risk – To identify older people with increased fracture risk, we provided evidence that in men handgrip strength and physical performance are useful measurements, whereas in women, a low level of physical activity is an important risk factor. Future training studies should investigate whether improving these parameters of physical functioning may result in a reduced fracture risk and whether these tests can be used to evaluate the effectiveness of training programs in reducing fracture risk.

Improving muscle performance – To improve training strategies for increasing maximal muscle force and oxidative capacity simultaneously, future studies should focus on the intensity- and frequency-dependent interference effect of endurance training with fibre size. The question is whether the increase in fibre size of mainly low oxidative type II fibres by peak power training is not attenuated when endurance training is performed at very low intensities (neither inducing catabolic processes nor recruiting low oxidative type II muscle fibres). In addition, research is needed to further elucidate the effects of hypoxia on the adaptation of muscle fibre size (as shown in studies performing hypertrophic exercise with occlusion) and oxidative capacity (as performed with high intensity training). Fibre type specific adaptation should be considered in those studies. Furthermore, age-related differences in the adaptive responses to combined peak power and endurance training is also subject for future research.

Training and measuring bone strength – Future studies should investigate differences in training adaptation of immature and mature trabecular bone and which type of training may be most effective to increase bone formation. Furthermore, to obtain more insight into the adaptive responses of bone tissue to different types of training, bone strength should more often be assessed in addition to DXA or histomorphometry measurements. In animal studies, analyses of ultimate force and energy to failure may give a good indication of the site-specific adaptation of bone tissue in response to mechanical loading and the reduction in fracture risk. These measures possibly to-
gether with collagen content and cross-linking will give comprehensive insight in the training-induced adaptation of bone strength. In addition, pQCT could be performed for pre- and post-measures. In human studies, pQCT could be used to assess structural changes in cortical and trabecular bone, as well as bone quality as measured by the second moment of inertia.