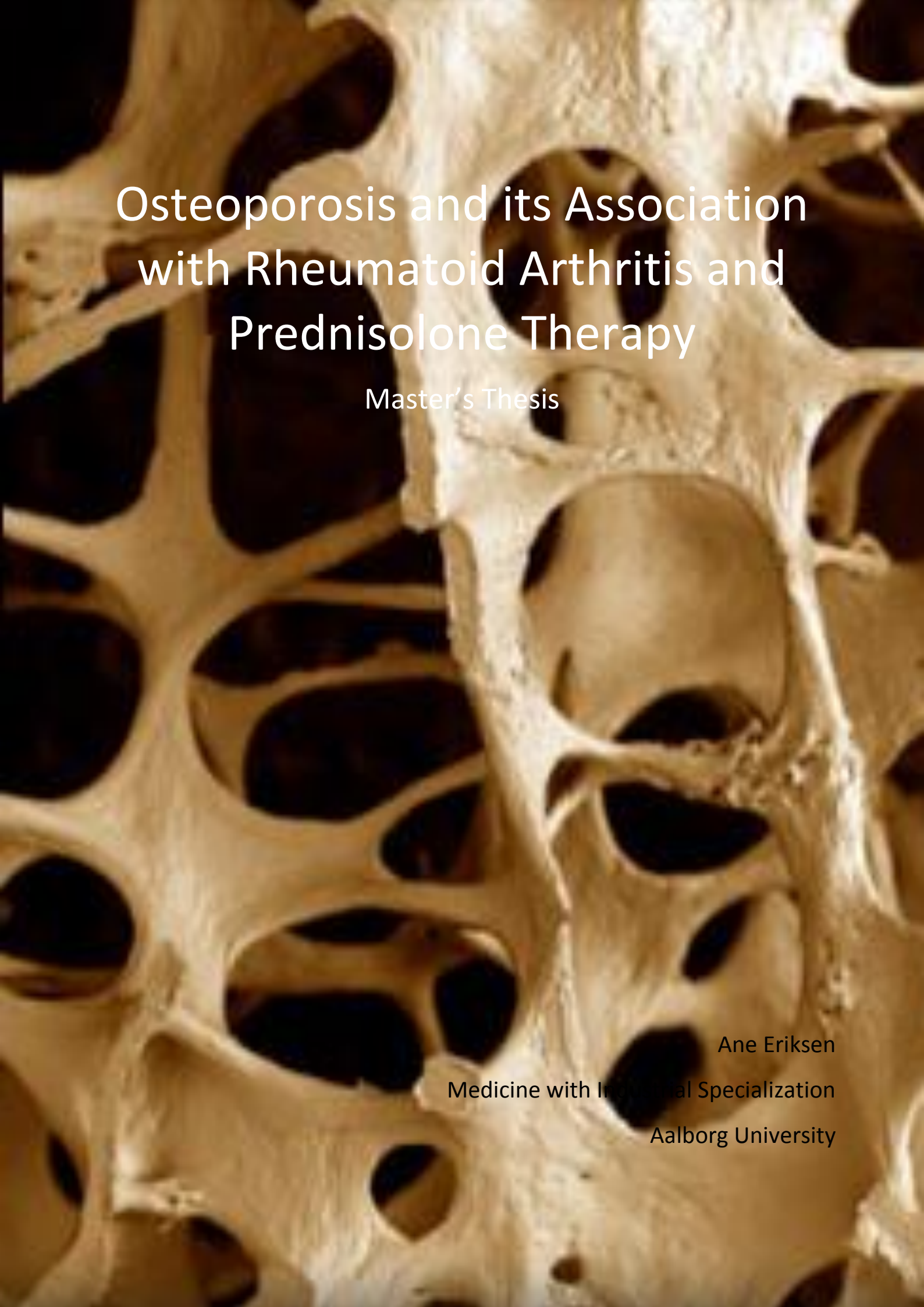

Osteoporosis and its Association with Rheumatoid Arthritis and Prednisolone Therapy

Master's Thesis

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Abstract

Osteoporosis is a common systemic skeletal disease characterized by reduced bone mass and consequently enhanced susceptibility to fractures. Osteoporotic fractures are associated with increased morbidity and mortality as well as large socioeconomic costs. Several studies have demonstrated that patients receiving long-term glucocorticoid therapy or patients suffering from rheumatoid arthritis (RA) have an increased risk of developing osteoporosis. Therefore, the aim of the present study was to investigate the association between prednisolone therapy, RA and osteoporosis. The study included all patients referred to a dual-energy x-ray absorptiometry (DXA) scan at Aalborg University Hospital during the time period 1st of January 2013 to 31st of December 2013. A total of 3557 patients participated in the study. Data were obtained from a database that contained results from DXA measurements, i.e. T-scores for lumbar spine (L1-L4) and total hip, as well as information about various factors that potentially may affect bone strength. DXA measurements were performed by means of a Hologic Discovery QDR device, whereas the remaining information was obtained from self-completion questionnaires. The results revealed that patients receiving prednisolone therapy had higher T-scores of both lumber spine and total hip compared to non-treated patients. Additionally, no significant correlation between prednisolone dose and T-score was demonstrated. Furthermore, RA patients had higher lumbar spine T-scores compared to patients without RA, whereas total hip T-scores were similar between the two groups. Stepwise multiple linear regression analysis further demonstrated that prednisolone therapy and RA were positive predictors of lumbar spine T-score, whereas they were not predictive of total hip T-score. These results were contrary to the hypothesis as well as the findings of other studies. This was thought to be related to limitations of the present study, including the lack of information regarding treatment time of prednisolone as well as disease duration and severity of RA.

1. Introduction

Osteoporosis is a systemic skeletal disease characterized by reduced bone mass and altered bone structure and consequently increased bone fragility and porosity (1). Therefore osteoporosis is associated with an increased susceptibility to fractures, especially fractures of the vertebrae, proximal hip and distal forearm (2). In Denmark approximately 500,000 patients suffer from osteoporosis (3). Further, about 10,000 hip fractures, 7,000 fractures of the distal forearm and 2,000 vertebral fractures attributable to osteoporosis occur annually in Denmark (4). The number of vertebral fractures is probably even higher, since a significant proportion of these fractures is asymptomatic and therefore undiagnosed (5, 6). The prevalence of osteoporotic fractures generally increases with age and is markedly higher in women compared to men (4, 6, 7). The consequences of osteoporotic fractures include increased morbidity and mortality as well as large socioeconomic costs (8, 9, 10). Several studies (11, 12, 13) have demonstrated that fractures of the hip and vertebrae are associated with increased mortality. Moreover, osteoporotic fractures are associated with increased morbidity in the form of pain and decreased functional ability, which may lead to institutionalization and reduced quality of life (8, 9, 14). Additionally, a previous osteoporotic fracture also increases the risk of subsequent fractures (15, 16, 17, 18). Thus, osteoporosis

represents a major health problem that affects several people and has a substantial impact on both the individual patient as well as the society.

1.1 Bone Structure and Physiology

Bone tissue is a specialized connective tissue, demonstrating extensive hardness and strength (19). Basically, two different types of bone tissue exist, namely cortical and trabecular bone tissue. Cortical bone represents approximately 80 % of the skeleton, and it is characterized by considerable compactness and density (19-21). Cortical bone constitutes the surface of all bones in the skeleton, but its quantity is particularly large in the diaphyses of long bones (20, 22, 23). Trabecular or cancellous bone comprises the remaining 20 % of the skeleton, and it is primarily found in the vertebral bodies and inside the long bones (20, 22, 23). This bone type forms an interconnected network of horizontally and vertically arranged bone beams leading to a large surface area (19, 23). The space between the trabeculae is filled with red bone marrow with hematopoietic properties (19, 21). The outer bone surfaces, except for the joints, are covered with a layer of dense connective tissue called periost, whereas the inner bone surfaces are encased with a thin membrane of connective tissue named endost (19).

Both cortical and trabecular bone tissue are composed of extracellular bone matrix as well as bone cells (19, 21, 23). The organic part of the bone matrix is primarily composed of type I collagen, which is responsible for the tensile strength of bone (19, 21-23). Other non-collagenous proteins present in the organic bone matrix include proteoglycans, bone sialoprotein (BSP), osteocalcin, osteonectin and osteopontin (19-22). The function of these non-collagenous proteins is not fully elucidated, but they are thought to play a role in mineralization, anchoring of osteoclasts to bone and remodeling of bone (24-27). The inorganic part of bone matrix consists mainly of depositions of calcium and phosphate ions in the form of hydroxyapatite (19, 22). These mineral depositions in bone are responsible for its compressive strength, and they are also essential for the maintenance of the calcium homeostasis in serum (21, 22).

1.1.1 Bone Cells

In general, two morphologically and functionally distinct bone cell types exist; osteoblasts and osteoclasts that are responsible for bone formation and bone resorption, respectively. The characteristics and functions of these two cell types will be described in the following sections.

1.1.1.1 Osteoblasts

The osteoblasts originate from pluripotent mesenchymal stem cells of the bone marrow, which also have the potential to differentiate into fibroblasts, chondrocytes, adipocytes and myoblasts (28, 29). The commitment of the mesenchymal stem cells into the osteoblast cell lineage is controlled by several transcription factors. One essential transcription factor includes the runt-related transcription factor 2 (Runx2) that is also known as core-binding factor alpha 1 (Cbfa1), which is highly expressed in cells of the osteoblast lineage (30-32). Runx2 up-regulates the expression of different osteoblast-specific genes including type I collagen, BSP and osteocalcin, and it thus promotes the development of the osteoblastic phenotype (31-33). Additionally, Runx2 negatively influences the proliferation of osteoblasts, thereby controlling their growth (34). Another important transcription factor involved in the osteoblastogenesis is called Osterix. Studies (35, 36) have demonstrated

defective bone formation secondary to an absence of osteoblasts in Osterix-deficient mice, underpinning the indispensable role of Osterix in osteoblast differentiation. Furthermore, the expression of osteoblast markers such as type I collagen, BSP, osteocalcin and osteopontin are significantly decreased in Osterix-deficient mice (35, 37, 38). It has been suggested that Osterix acts downstream of Runx2, but other studies indicate that Osterix expression is mediated independently of Runx2 (35, 39, 40). Other transcription factors that also participate in the osteoblastogenesis include homeobox proteins such as Msx2, Dlx3 and Dlx5 as well as helix-loop-helix proteins like Id and Twist (31, 39). The expression and activity of the transcription factors involved in the osteoblastogenesis is regulated by both systemic and local factors. These include growth factors such as bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor (FGF), hormones like parathyroid hormone (PTH) as well as the canonical Wnt/β-catenin signaling pathway (30, 38, 39, 41-45).

The differentiation of mesenchymal stem cells into mature osteoblasts involves the following stages of progressive development; mesenchymal stem cells, osteoprogenitor cells, preosteoblasts and osteoblasts (46, 47). The initial developmental phases are characterized by massive proliferation, but gradually the proliferation ceases and the production of osteoblast-specific proteins such as alkaline phosphatase, type I collagen and osteocalcin increases (48, 49). The mature bone-forming osteoblasts are responsible for the synthesis of extracellular matrix proteins and the subsequently mineralization of the matrix. During bone formation the osteoblasts synthesize and secrete type I collagen as well as other non-collagenous proteins such as BSP, osteocalcin and osteopontin which are associated with the mineralization process (26, 33, 49). These non-collagenous proteins are capable of binding calcium, thereby promoting the deposition of hydroxyapatite (25, 49). The production of osteocalcin is stimulated by 1,25-dihydroxycalciferol ($1,25(OH)_2D_3$), the biologically active form of vitamin D, whereas mechanical strain enhances the expression of both osteopontin and BSP in osteoblasts (50-54). During bone formation and mineralization some of the osteoblasts are trapped in the matrix and they subsequently differentiate into osteocytes, whereas the remaining osteoblasts either become quiescent lining cells or undergo apoptosis (55).

Osteocytes represent the final stage of differentiation in the osteoblast lineage and they are the most abundant cell type in bone (56). Osteocytes are found in spaces called lacunae in the bone tissue and have several cytoplasmic processes, which extend through small channels denominated canaliculi (57, 58). The osteocytes are thought to communicate with other osteocytes as well as with osteoblasts and bone lining cells on the bone surface via these processes and intercellular gap junctions (57-59). It has been suggested that osteocytes are capable of sensing mechanical strain on bone and subsequently communicate this information to the osteoblasts on the bone surface, leading to bone remodeling (57, 59-61). In this way, the mass and architecture of bones are adapted to the mechanical loading in accordance to the law of Wolff (62).

Bone lining cells are inactive, flattened cells that originate from osteoblasts, which have completed bone formation (19, 63, 64). The lining cells cover the non-remodeling bone surfaces, where they rest on a thin layer of unmineralized bone matrix (19, 64). The bone-resorbing osteoclasts are not capable of attaching to this unmineralized matrix, and therefore resorption cannot take place until the matrix has been degraded by means of the enzyme collagenase that is produced by the lining cells (19, 64). Bone lining cells are as aforementioned inactive, but they can be reactivated into

osteoblasts under the influence of certain stimuli such as estrogen, intermittent PTH or mechanical strain, leading to bone formation (65-67).
In addition to their essential role in bone formation, osteoblasts also control the differentiation and activity of osteoclasts through the production of various cytokines involved in osteoclastogenesis (see section 1.1.1.2) (29, 68, 69). Hence, osteoblasts also play a central role in the regulation of bone remodeling. Osteoblasts have surface receptors for various factors that regulate bone metabolism, including PTH, $1,25(OH)_2D_3$, prostaglandin E_2 (PGE_2), estrogen and androgen (19, 70, 71).

1.1.1.2 Osteoclasts

Osteoclasts originate from hematopoietic cells of the monocyte/macrophage lineage (72, 73). The transcription factor PU.1 is essential for the initial differentiation of macrophages, hence loss-of-function mutations in the *PU.1* gene prevents formation of both macrophages and osteoclasts (69, 73). Additionally, the transcription factor c-Fos plays a pivotal role in early osteoclast differentiation and commitment to the osteoclast lineage (74-76). The further differentiation of osteoclast precursors into mature osteoclasts is highly dependent on the two cytokines macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL), which are both expressed in osteoblast lineage cells (77-81). Actually, it has been demonstrated that a combination of M-CSF and RANKL is both necessary and sufficient for osteoclast generation in vitro (77). The pivotal role for M-CSF in osteoclast development is supported by studies (78, 82) conducted in mice lacking functional M-CSF due to a mutation in the *op/op* gene. These *op/op* mice fail to generate osteoclasts, which leads to the development of osteopetrosis that is a disease characterized by abnormally high bone mass caused by dysfunctional osteoclasts and decreased bone resorption and remodeling. The *op/op* osteopetrotic mice can be cured by administration of M-CSF (78, 82). M-CSF exerts its biological effect through binding to its receptor c-fms, which is expressed on osteoclast precursors and mature osteoclasts (83). Binding of M-CSF to c-fms induce intracellular signaling important for the proliferation and survival of osteoclast precursors (84, 85).
RANKL is a transmembrane protein that belongs to the tumor necrosis factor (TNF) superfamily (86, 87). It is highly expressed on osteoblast lineage cells as well as on activated T lymphocytes (87-89). In addition to its membrane-bound form, RANKL also exists in a soluble form. The latter is produced via proteolytic cleavage of the membrane-bound RANKL by a disintegrin and metalloprotease domain (ADAM) family members and matrix metalloproteases (MMPs) (86, 87). Studies (90-92) have demonstrated that ADAM17, ADAM19 and MMP-7 are involved in the generation of soluble RANKL. As mentioned above, RANKL is essential for osteoclastogenesis, and RANKL knockout mice develop osteopetrosis and defective tooth eruption secondary to a complete lack of osteoclasts (93, 94). Furthermore, RANKL promotes the activation of mature osteoclasts and inhibits osteoclast apoptosis, thereby prolonging their lifespan (79, 95). RANKL exerts its function through binding to its receptor RANK that belongs to the TNF receptor superfamily and is expressed on mature osteoclasts and their precursors (86, 88, 96). Thus, direct cell-cell contact between osteoblast lineage cells and osteoclast precursors is necessary for osteoclastogenesis to take place (72). Binding of RANKL to RANK causes activation of

intracellular signaling pathways, leading to the activation of several transcription factors involved in the regulation of osteoclast lineage commitment, activation of mature osteoclasts and osteoclast survival (86, 87, 97). A central initial step in RANK signaling involves recruitment of various TNF-receptor associated factors (TRAFs) that bind to different cytoplasmic domains on RANK (98). RANK has the ability to bind TRAF2, TRAF5 and TRAF6, of which TRAF6 is thought to be the most important for the osteoclastogenesis (86, 98-101). The interaction between RANK and TRAF leads to the activation of the transcription factors NF-κB and activator protein 1 (AP-1), which are both essential for the osteoclastogenesis. Additionally, TRAF6 activates a signaling pathway involving the tyrosine kinase c-Src through which the anti-apoptotic serine/threonine kinase Akt/PKB is activated, mediating osteoclast survival (102). RANK signaling also results in the up-regulation of the transcription factor nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (NFATc1), which plays an important role in the terminal osteoclast differentiation (103). Thus, binding of RANKL to RANK and the subsequent intracellular signaling is necessary for osteoclast differentiation and promotes the expression of osteoclast-specific markers such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor and carbonic anhydrase II (103-108).

The biological effect of RANKL is negatively regulated by its natural decoy receptor called osteoprotegerin (OPG), which is capable of binding RANKL (109). OPG is a soluble receptor, belonging to the TNF receptor superfamily (86, 110). It is secreted by osteoblasts and inhibits the function of RANKL by preventing the interaction between RANKL and its receptor RANK (86, 97). Hence, OPG inhibits the formation and activation of osteoclasts and thus bone resorption (110, 111). This is supported by studies (110, 112, 113) that have demonstrated that overexpression of OPG leads to osteopetrosis, whereas deletion of the OPG gene results in osteoporosis. The RANKL/OPG ratio controls the osteoclastogenesis and thus the degree of bone resorption, and numerous hormones and cytokines affect the osteoclastogenesis by modulating the expression of RANKL and/or OPG on osteoblasts (68, 73, 97). Hence, PTH, $1,25(OH)_2D_3$, PGE_2 and glucocorticoids stimulates the osteoclastogenesis by increasing the expression of RANKL and simultaneously decreasing the expression of OPG, leading to an increased RANKL/OPG ratio (114-120). On the other hand, estrogen and TGF-β inhibits osteoclast formation and activity through an increment of the OPG expression and a reduction of the RANKL expression (121-123). Additionally, it has been demonstrated that various cytokines such as interleukin (IL)-1, IL-6, IL-11 and TNF-α are capable of stimulating osteoclast differentiation and bone resorption (124-128).

Mature osteoclasts are large multinucleated cells formed via fusion of several mononucleated precursors (19, 20, 69). The fusion process is mediated by the transcription factors microphthalmia-associated transcription factor (MITF) and NFATc1 (129, 130). The mature osteoclasts have a unique ability to resorb bone (131, 132). The initial step of bone resorption involves the adhesion of osteoclasts to the underlying bone, which is facilitated by the integrin molecules β_1 integrin and $\alpha_v\beta_3$ integrin (68, 69, 73, 133). The β_1 integrin binds to type I collagen and fibronectin, whereas the $\alpha_v\beta_3$ integrin recognizes and binds to the amino acid sequence Arg-Gly-Asp (RGD), which is present in the bone matrix proteins osteopontin and BSP (68, 134, 135). The attachment of $\alpha_v\beta_3$ integrin to bone matrix proteins is further thought to mediate transmission of signals, ultimately

leading to intracellular cytoskeletal organization and polarization of osteoclasts, which is crucial for bone resorption (69, 73, 136). Thus, $\alpha_v\beta_3$ integrin plays an important role for normal osteoclast function and bone resorption. This is supported by a study conducted by McHugh et al. (137), who found that osteoclasts from β_3-deficient mice were dysfunctional and failed to resorb bone caused by abnormal cytoskeletal organization. Additionally, the β_3-deficient mice exhibited increased bone mass and hypocalcemia.

Binding of osteoclasts to bone matrix proteins causes polarization of the cells, which involves formation of a so-called ruffled border that is considered the resorptive organel of the osteoclast (69, 73). The ruffled border is characterized by several infoldings of the plasma membrane and is a product of massive exocytosis of intracellular vesicles or lysosomes (69, 73, 138). The ruffled border is surrounded by an actin ring or sealing zone, which is formed through cytoskeletal organization in response to osteoclast contact with bone (68, 135). The actin ring isolates the resorptive microenvironment beneath the osteoclast from the general extracellular space (68, 73). Both the ruffled border and the actin ring are essential for bone resorption (137, 139, 140). Their function is highly dependent on the c-Src gene, which is also necessary for $\alpha_v\beta_3$ integrin formation. Therefore, c-Src-deficient mice fail to resorb bone caused by defective ruffled border and actin ring formation, leading to osteopetrosis (140-143).

Bone resorption includes degradation of the inorganic hydroxyapatite, followed by degradation of the organic bone matrix (69). The former involves acidification of the resorptive microenvironment, which is mediated by a vacuolar proton pump (H^+-ATPase) that is present in the ruffled border membrane, see figure 1 (144). The H^+-ATPase pumps protons out of the osteoclast and into the resorptive lacuna via an ATP-dependent mechanism. In order to maintain an electroneutral intracellular environment, the osteoclast must pump out an equivalent number of negatively charged anions. This is achieved by a ClC-7 chloride channel in the ruffled border membrane that is associated with the H^+-ATPase, see figure 1 (138, 145). The H^+-ATPase is supplied with protons via the actions of the enzyme carbonic anhydrase II that converts carbon dioxide and water into bicarbonate and proton ions, see figure 1 (138). The excess bicarbonate is removed from the cell via a chloride-bicarbonate exchanger localized in the basolateral osteoclast membrane (69, 73, 138). The exchanger secretes bicarbonate out from the cell in exchange for chloride ions, and thus the intracellular pH value is maintained at a physiological level and the ClC-7 channel is provided with chloride ions (69, 73). The net result of the abovementioned mechanisms is secretion of hydrochloride acid (HCl) into the resorptive lacuna, producing a highly acidic microenvironment with a pH value of about 4.5 (68, 69, 73). The acidic environment facilitates the degradation of the inorganic bone mineral (68, 146).

Subsequently, the organic bone matrix, which primarily consists of type I collagen, is degraded by proteases produced by the osteoclasts. One of these is the lysosomal protease cathepsin K, which is highly expressed in osteoclasts (105, 147). Cathepsin K is capable of degrading collagen fibers at a low pH, and the enzyme plays a central role in the process of bone matrix degradation (88, 148-150). This has been demonstrated in several studies (149-151) which found that a mutation in the gene encoding cathepsin K was associated with deficient bone resorption and development of the rare bone disease pycnodysostosis. In addition, TRAP is a contributing factor in bone resorption, since this enzyme is capable of dephosphorylating osteopontin and BSP (152). A study by Angel et

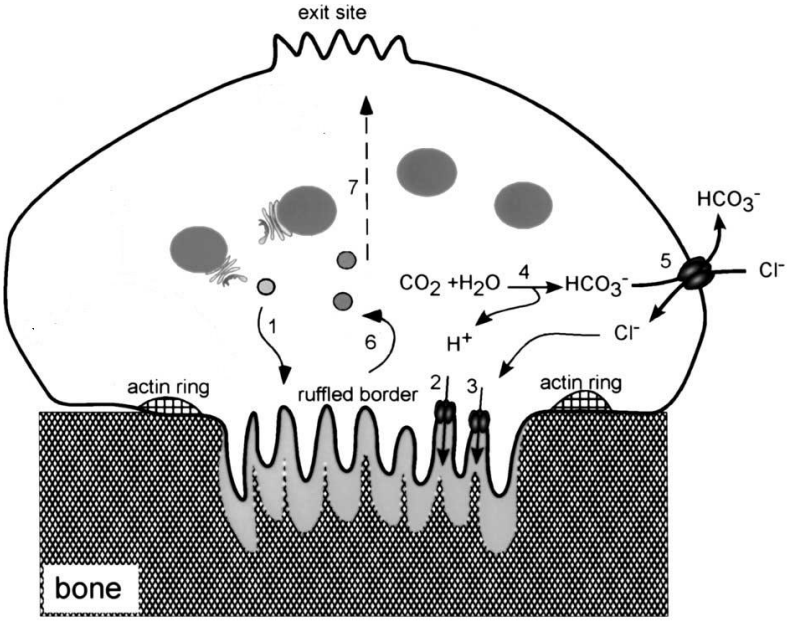

Figure 1: An overview of the main pathways involved in bone resorption (138). 1) Transport vesicle carrying lysosomal enzymes such as cathepsin K towards the ruffled border. 2) Vacuolar proton pump (H^+-ATPase) localized in the ruffled border membrane that pumps protons out of the osteoclast and into the resorptive lacuna, thereby mediating acidification of the extracellular microenvironment. 3) ClC-7 chloride channel localized in the ruffled border membrane in association with the H^+-ATPase. The ClC-7 chloride channel pumps out negatively charged chloride ions in order to maintain an electroneutral intracellular environment. 4) The enzyme carbonic anhydrase II converts carbon dioxide and water into bicarbonate and proton ions, thereby generating protons for the H^+-ATPase. 5) Chloride-bicarbonate exchanger. 6) Endocytosis of degraded matrix proteins. 7) Removal of bone resorption product by transcytosis.

al. (153) has demonstrated that mice overexpressing TRAP have an increased rate of bone turnover and mild osteoporosis, underpinning the importance of TRAP in bone degradation. Furthermore, osteoclasts produce MMPs, of which MMP-1, MMP-9 and membrane type 1 MMP (MT1-MMP) have been localized near the ruffled border, for which reason they are thought to play a role in bone degradation (154-157). However, inhibition of MMP-9 and MT1-MMP has only minimal effect on osteoclast-mediated bone resorption, indicating that the activity of these MMPs is not essential for normal bone degradation (138, 158). The bone degradation products are removed from the resorption site via transcytosis and released to the extracellular space (159).

As aforementioned, osteoclasts express calcitonin receptors, and thus calcitonin directly affects the activity of osteoclasts (106, 107). Calcitonin is released from the thyroid gland in response to elevated serum calcium concentrations, and binding of calcitonin to its receptor leads to inhibition of osteoclast activity and bone resorption (23). Calcitonin is thought to inhibit the osteoclast-mediated bone resorption through changes in cytoskeletal organization, actin ring disruption and inhibition of osteoclast protease production (20, 160, 161).

1.1.2 Bone Remodeling

Bone remodeling involves the replacement of existing bone tissue with newly formed bone. It is a continuous process that occurs throughout life and serves to maintain bone integrity and strength by replacing old bone containing microfractures with new bone of high quality (20, 29, 68, 162). Furthermore, bone remodeling is important for the maintenance of the calcium homeostasis in serum (19, 68). Remodeling involves coordinated activities of osteoclasts and osteoblast, which

form so-called basic multicellular units (BMUs). The lifespan of a BMU is 6-9 months, which is considerably longer than the average lifespan of both osteoclasts and osteoblasts, which is approximately 2 weeks and 3 months, respectively (33, 64, 163). Thus, the bone cells constituting the BMU must be continuously replaced in order to sustain a functional BMU. During bone remodeling, the BMU excavates a tunnel in cortical bone, whereas it excavates a trench on the bone surface of trabecular bone. The rate of excavation is about 25 µm per day in adult humans (64, 163). The initiation of bone remodeling and the formation of the BMU are thought to be mediated by signals from osteocytes via their canalicular network through which they are in direct contact with osteoblasts and bone lining cells on the bone surface (57, 59-61, 163).

Bone remodeling includes four phases; activation, resorption, reversal and formation, see figure 2 (33, 68). The activation phase involves recruitment of mononucleated osteoclast precursors from the circulation and bone marrow and subsequent differentiation of these precursors into mature multinucleated osteoclasts (33, 68). The differentiation and activation of osteoclasts is mediated by osteoblast lineage cells via direct cell-cell contact, facilitating RANKL-RANK interaction. Once the osteoclasts have been activated, they bind to the underlying bone matrix via integrins and form ruffled borders and actin rings, thereby initiating the resorption phase (138). The binding of osteoclast to the underlying bone is thought to involve the actions of bone lining cells. These cells produce proteolytic enzymes such as collagenase and MMPs, mediating degradation of the unmineralized bone matrix on the bone surface, thus facilitating attachment of osteoclasts to bone matrix proteins (19, 64, 162). During the resorption phase, the osteoclasts demineralize the bone matrix by acidification of the sub-osteoclastic microenvironment, and subsequently they degrade the bone matrix proteins through production of proteolytic enzymes, see figure 2. The following reversal phase represents the transition from bone resorption to bone formation (68). During this phase, the osteoclasts undergo apoptosis, and osteoblast precursor cells proliferate and differentiate into mature bone-forming osteoblast (33). The recruitment and differentiation of osteoblast precursors is thought to be mediated by local factors derived from the bone matrix, including TGF-β, IGF-1, BMPs, PDGF and FGF, see figure 2 (68, 162, 164-166). TGF-β inhibits the activity of osteoclasts by decreasing the expression of RANKL on osteoblasts and by inducing apoptosis of osteoclasts (167-170). In addition to its inhibitory effect on osteoclasts, TGF-β stimulates osteoblasts-mediated collagen synthesis and bone formation, thus favoring the transition from bone resorption to bone formation (171, 172). The growth factors IGF-1, PDGF, FGF and BMPs are all involved in the proliferation and differentiation of osteoblast precursors, thereby stimulating bone formation (162, 164, 166, 173-175). The following and final phase involves osteoblasts synthesizing organic bone matrix that fills in the resorption cavity and subsequently undergoes mineralization, see figure 2 (20, 33, 68, 162). During this phase, some of the osteoblasts will become trapped in the bone matrix and differentiate into osteocytes, some will undergo apoptosis and the rest will become quiescent lining cells, covering the resting bone surface (64).

On average, approximately 10 % of the entire skeleton is remodeled each year (22, 64). However, the turnover rate is much higher in trabecular bone (28 %) than in cortical bone (4 %) due to its greater surface area (20, 29, 64, 68). Bone turnover is closely regulated by local factors such as cytokines and growth hormones and systemic factors such as PTH, $1,25(OH)_2D_3$, calcitonin and sex

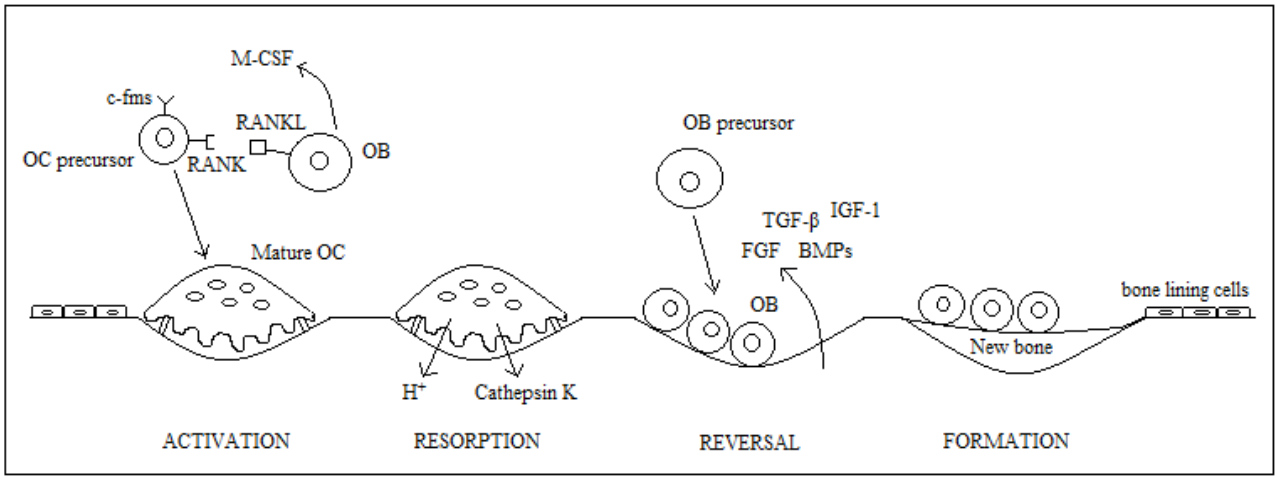

Figure 2: The bone remodeling process. During the activation phase, osteoclast precursors differentiate into mature osteoclast through direct interaction with osteoblast lineage cells. Osteoblast lineage cells produce the two cytokines M-CSF and RANKL, which are both necessary and sufficient for the development and activation of osteoclasts. The activated osteoclasts bind to the underlying bone by means of integrins, and subsequently they form ruffled borders and actin rings. During the resorption phase, the osteoclasts demineralize the bone matrix by acidification of the sub-osteoclastic microenvironment, and subsequently they degrade the bone matrix proteins through production of proteolytic enzymes such as cathepsin K. During the reversal phase, the osteoclasts undergo apoptosis, and osteoblast precursor cells proliferate and differentiate into mature bone-forming osteoblasts. The recruitment and differentiation of osteoblast precursors are thought to be mediated by local growth factors derived from the bone matrix, including TGF-β, IGF-1, BMPs and FGF. During the formation phase, the osteoblasts synthesize organic bone matrix that fills in the resorption cavity and subsequently undergoes mineralization. The resting bone surface is covered by quiescent bone lining cells. OC = osteoclast, OB = osteoblast, M-CSF = macrophage colony stimulating factor, RANK = receptor activator of nuclear factor kappa-B, RANKL = receptor activator of nuclear factor kappa-B ligand, TGF-β = tumor growth factor beta, IGF-1 = insulin-like growth factor 1, FGF = fibroblast growth factor, BMPs = bone morphogenetic proteins.

hormones (20, 33, 162, 176). Novel studies (177-182) suggest that the hormone leptin also may play a role in the regulation of bone turnover. Leptin is produced by adipocytes and it is known to regulate appetite, energy expenditure, body weight, fertility and growth (183). Studies (178, 182) have found that leptin-deficient *ob*/*ob* mice exhibit a high bone mass phenotype despite of both hypogonadism and hypercortisolism, although these conditions normally favor bone loss. Furthermore, intracerebroventricular administration of leptin reverses the high bone mass phenotype observed in *ob/ob* mice and induces bone loss in wild type mice through a central pathway involving the hypothalamus and sympathetic nervous system (178, 182). These results suggest that leptin is a potent inhibitor of bone formation. However, other studies (177, 179-181) have obtained conflicting results, suggesting that leptin stimulates bone formation. Cornish and colleagues (180) demonstrated that leptin stimulates osteoblast proliferation and inhibits the osteoclastogenesis, thus favoring bone formation. Leptin has also been shown to increase the bone mass and reduce bone fragility and susceptibility to fractures (179-181). The stimulatory effect of leptin is thought to be mediated through leptin receptors, which are present on osteoblasts as well as on chondrocytes (180, 184). Thus, so far it is uncertain whether leptin inhibits or stimulates bone formation.

Abnormal bone remodeling, i.e. an imbalance between bone resorption and bone formation, is associated with pathological conditions such as osteoporosis. The pathophysiology of osteoporosis will be addressed in the following sections.

1.2 Osteoporosis

Osteoporosis is generally defined as "a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (1, 3, 185, 186). The condition is caused by an imbalance between bone resorption and bone formation, in which the former exceeds the latter. From an etiological view, osteoporosis is divided into primary (idiopathic) and secondary osteoporosis. The two most frequent causes of primary osteoporosis include senile and postmenopausal osteoporosis. On the other hand, in secondary osteoporosis it is possible to identify another disease or a pharmacological intervention as the underlying cause.

1.2.1 Senile Osteoporosis

Senile osteoporosis primarily affects the elderly above the age of 75 years, and it is a consequence of the age-related progressive bone loss of 0.5 % per year, which is initiated after the attainment of peak bone mass (PBM) in the third decade of life (23, 187-189). Thus, the PBM, which is defined as the amount of bone tissue present at the end of skeletal maturation, has a great influence on the lifetime risk of developing osteoporosis (3, 23, 190). The size of PBM depends on heredity, sex, nutritional factors such as intake of calcium and protein, mechanical factors such as exercise and body weight as well as endocrine factors such as sex hormones, IGF-1 and $1,25(OH)_2D_3$ (190-195). Of these factors, heredity is the most important, accounting for about 75 % of the variation in PBM (190, 196). All the involved genes have not yet been identified, but polymorphisms in the gene encoding the vitamin D receptor (VDR) are thought to play a significant role (190, 195).

Senile osteoporosis is thought to be related to alterations in the $1,25(OH)_2D_3$ and calcium metabolism (189, 190, 197, 198). Deficiency of $1,25(OH)_2D_3$ is common among the elderly caused by decreased sun exposure, an insufficient dietary intake of vitamin D as well as age-related reduced cutaneous and/or renal synthesis of $1,25(OH)_2D_3$ (188, 189, 197, 198). The $1,25(OH)_2D_3$ deficiency leads to reduced intestinal absorption of calcium, and thus a decreased concentration of calcium in serum (189, 197). In some people, the intestinal absorption of calcium is reduced despite a normal level of $1,25(OH)_2D_3$ due to a reduced number of VDRs in the intestines or reduced responsiveness to $1,25(OH)_2D_3$ (189, 198). Additionally, insufficient intake of calcium in the diet may be a contributing factor to the low serum calcium often observed in the elderly. In order to maintain a physiological level of calcium in serum, a compensatory increase in the production of PTH takes place, resulting in secondary hyperparathyroidism (188, 189, 198). This ultimately stimulates the osteoclast differentiation and activation, leading to increased bone resorption and bone loss.

The pathogenesis of senile osteoporosis also involves suppressed osteoblastogenesis and decreased osteoblast function (188). With increasing age, the differentiation of mesenchymal stem cells in the bone marrow shifts towards adipogenesis in the expense of osteoblastogenesis, leading to fat accumulation in the bone marrow (188, 199). Furthermore, increased apoptosis reduces the life span

of osteoblasts (200). Hence, the number and activity of osteoblasts decrease, leading to diminished bone formation and increased risk of osteoporosis.
The prevalence of senile osteoporosis is approximately twice as high in women compared to men, which is explained by women's lower PBM and longer life expectancy (23, 187, 189, 196). Senile osteoporosis affects both cortical and trabecular bone, and the disease is typically manifested by hip fractures (187-189).

1.2.2 Postmenopausal Osteoporosis

Postmenopausal osteoporosis is caused by estrogen deficiency associated with the onset of natural or surgically induced menopause (201-203). Estrogen deficiency leads to markedly increased osteoclastogenesis and bone resorption, resulting in bone loss and enhanced susceptibility to fractures (202, 203). Thus, estrogen exerts a protective effect on the skeleton, which is mediated through the estrogen receptor (ER) that is found in the cytosol of both osteoblasts and osteoclasts (204). Two subtypes of the ER exist, namely ERα and ERβ, and they are both expressed in osteoblasts, whereas osteoclasts only express ERα (204-206). Estrogen is thought to prevent bone loss by decreasing the production of osteoclast-stimulating cytokines such as IL-1, IL-6, TNF-α, M-CSF and PGE2, leading to inhibition of the differentiation and the activity of osteoclasts (202-204, 207-210). Furthermore, estrogen stimulates the production of TGF-β, which mediates osteoclast apoptosis, thereby shortening the lifespan of osteoclasts (170, 202, 205). Hence, estrogen deficiency results in increased levels of the abovementioned bone resorbing cytokines in the bone marrow microenvironment as well as increased osteoclast survival, which form the basis of the pathogenesis of postmenopausal osteoporosis.
IL-1 and TNF-α stimulate osteoclast differentiation and activation by up-regulating the expression of RANKL on osteoblasts (127, 211). They further stimulate the production of other bone resorbing cytokines, including IL-6 and M-CSF (212, 213). Studies have demonstrated that blockage of the IL-1 activity by means of an IL-1 receptor antagonist resulted in decreased osteoclast formation and bone resorption following ovariectomy or menopause (208, 209, 214). Similar results were obtained by blocking the activity of TNF-α via administration of TNF binding protein (TNFbp) or use of transgenic mice overexpressing the decoy receptor TNF-α receptor 1 (TNFR1) (208, 214, 215). However, in order to completely prevent bone loss following ovariectomy, simultaneous blockage of IL-1 and TNF-α is necessary (216). Thus, both IL-1 and TNF-α play a critical role in the bone loss associated with estrogen deficiency. Another important cytokine involved in the pathogenesis of postmenopausal osteoporosis is IL-6. IL-6 is produced by osteoblasts and bone marrow stromal cells and stimulates the early stages of the osteoclastogenesis (203). IL-6 exerts its effect via binding to its surface receptor IL-6R, leading to intracellular signaling that involves the glycoprotein 130 (gp130) pathway (217). Estrogen inhibits the expression of gp130, and thus gp130 is up-regulated in estrogen deficiency, thereby potentiating the effects of IL-6 on the osteoclastogenesis (218). A study by Jilka and colleagues (219) found that injection of neutralizing anti-IL-6 antibody in mice caused a reduction in the number of osteoclast precursors and mature osteoclasts in ovariectomized mice. Another study conducted by Poli et al. (220) further demonstrated that IL-6 knockout mice were protected from bone loss caused by estrogen

deficiency. These studies thus substantiate the importance of IL-6 in mediating the bone loss observed in postmenopausal women.

There is also evidence suggesting that T-cells are involved in the development of postmenopausal osteoporosis (221, 222). A study by Cenci et al. (222) demonstrated that T-cells from ovariectomized mice exhibit increased production of TNF-α, leading to increased osteoclastogenesis and bone resorption. They further showed that the enhanced production of TNF-α was a result of T-cell proliferation and not due to an increased production of TNF-α per T-cell. Additionally, they found that T-cell deficient mice were resistant to the increased osteoclastogenesis, bone resorption and bone loss otherwise induced by ovariectomy.

The RANKL/RANK/OPG system is also influenced by the estrogen deficiency following menopause (202). Estrogen deficiency leads to decreased expression of OPG mRNA, thereby stimulating the osteoclastogenesis (206, 223). Furthermore, estrogen deficiency causes increased production of cytokines such as TNF-α, IL-1 and PGE2, which are known to up-regulate the expression of RANKL (211, 224). The net result is an increased RANKL/OPG ratio, which facilitates the differentiation and activation of osteoclasts, leading to increased bone resorption and bone loss.

To summarize, postmenopausal osteoporosis results from increased osteoclastogenesis and bone resorption caused by estrogen deficiency. Thus, after the menopause an accelerated bone loss is observed in women, especially during the first decade (23, 29, 201, 203, 204). The bone loss is most pronounced in trabecular bone, which predisposes to fractures in the vertebrae that predominantly consist of this bone type (187, 189, 204).

1.2.3 Secondary Osteoporosis

Secondary osteoporosis is caused by an underlying disease or a pharmacological intervention. Several diseases are associated with osteoporosis, including anorexia nervosa, osteogenesis imperfecta, Morbus Cushing, cancer, prolonged immobilization, rheumatoid arthritis (RA), malabsorption (e.g. Morbus Crohn, coeliac disease, gastrectomy), chronic kidney disease and primary hyperparathyroidism (3). Pharmacological interventions that increase the risk of developing osteoporosis include glucocorticoids, antiepileptic drugs and aromatase inhibitors (225-233). Additionally, various lifestyle factors such as smoking, excessive alcohol consumption and an inadequate dietary intake of calcium and vitamin D increase the risk of osteoporosis (234-241).

In the following sections, the association between RA and osteoporosis as well as the link between use of glucocorticoids and osteoporosis will be further elucidated.

1.2.3.1 Rheumatoid Arthritis and Osteoporosis

RA is a chronic inflammatory autoimmune disease that typically affects multiple peripheral synovial joints, including the joints of the hands, feet, wrists, shoulders and knees (242, 243). It leads to progressive destruction of the articular cartilage and bone, ultimately resulting in immobility, disability and pain (244, 245). RA is a common disease, affecting approximately 1 % of the population (242, 243, 246). The frequency is higher in women compared to men with a female to male ratio of 3:1 (21, 247). The etiology of RA is unclear, but genetic predisposition contributes markedly to the risk of developing the disease (248). Studies (249-252) have established that

presence of the human leukocyte antigen (HLA)-DR4 haplotype is associated with an increased susceptibility to RA as well as enhanced disease severity. Additionally, a single-nucleotide polymorphism in the gene protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) has also been associated with RA (253-255).

The pathogenesis of RA is complex and involves several cell types as well as various pro-inflammatory cytokines (243, 246, 256). RA is thought to be a T cell-mediated disease based on the following evidence: Firstly, the abovementioned relationship between HLA-DR4 and RA suggests that T cells have a central role in the pathogenesis of the disease (242, 243, 246, 256). HLA-DR4 is a major compatibility complex (MHC) class II molecule, which primary role is to present peptides to $CD4^+$ T cells. Thus, it is believed that the molecule binds and presents an arthritogenic peptide in predisposed individuals, resulting in the activation of $CD4^+$ T cells and the initiation of an inflammatory response (242, 243). However, no specific reactive peptide has been identified so far. Secondly, animal studies have demonstrated that collagen-induced arthritis and adjuvant arthritis in rodents, which are similar to human RA, are T cell-dependent (242, 243, 257). Finally, presence of a large number of T cells in the inflamed synovium from RA patients has been demonstrated, underpinning the critical role of this specific cell type in the RA pathogenesis (246, 257). The $CD4^+$ T cells further activate other cell types involved in the maintenance of the inflammatory response and the joint destruction observed in RA, including B cells, synovial fibroblasts, macrophages, chondrocytes and osteoclasts (242, 246). The activation is mediated by T cell-dependent secretion of interferon-γ (IFN-γ) and IL-17 as well as by cell-to-cell contact.

B cells play a central role in RA, since selective blockage of these cells by use of the anti-CD20 monoclonal antibody rituximab causes amelioration of symptoms in RA patients (258, 259). Activated B cells differentiate into plasma cells that produce autoantibodies called rheumatoid factor (RF) (256). RF targets the Fc portion of immunoglobulin G (IgG), resulting in the formation of immune complexes that activate the complement system (242, 243, 246, 257). This leads to the recruitment of inflammatory cells such as macrophages and neutrofiles into the affected joints, thereby contributing to the inflammatory response (242, 260). Although autoantibodies thus are thought to play a central role in the RA pathogenesis, only about 80 % of RA patients are seropositive for RF (243, 247). Furthermore, some healthy individuals as well as patients with other rheumatic diseases such as Sjögren's syndrome or systemic lupus erythematous also produce RF, indicating that the presence of autoantibodies is insufficient for the induction of a RA specific pathogenesis (243, 261, 262). In addition to their autoantibody-producing properties, B cells are also involved in antigen presentation for and activation of $CD4^+$ T cells (246, 260).

Macrophages are an important source of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-12 and IL-18 that are all involved in the pathogenesis of RA (246, 256, 263-265). Especially TNF-α is thought to be a central mediator of the inflammatory response observed in RA, because anti-TNF-α therapy such as infliximab or etanercept significantly inhibits the progressive joint destruction and reduces the symptoms of RA (266-270). TNF-α normally up-regulates other pro-inflammatory cytokines such as IL-1, IL-6 and IL-8, which may explain the substantial effect of anti-TNF-α therapy in RA (256, 271, 272). IL-1 is likewise an important cytokine in RA, and it is a more potent mediator of the progressive articular cartilage and bone damage seen in RA than TNF-α (273-275). Joosten et al. (273) found that blockage of IL-1 completely prevented the bone and

cartilage destruction in collagen-induced arthritis in mice, whereas neutralization of TNF-α only had a minor effect on the tissue damage. IL-1 is thought to mediate the destruction of cartilage by stimulating the production of proteases such as MMPs, cathepsins and aggrecanases in chondrocytes and synovial fibroblasts, which facilitate the degradation of extracellular matrix proteins, including type II collagen and proteoglycans (243, 246, 256, 275, 276). Additionally, IL-1 inhibits the synthesis of new cartilage matrix proteins in chondrocytes, thereby causing a net degradation of articular cartilage (244, 275).

In RA, a subpopulation of activated synovial fibroblasts is characterized by an aggressive invasive behavior and uncontrolled proliferation, resulting in synovial hyperplasia and pannus formation, which both are key features of RA (242, 276). Pannus is invasive granulation tissue that arises from the junction between the synovial tissue and articular cartilage and may cause erosion of both cartilage and bone (277). The development of pannus tissue is dependent on angiogenesis that is stimulated by cytokines and growth factors such as TNF-α, basic FGF (bFGF) and vascular endothelial growth factor (VEGF) produced by synovial fibroblasts and macrophages (246, 278).

The activated synovial fibroblasts are along with activated T cells thought to stimulate the differentiation of synovial macrophages into mature osteoclasts (244, 276, 279-281). This differentiation is mediated by RANKL, which is expressed on both synovial fibroblasts and activated T cells (246, 282, 283). The expression of RANKL is up-regulated by several pro-inflammatory cytokines involved in the RA pathogenesis such as TNF-α, IL-1, IL-6, IL-17 and IL-18, thereby facilitating osteoclastogenesis and bone resorption (246, 284, 285). The increased bone resorption observed in RA is inhibited by means of OPG in a dose-dependent manner, and is thus dependent on the RANKL/OPG ratio (281, 286). However, some cytokines such as TNF-α and IL-1 are also capable of inducing osteoclastogenesis independently of the RANKL/RANK/OPG system (287).

The abovementioned underlying mechanisms explain why patients with RA have an increased risk of developing osteoporosis and experience bone fractures as demonstrated in several studies (288-290). In RA patients, two different forms of osteoporosis occur, namely local juxta-articular osteoporosis around affected joints as well as generalized osteoporosis with excessive bone loss in the appendicular and axial skeleton distant from inflamed joints (290). The former is characteristically found in the early course of the disease (290).

1.2.3.2 Glucocorticoids and Osteoporosis

Glucocorticoids are anti-inflammatory and immunosuppressive drugs that are used for the treatment of multiple diseases, including Addison's disease, RA, systemic lupus erythematosus, dermatitis, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease, organ transplantation and autoimmune hemolytic anemia (291, 292). Synthetic glucocorticoids are available in different formulations such as oral, injectable and topical (225, 292, 293). They are absorbed almost completely from the gastrointestinal tract, resulting in a high bioavailability (292). They are primarily metabolized in the liver by conjugation and have a half-life of approximately 4 hours, which is prolonged compared to that for endogenous cortisol (292, 293). Glucocorticoids mediate their biological effect through binding to the glucocorticoid receptor (GR), which is part of the nuclear receptor superfamily, see figure 3 (294). The GR contains a glucocorticoid-binding

domain and a DNA-binding domain, among others (295). In the absence of ligand, i.e. glucocorticoid, the inactive GR resides in the cytoplasm, where it is anchored with proteins, including two molecules of heat shock protein 90 (Hsp90) (292). Upon binding of glucocorticoid, the GR disassociates from the protein complex and undergoes conformational changes (292, 294). The GR-ligand complex then translocates into the nucleus, where it regulates the expression of various target genes through different mechanisms, see figure 3 (294). The GR-ligand complex may dimerize and bind to either glucocorticoid response elements (GREs) or negative GREs (nGREs) in the promotor region of certain target genes, resulting in transcriptional activation and repression, respectively (292, 294). Alternatively, the GR-ligand complex may interact directly with the transcription factors NF-κB and AP-1, thereby preventing the transcription of several pro-inflammatory cytokines and chemokines (292, 294, 296, 297). This interaction is thought to be responsible for the anti-inflammatory and immunosuppressive effects of glucocorticoids (296, 297).

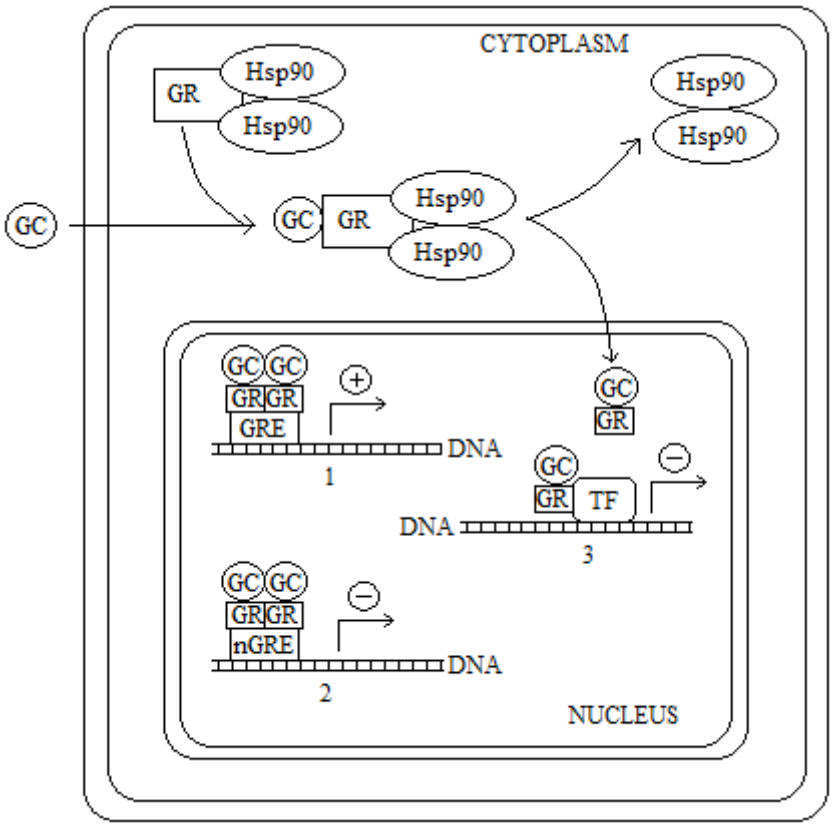

Figure 3: A simplified model of the interaction between glucocorticoid and its receptor that mediates the biological effects of the drug. Glucocorticoid is transported to the target cell via the blood circulation and enters the cell through simple diffusion. The inactive intracellular glucocorticoid receptor is bound to several proteins, including two molecules of Hsp90. When glucocorticoid binds to its receptor, the receptor dissociates from the protein complex and undergoes conformational changes that lead to its activation. The ligand-receptor complex translocates to the nucleus, where it is capable of regulating the expression of various target genes through different mechanism; 1) The ligand-receptor complex dimerizes and binds to GREs in the promotor region, resulting in transcriptional activation. 2) The dimerized ligand-receptor complex binds to negative GREs in the promotor region, which leads to transcriptional repression. 3) Directly interaction between the ligand-receptor complex and transcription factors such as NF-κB and AP-1 prevents the transcription of several pro-inflammatory cytokines. GC = glucocorticoid, GR = glucocorticoid receptor, Hsp90 = heat shock protein 90, GRE = glucocorticoid response element, nGRE = negative glucocorticoid response element, TF = transcription factor.

Two isoforms of the human GR exist, and they are termed hGRα and hGRβ (292, 298). The hGRα is a ligand-activated transcription factor that regulates the expression of various target genes (292, 298). On the other hand, hGRβ is not capable of binding glucocorticoid and is therefore transcriptionally inactive (298). However, hGRβ is able to inhibit the biological effects mediated by hGRα, thereby functioning as a physiological endogenous inhibitor of glucocorticoid action (298).

Long-term treatment with glucocorticoids is associated with various severe side effects, including development of osteoporosis (298, 299). Several studies have demonstrated that treatment with glucocorticoids leads to reduced bone mass as well as increased fracture risk (225-227, 300, 301). About 30-50 % of the patients receiving long-term glucocorticoid therapy experience an osteoporotic fracture and glucocorticoid-induced osteoporosis is currently the most frequent cause of secondary osteoporosis (294, 302-305). The bone loss is dose- and time-dependent, and it is more severe in patients receiving systemic glucocorticoids compared to topical preparations (225-227, 306, 307). The bone loss is usually most pronounced during the first year of treatment, whereupon it continues at a slower rate (294, 299, 303, 306).

The mechanisms underlying glucocorticoid-induced osteoporosis are multifarious and include both direct and indirect effects on the skeleton (299, 303, 304, 308). Firstly, glucocorticoids inhibit the intestinal absorption of calcium by reducing the active transcellular transport of calcium in the duodenum as well as by reducing the synthesis of calcium-binding proteins (304, 306, 308-310). In addition, glucocorticoids decrease the renal reabsorption of calcium, leading to increased excretion of calcium and hypercalciuria (304, 306, 308, 311). Consequently, the level of calcium in serum decreases, which leads to the development of secondary hyperparathyroidism (304, 306, 308). Hyperparathyroidism further leads to increased osteoclastogenesis and bone resorption, thereby facilitating an excessive bone loss.

Moreover, treatment with glucocorticoids also affects the synthesis of sex hormones through inhibition of the hypothalamic-pituitary-gonadal axis in both sexes (303, 304, 306, 308). Glucocorticoids inhibit the secretion of luteinizing hormone (LH) from the pituitary gland in response to hypothalamic gonadotropin-releasing hormone (GnRH) (304, 312, 313). This inhibitory effect of glucocorticoids on the gonadotropin secretion may also take place on a suprapituitary level, i.e. be mediated by disruption of the secretion of GnRH from the hypothalamus (314). The low plasma levels of LH further result in decreased production of estrogen and testosterone in the ovaries and the testes, respectively (304). The decreased levels of sex hormones in plasma subsequently result in increased osteoclastogenesis and bone resorption (202, 203, 315).

Furthermore, glucocorticoids also directly affect the activity of bone cells, thereby disrupting the balance between bone resorption and bone formation. Glucocorticoids inhibit the proliferation and differentiation of osteoblast precursors, which leads to a reduced number of mature osteoblasts and thus decreased bone formation (305, 316, 317). The underlying mechanism includes up-regulation of the transcription factor peroxisome proliferator-activated receptor gamma 2 (PPARγ2), which stimulates adipogenesis in the bone marrow at the expense of osteoblastogenesis (294, 303, 318, 319). In addition, glucocorticoids decrease the lifespan of mature osteoblasts by inducement of apoptosis (305, 317). This further contributes to the decreased bone formation observed in glucocorticoid-induced osteoporosis. Glucocorticoids also inhibit the function of osteoblasts by

decreasing their synthesis of type I collagen (320, 321). Additionally, they reduce the expression of other extracellular matrix proteins such as osteocalcin, osteopontin and BSP (306, 308). Glucocorticoids also have a more indirect effect on osteoblasts, which is mediated by growth factors such as TGF-β and IGF-1. TGF-β usually increases the replication and synthesis of type I collagen in osteoblasts, leading to enhanced bone formation (171, 172). However, administration of glucocorticoid causes decreased binding of TGF-β to its signal-transducing receptors, thereby inhibiting the anabolic effects of TGF-β on bone (322). Like TGF-β, the growth factor IGF-1 also stimulates the osteoblastogenesis and the synthesis of type I collagen, leading to increased bone formation (299, 303, 308). But glucocorticoids inhibit the transcription of IGF-1 and thus bone formation (323). The activity of IGF-1 is regulated by six different IGF binding proteins (IGFBPs) (299, 303, 308). The IGFBP-5 stimulates skeletal growth, but this protein is down-regulated by glucocorticoids (299, 303, 308).

Glucocorticoids also influence the activity of osteocytes by reducing their lifespan due to increased apoptosis (305, 317). O'Brien et al. (317) found that the glucocorticoid-induced death of osteocytes was associated with reduced bone strength that was independent of bone loss. The underlying cause may be that microfractures accumulate in the bone tissue due to compromised mechanosensing of shear stress, resulting in impaired bone quality and mechanical integrity (59, 60, 303, 308).

Moreover, glucocorticoids have an impact on osteoclast activity, although the exact effect remains inconclusive, because studies have obtained conflicting results (294, 304, 308). Some studies (119, 120, 324-326) showed that glucocorticoids increased the osteoclast activity and bone resorption as opposed to other studies (327-329) which demonstrated that glucocorticoids inhibited bone resorption in vitro. These discrepancies may be explained by diverse effects of glucocorticoids in different species or by different effects of the drug in vitro compared to in vivo. However, the majority of in vivo studies (324, 326, 330) suggest that glucocorticoids in fact do induce osteoclast differentiation and increased bone resorption, which is consistent with the increased bone loss observed in patients receiving glucocorticoid therapy. The mechanisms underlying the glucocorticoid-induced osteoclastogenesis and osteoclast activation include increased expression of RANKL and M-CSF as well as decreased expression of OPG (119, 120, 303, 325). Additionally, glucocorticoids inhibit the apoptosis of mature osteoclasts, thereby enhancing the lifespan of these cells and stimulating the bone resorption process (324, 330). The bone resorption is further facilitated by the glucocorticoid-induced expression of specific MMPs, which are involved in the degradation of type I collagen (299, 303, 331).

To summarize, glucocorticoid therapy increase the risk of developing osteoporosis through various mechanisms that include indirect effects on the skeleton such as altered calcium metabolism and decreased synthesis of sex hormones as well as direct effects on bone cells and their differentiation, function and survival.

1.2.4 Dual-Energy X-ray Absorptiometry

The diagnosis of osteoporosis is primarily based on so-called dual-energy X-ray absorptiometry (DXA), which is considered a simple, safe and precise method for determination of bone mass density (BMD) (332-334). BMD is defined as the bone mineral mass, i.e. the amount of hydroxyapatite, per square centimeter of bone, and it is measured in g/cm^2 (332, 334).

Approximately 80 % of the bone strength is directly related to the BMD, while the remaining 20 % depends on the elasticity of bones as well as the three-dimensional structure of the bone tissue (3, 196). BMD is therefore a useful predictor of osteoporotic fracture risk and thus a clinically relevant measure (7, 335, 336). The determination of BMD by DXA scanning is based on the fact that transmission of X-rays through the body results in greater attenuation in bone compared to soft tissue (332-334). The attenuation of X-rays depends on the thickness of the tissue, the atomic number of its constituents as well as the photon energy (332, 334). DXA scanners use an energy source that generates X-rays at two different energies, thereby enabling determination of two tissue components such as bone tissue and soft tissue. There are two different methods to generate a dual energy spectrum; the use of a K-edge filter and the application of a switching pulse system. A K-edge filter can be made of either cerium or samarium and is used to filter polyenergetic X-rays into two photon peaks with different energies (333, 337). On the other hand, the switching pulse system rapidly alternates the voltage of the X-ray generator, thereby producing two beams of high and low energy, respectively (332, 333). During the DXA scanning, the subject is placed on a bed, below which the energy source is placed (333). The attenuated X-rays that pass through the body during the scanning session are measured by a detector localized above the patient (333). A DXA measurement lasts for about 20 minutes (333, 334).
In order to calculate BMD, the bone mineral content (BMC) as well as the projected bone area must be determined. However, the determination of BMC is complicated because of the variable distribution of soft tissue in bone regions (332, 333). The soft tissue adjacent to bone is used as a reference area with similar thickness and soft tissue composition (332). Thus, the attenuation measured in the adjacent soft tissue is used to extrapolate the tissue composition in the bone region (333, 334). The projected bone area is found by use of an edge detection algorithm. This algorithm locates the bone edges, and subsequently the total projected bone area is determined by summarizing all the pixels identified as bone (332). Then, BMD is calculated by dividing BMC with the projected bone area (332). BMD is most frequently measured in the lumbar spine (L1-L4) and the proximal hip (total hip or femoral neck), because BMD measured at these specific sites is predictive of fractures of the vertebrae and hip, respectively, and thus has clinical relevance (3, 338-340).
The radiation dose from DXA measurements is very low compared to the background radiation and other examination methods such as X-ray (3, 196, 332-334). Another advantage of DXA is the relatively high reproducibility that is demonstrated by a coefficient of variance (CV) of only 1-2 % dependent on the site of measurement (288, 332, 333). However, the CV is higher in subjects with low BMD, i.e. patients with osteoporosis, and in obese subjects (333, 334).

BMD measurements can be used to calculate a so-called T-score, which states the number of standard deviations (SD) that the BMD measurement deviates from the mean value in younger healthy subjects of the same sex, i.e. from the PBM (3, 186, 196, 338). According to the World Health Organization (WHO), a T-score >-1 is defined as normal, a T-score between -1 and -2.5 is defined as osteopenia or low bone mass, and a T-score ≤-2.5 is diagnostic for osteoporosis (185, 338, 341). Furthermore, severe osteoporosis is defined as a T-score ≤-2.5 and a concomitant fragility fracture (341). The T-score is a significant predictor of fracture risk, and a decrease in T-

score of 1 in the hip or the lumbar spine causes an increment in fracture risk of 2.6 and 2.3, respectively (340). The BMD measurements can also be used to calculate a Z-score, which states the number of SDs that the BMD measurement deviates from the age- and gender-matched average (196, 338). A Z-score ≤ -2.0 is considered below the expected range for age, whereas a Z-score > -2.0 per definition is within the expected range for age (341). T-score is applied in postmenopausal women and men aged 50 years or older, whereas Z-score preferably is used in premenopausal women and younger men < 50 years old as well as in children (341).

2. Aim

The aim of the present study was to investigate if RA patients or patients receiving prednisolone therapy were more likely to develop osteoporosis, i.e. had lower T-scores. The hypothesis was that patients diagnosed with RA or patients treated with prednisolone had lower T-scores compared to patients without RA and patients receiving no prednisolone therapy, respectively.

3. Methods

3.1 Study population

The study population included all patients that had a DXA examination at Aalborg University Hospital during the time period 1st of January 2013 to 31st of December 2013. A total of 3557 patients participated in the study. Of these, 2842 were women and 714 were men, resulting in a female to male ratio of 4.0. The mean age of the patients was 62.8 ±13.3 years. The study was approved by The Danish Data Protection Agency.

3.2 Database

The data used for statistical analysis were obtained from a database from the Department of Endocrinology at Aalborg University Hospital. The database contained results from DXA measurements, i.e. T-scores, as well as information about height, weight, age, gender, previous fractures, genetic disposition, smoking status, alcohol consumption, intake of calcium and vitamin D, prednisolone treatment including dose, severe diseases including RA, use of anti-osteoporotic drugs, age at menopause and sun exposure. This information was obtained from self-completion questionnaires that were filled in by all patients referred to a DXA examination at Aalborg University Hospital. Two slightly different self-completion questionnaires were used, because a newer and more detailed questionnaire was introduced in the summer 2013 and subsequently replaced the older one. As opposed to the old questionnaire (appendix I), the new questionnaire (appendix II) provided information about RA prevalence and dose of prednisolone. In total, 1882 patients filled in the old questionnaire, whereas 1675 patients completed the new questionnaire.
The DXA measurements were performed by trained personnel using a Hologic Discovery QDR device (Waltham, Massachusetts, USA). T-scores were obtained for both lumbar spine (L1-L4) and total hip. A DXA measurement lasted approximately 20 minutes.

3.4 Statistical Analysis

All data were presented as means and SD of the means (mean±SD). Two-sample t-test was used for analysis when appropriate, whereas Mann-Whitney U test was performed when data were not normally distributed. Non-metric data were analyzed by means of the Chi-squared test. The association between prednisolone dose and T-score was investigated by use of Pearson's correlation analysis. Stepwise multiple linear regression analysis was used to identify independent predictors of T-scores in the hip and lumbar spine. Data obtained from the two different questionnaires were analyzed separately. All missing data were omitted from the statistical analyses. The statistical analyses were performed in SPSS Statistics version 21. *P*-values less than 0.05 were considered significant.

4. Results

	Old questionnaire (n=1882)	New questionnaire (n=1675)
Time period	1st of January to 5th of July 2013	6th of July to 31st of December 2013
Subjects (%)	52.9 %	47.1 %

Table 1: The table provides an overview of the old and the new questionnaire. The old questionnaire covered the time period from 1st of January to 5th of July, and it was filled in by 1882 subjects, corresponding to 52.9 %. The new questionnaire covered the time period from 6th of July to 31st of December 2013, and it was completed by 1675 subjects, which was equivalent to 47.1 % of the study population.

The features of the two questionnaires are outlined in table 1. The old questionnaire covered the time period from 1st of January to 5th of July 2013, and it was completed by a total of 1882 subjects, corresponding to 52.9 % of the study population. The new questionnaire covered the time period from 6th of July to 31st of December 2013, and it was filled in by the remaining 1675 subjects, which was equivalent to 47.1 % of the study population.

According to the old questionnaire, 8.7 % of the subjects received > 5 mg of prednisolone daily, whereas the remaining 91.3 % received no or ≤ 5 mg prednisolone daily. These two groups of subjects were compared with regard to various factors that might influence bone strength, see table 2. The subjects receiving >5 mg prednisolone had higher T-scores of both the lumbar spine and the hip compared to the subjects receiving no or ≤ 5 mg prednisolone (T-score lumbar spine -1.0 ± 1.5 vs. -1.4 ± 1.5, *P*=0.003; T-score hip -1.0 ± 1.1 vs. -1.2 ± 1.1, *P*=0.037). Furthermore, both the mean

	Prednisolone >5 mg (n=159)	No or ≤5 mg prednisolone (n=1661)	*P*-value
Females (%)	64.2	82.4	<0.001*
Age (years)	60.9 ± 14.7	62.6 ± 13.0	0.272
Height (cm)	167.1 ± 9.2	164.6 ± 8.6	0.001*
Weight (kg)	74.3 ± 12.9	69.0 ± 13.2	<0.001*
Age at menopause (years)	48.9 ± 4.4	47.8 ± 5.5	0.097
T-score lumbar spine	-1.0 ± 1.5	-1.4 ± 1.5	0.003*
T-score hip	-1.0 ± 1.1	-1.2 ± 1.1	0.037*
Previous fracture (%)	29.9	33.8	0.119
Smoking (%)	20.8	21.1	0.919
Alcohol ≥ 3 units per day (%)	3.1	3.2	0.972
Anti-osteoporotic therapy (%)	21.2	18.7	0.447
Milk and cheese intake (%)			
- Rarely	3.8	4.5	0.540
- Every week	9.5	12.2	
- Every day	86.7	83.3	
Sun exposure (%)			
- Low	24.1	16.7	0.065
- Normal	69.6	75.6	
- High	6.3	7.6	

Table 2: Patients receiving > 5 mg prednisolone (n=159) were compared to patients receiving no or ≤ 5 mg prednisolone (n=1661) with regard to various parameters that may affect bone strength. Data are presented as means ± SD or percentages. * Indicates a statistical significant difference between the two groups (*P*<0.05). The table is made based on data from the old questionnaire as well as results obtained from DXA measurements.

	Prednisolone (n=231)	No prednisolone (n=1380)	*P*-value
Females (%)	57.1	82.8	<0.001*
Age (years)	65.0 ± 14.0	62.7 ± 13.5	0.002*
Height (cm)	168.1 ± 9.6	164.8 ± 8.5	<0.001*
Weight (kg)	76.9 ± 19.0	70.6 ± 16.1	<0.001*
Age at menopause (years)	48.1 ± 8.5	48.1 ± 5.5	0.088
T-score lumbar spine	-0.9 ± 1.6	-1.5 ± 1.4	<0.001*
T-score hip	-1.1 ± 1.1	-1.2 ± 1.1	0.039*
Osteoporosis diagnosis (%)	22.1	29.3	0.025*
Previous fracture (%)			
- None	61.4	60.5	0.259
- Vertebrae	2.8	4.0	
- Hip	2.8	3.4	
- Forearm	11.2	15.2	
- Other	21.9	17.1	
Parent with osteoporosis (%)	12.4	27.3	<0.001*
RA (%)	14.8	11.1	0.124
Severe disease (%)	63.8	36.3	<0.001*
Smoking (%)	17.8	20.5	0.350
Alcohol ≥ 3 units per day (%)	5.2	4.3	0.519
Anti-osteoporotic therapy (%)	28.8	21.7	0.020*
- Treatment time (years)	3.4 ± 3.1	5.2 ± 4.3	0.002*
Calcium and vitamin D (%)	92.9	77.3	<0.001*
Milk (glasses/day)	1.3 ± 1.2	1.0 ± 1.1	<0.001*
Cheese (sandwiches/day)	1.2 ± 0.9	1.2 ± 0.9	0.656
Cereal with milk, yogurt, A38 etc. (portions/day)	0.5 ± 0.6	0.6 ± 0.6	0.039*
Sun exposure (%)			
- Low	22.0	16.4	0.118
- Normal	69.5	73.6	
- High	8.5	10.0	

Table 3: Patients currently treated with prednisolone (n=231) were compared to patients not receiving prednisolone therapy (n=1380) with regard to different factors that potentially affect bone strength. Data are presented as means ± SD or percentages. * Indicates a statistical significant difference between the two groups. The table is made based on data from the new questionnaire as well as results obtained from DXA measurements.

height and weight were significantly larger in the group of subjects receiving > 5 mg of prednisolone compared to the group receiving no or ≤ 5 mg of prednisolone ($P<0.05$). Additionally, the gender distribution differed between the two groups with a significantly lower percentage of women present in the group of patients receiving >5 mg of prednisolone compared to the other group ($P<0.001$). The other investigated parameters did not reach statistical significance between the two groups ($P>0.05$), see table 2.

The association between prednisolone therapy and T-score was also investigated by means of data obtained from the new questionnaire. Based on this questionnaire, the subjects were divided into two groups, of which the first group was comprised of subjects currently treated with prednisolone at any dose (14.3 %), whereas the other group was comprised of subjects that did not receive any prednisolone therapy (85.7 %). The two groups were compared regarding different factors that

potentially affected bone strength, see table 3. The mean T-score of the lumbar spine and the hip were significantly higher in the prednisolone group compared to the group receiving no prednisolone (T-score lumbar spine -0.9 ± 1.6 vs. -1.5 ±1.4, *P*<0.001; T-score hip -1.1 ± 1.1 vs. -1.2 ± 1.1, *P*=0.039). This result was consistent with the lower percentage of subjects diagnosed with osteoporosis in the prednisolone group compared to the group receiving no prednisolone (22.1 % vs. 29.3 %, *P*=0.025). Additionally, patients receiving prednisolone therapy were less likely to have a parent diagnosed with osteoporosis compared to subjects that did not receive any prednisolone therapy (12.4 % vs. 27.3 %, *P*<0.001). Subjects in the prednisolone group more often received anti-osteoporotic therapy than the group receiving no prednisolone (28.8 % vs. 21.7 %, *P*=0.020), although their treatment time was shorter on average (3.4 ± 3.1 years vs. 5.2 ± 4.3 years, *P*=0.002). Likewise, the prednisolone-treated subjects more often took supplements of calcium and vitamin D compared to the non-treated subjects (92.9 % vs. 77.3 %, *P*<0.001). In addition, subjects in the prednisolone group had a higher daily intake of milk, but a lower daily intake of cereal, yogurt, A38 etc. The Danish Bone Society had formulated an equation (3), which makes it possible to calculate the daily intake of calcium in the two groups;

$$Calcium(mg/day) = 350 + (150 \cdot milk) + (200 \cdot cheese) + (250 \cdot cereal)$$

By means of this equation, it was found that the patients receiving prednisolone therapy had an average calcium intake of 910 mg/day, whereas the non-treated subjects had an average calcium intake of 890 mg/day.

The subjects treated with prednisolone more often suffered from a severe disease compared to subjects not receiving any prednisolone treatment (63.8 % vs. 36.3 %, *P*<0.001). Examples of severe diseases included Morbus Crohn, coeliac disease, COPD, asthma, heart disease, anorexia nervosa, breast cancer, epilepsy and Sjögren's syndrome.

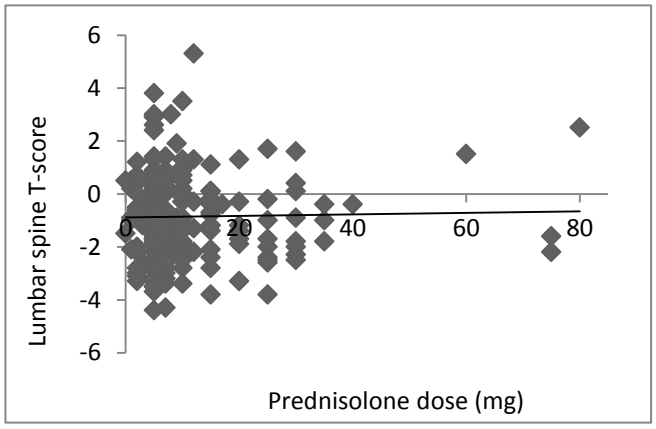

Figure 4: Correlation between lumbar spine T-score determined by DXA scan of the L1-L4 vertebrae and dose of prednisolone measured in mg (n=229). Pearson's correlation coefficient was 0.019, but the correlation was not statistically significant (*P*=0.783). The data used to complete this figure are derived from the new questionnaire.

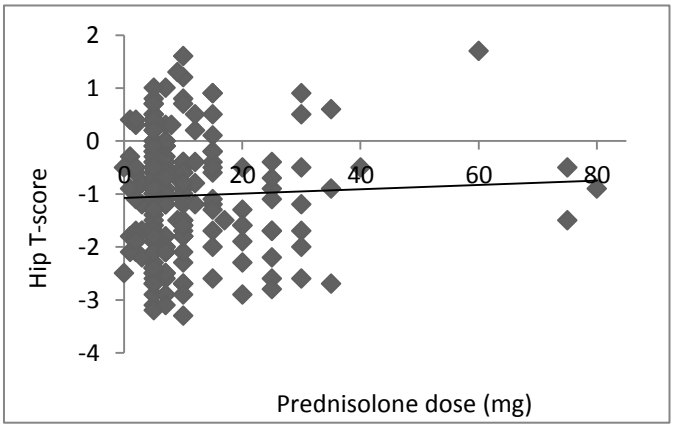

Figure 5: Correlation between hip T-score determined by DXA scan of total hip and dose of prednisolone measured in mg (n=227). Pearson's correlation coefficient was 0.036, but the correlation did not reach statistical significance (*P*=0.603). The data used to complete this figure are derived from the new questionnaire.

The two groups also differed with regard to demographic parameters, including gender distribution, age, height and weight, see table 3. Thus, the percentage of women was significantly lower in the prednisolone group compared to the other group (P<0.001). Furthermore, the subjects treated with prednisolone were older, higher and weighed more than the other group. The remaining investigated parameters did not reach statistical significance between the two groups (P>0.05), see table 3.

The association between use of prednisolone and T-score was further investigated by means of Pearson's correlation analysis. The correlation between lumbar spine T-score and dose of prednisolone was 0.019, but it did not reach statistical significance (P=0.783), see figure 4. Likewise, the correlation between hip T-score and dose of prednisolone of 0.036 was not statistically significant, see figure 5. The two graphs further demonstrated that the majority of patients treated with prednisolone received relatively low doses of the drug.

	RA (n=179)	No RA (n=1540)	*P*-value
Females (%)	76.5	79.0	0.452
Age (years)	62.5 ± 13.6	63.1 ± 13.6	0.744
Height (cm)	166.1 ± 9.2	165.2 ± 8.7	0.184
Weight (kg)	74.1 ± 18.0	71.2 ± 16.3	0.031*
Age at menopause (years)	49.1 ± 5.0	47.9 ± 6.0	0.092
T-score lumbar spine	-1.0 ± 1.6	-1.4 ± 1.5	0.001*
T-score hip	-1.2 ± 1.2	-1.2 ± 1.1	0.456
Osteoporosis diagnosis (%)	23.5	29.5	0.092
Previous fracture (%)			
- None	63.9	61.1	0.165
- Vertebrae	0.6	3.9	
- Hip	4.7	2.9	
- Forearm	13.6	14.9	
- Other	17.2	17.1	
Parent with osteoporosis (%)	21.9	25.6	0.326
Severe disease (%)	37.8	40.1	0.582
Smoking (%)	22.6	19.6	0.343
Alcohol ≥ 3 units per day (%)	3.4	4.7	0.435
Anti-osteoporotic therapy (%)	21.8	23.0	0.729
- Treatment time (years)	5.1 ± 5.9	4.8 ± 4.0	0.313
Prednisolone therapy (%)	17.3	13.1	0.124
- Prednisolone dose (mg)	7.3 ± 3.6	11.5 ± 12.6	0.083
Calcium and vitamin D (%)	84.7	79.3	0.089
Milk (glasses/day)	1.0 ± 1.1	1.0 ± 1.1	0.924
Cheese (sandwiches/day)	1.1 ± 0.9	1.2 ± 0.9	0.330
Cereal with milk, yogurt, A38 etc. (portions/day)	0.5 ± 0.5	0.6 ± 0.6	0.962
Sun exposure (%)			
- Low	14.0	17.4	0.404
- Normal	74.2	73.0	
- High	11.8	9.6	

Table 4: Patients with RA (n=179) were compared to patients without RA (n=1540) with regard to various factors that affect bone strength. Data are presented as means ± SD or percentages. * Indicates a statistical significant difference between the two groups (P<0.05). RA = rheumatoid arthritis. The table is made based on data from the new questionnaire as well as results obtained from DXA measurements.

Variable	B coefficient	SE	*P*-value
Constant	-2.643	0.336	<0.001*
T-score hip	0.742	0.040	<0.001*
Age	0.019	0.004	<0.001*
Weight	0.012	0.003	<0.001*
Anti-osteoporotic therapy	-0.212	0.072	0.003*
Alcohol	0.612	0.218	0.005*
Smoking	0.188	0.084	0.025*
Previous fracture	-0.047	0.021	0.026*

Table 5: Stepwise multiple linear regression analysis was used to find significant predictors of lumbar spine T-score (n=975). R^2 was 0.403. * Indicates a statistically significant predictor of lumbar spine T-score (P<0.05). SE = standard error. The data are obtained from the old questionnaire.

The new questionnaire further provided information about the prevalence of RA, which was 11.6 %. The patients with RA were compared to the patients without RA with regard to different parameters that may affect bone strength, see table 4. The mean T-score of the lumbar spine was significantly higher in patients with RA compared to patients without RA (-1.0 ± 1.6 vs. -1.4 ± 1.5, P=0.001). On the contrary, the T-score of the hip was similar between the two groups (-1.2 ± 1.2 vs. -1.2 ± 1.1, P=0.456). The mean weight was significantly higher in patients diagnosed with RA compared to patients without RA (P=0.031). The remaining investigated parameters did not reach statistical significance (P>0.05), see table 4.

The average daily intake of calcium in the two groups was calculated by means of the equation formulated by Danish Bone Society (3). RA patients had a calcium intake of 845 mg/day, whereas the patients without RA had a calcium intake of 890 mg/day.

Stepwise multiple linear regression analysis was performed in order to identify significant predictors of the T-scores of the lumbar spine and total hip. The results obtained by means of the old questionnaire are presented in table 5 and table 6, where the dependent variable is lumbar spine T-score and hip T-score, respectively. For lumbar spine T-score significant predictors included T-score of the hip, age, weight, anti-osteoporotic therapy, alcohol consumption, smoking, and previous fracture, see table 5. The R^2 was 0.403, meaning that this model could explain about 40 % of the variation in lumbar spine T-score. Significant predictors of hip T-score included lumbar spine T-score, weight, age, smoking, alcohol consumption and sun exposure, see table 6. In this case, R^2 reached a value of 0.553, and thus this model could explain approximately 55 % of the variation in hip T-score.

Variable	B coefficient	SE	*P*-value
Constant	-0.612	0.250	0.015*
T-score lumbar spine	0.367	0.019	<0.001*
Weight	0.024	0.002	<0.001*
Age	-0.029	0.002	<0.001*
Smoking	-0.204	0.058	<0.001*
Alcohol	-0.396	0.152	0.009*
Sun exposure	0.125	0.050	0.012*

Table 6: Stepwise multiple linear regression analysis was used to find significant predictors of hip T-score (n=975). R^2 was 0.553. * Indicates a statistical significant predictor of hip T-score (P<0.05). SE = standard error. The data are derived from the old questionnaire.

Variable	B coefficient	SE	*P*-value
Constant	-1.535	0.232	<0.001*
T-score hip	0.527	0.041	<0.001*
Osteoporosis diagnosis	-1.372	0.091	<0.001*
Weight	0.009	0.002	<0.001*
Prednisolone therapy	0.270	0.098	0.006*
RA	0.260	0.104	0.013*
Age	0.007	0.003	0.009*
Smoking	0.210	0.083	0.011*

Table 7: Stepwise multiple linear regression analysis was used to identify significant predictors of lumbar spine T-score (n=872). R^2 was 0.586. * Indicates a statistical significant predictor of lumbar spine T-score (P<0.05). SE = standard error. The data are derived from the new questionnaire.

The results obtained from stepwise multiple linear regression analysis using the new questionnaire are presented in table 7 and table 8, where the dependent variable is lumbar spine T-score and hip T-score, respectively. Significant predictors of lumbar spine T-score included T-score of the hip, osteoporosis diagnosis, weight, prednisolone therapy, RA, age and smoking, see table 7. R^2 was 0.586, meaning that 58.6 % of the variation in lumbar spine T-score could be explained by this model. The significant predictors of hip T-score included T-score of the lumbar spine, age, weight, osteoporosis diagnosis, smoking, sex, previous fracture, as well as intake of cereal, yogurt, A38 etc., see table 8. In this case R^2 was 0.606, and thus this model could explain about 60 % of the variation observed in hip T-score.

Variable	B coefficient	SE	*P*-value
Constant	-0.580	0.184	0.002*
T-score lumbar spine	0.291	0.023	<0.001*
Age	-0.021	0.002	<0.001*
Weight	0.018	0.002	<0.001*
Osteoporosis diagnosis	-0.462	0.076	<0.001*
Smoking	-0.218	0.063	0.001*
Sex	-0.182	0.064	0.004*
Previous fracture	-0.041	0.015	0.005*
Intake of cereal with milk, yogurt, A38 etc.	0.090	0.044	0.043*

Table 8: Stepwise multiple linear regression analysis was used to identify significant predictors of hip T-score (n=872). R^2 was 0.606. * Indicates a statistical significant predictor of hip T-score (P<0.05). SE = standard error. The data are derived from the new questionnaire.

5. Discussion

In this study, the effect of prednisolone therapy on T-score was investigated. The hypothesis was that patients treated with prednisolone had lower T-scores compared to patients not receiving prednisolone therapy. However, when analyzing the data from the old questionnaire, it was found that patients treated with > 5 mg of prednisolone had higher T-scores of both the lumbar spine and the hip compared to patients receiving no or ≤ 5 mg of prednisolone. These results were comparable to those obtained from the new questionnaire, which likewise revealed that patients treated with prednisolone at any dose had higher mean T-scores of both the lumbar spine and the hip compared to patients not receiving prednisolone therapy. Furthermore, no significant correlation was observed between prednisolone dose and T-score of the lumbar spine and hip, respectively. This indicates that prednisolone therapy is not associated with osteoporosis. These findings are contrary to those of other studies (225-227, 342, 343), which have demonstrated a clear association between treatment with glucocorticoids and low BMD, i.e. low T-score, as well as increased fracture risk. A possible explanation for the missing association between prednisolone therapy and low T-score in the present study is that the questionnaires used did not provide any information about the treatment time. Therefore, some of the subjects included in the present study might have been referred to a DXA scan shortly after the initiation of prednisolone therapy in order to evaluate their bone mineral status. Thus, these subjects would not yet have developed any skeletal side effects, i.e. they would have normal T-scores. Furthermore, the questionnaires only provide information about the patients' current treatment status. In other words, some of the patients in the control group might previously have undergone a prolonged treatment course with prednisolone, leading to bone loss and decreased T-score. Additionally, the higher T-score found in the group receiving > 5 mg of prednisolone, when analyzing data from the old questionnaire, might also partly be due to the significantly higher weight and lower percentage of women found in the this group compared to the group receiving no or ≤ 5 mg prednisolone, since both low body weight and female sex are known risk factors for bone loss and osteoporosis (196, 344-346). Likewise, the higher T-score observed in the group receiving prednisolone, when analyzing data from the new questionnaire, might be caused by the lower percentage of women and the larger height and weight observed in this group compared to the group receiving no prednisolone. Further, the patients from the prednisolone group were less likely to have a parent with osteoporosis, and thus they were only rarely genetically predisposed to osteoporosis compared to the other group. Additionally, the patients receiving prednisolone more often received anti-osteoporotic drugs as well as supplements of calcium and vitamin D compared to the patients receiving no prednisolone. Anti-osteoporotic drugs, e.g. alendronate, prevent bone loss and decrease the risk of osteoporotic fractures (347-349). Thus, the more frequent use of anti-osteoporotic drugs in the group receiving prednisolone therapy might explain the higher T-scores found in this group compared to the non-treated group, although the mean treatment time was lower. Anti-osteoporotic drugs are possibly more often prescribed to patients treated with prednisolone in order to prevent the inevitable bone loss associated with glucocorticoid treatment. Furthermore, the patients receiving prednisolone therapy had a significantly higher intake of milk compared to the patients receiving no prednisolone, but this was probably counterbalanced by their lower intake of cereal with milk, yogurt, A38 etc., and thus of minor importance. The average calcium intake was 910 mg/day for the patients receiving prednisolone therapy, whereas the non-

treated patients had an average calcium intake of 890 mg/day. These values only differed by 20 mg, which also suggested that the calcium intake was not responsible for the discrepancy in T-score between the two groups. Further, both values were above the dietary reference intake of calcium of 800 mg/day, and thus both groups of patients received sufficient calcium through their diet (3).

Based on the new questionnaire, it was also demonstrated that the patients receiving prednisolone therapy more often suffered from a severe disease compared to the patients not receiving any prednisolone. This may be explained by the fact that prednisolone is used in the treatment of various severe diseases such as asthma, COPD, systemic lupus erythematosus, inflammatory bowel disease etc. (292). However, although prednisolone is often used to treat RA, the prevalence of RA was similar between the group receiving prednisolone and the non-treated group. This might be explained by the fact that several other treatment options are available for RA patients, including disease modifying anti-rheumatic drugs (DMARDs), analgesics and biological agents such as anti-TNF-α and IL-1 receptor antagonist as well as non-pharmacological treatments such as orthopedic surgery, physiotherapy and occupational therapy (247, 267, 350).

As aforementioned, the present study found no significant correlation between prednisolone dose and T-score, although an inversely proportional relationship between prednisolone dose and T-score was expected. This was opposed to other studies (226, 227, 300) that demonstrated that the fracture risk increased, i.e. the T-score decreased, with increasing cumulative dose of glucocorticoids. However, in this study it was not possible to calculate the cumulative dose of prednisolone, because the questionnaires did not provide any information regarding treatment time, and this could possibly explain the conflicting results.

The patients included in this study were mainly treated with relatively low doses of prednisolone ≤ 10 mg. A study by Sambrook et al. (307) found that low doses of glucocorticoids do not increase the risk of osteoporosis among women suffering from RA. This conclusion was based on the similar BMDs observed between RA patients treated with a mean dose of 8.0 mg prednisolone per day in 89.6 months on average and RA patients receiving no prednisolone treatment. These results were supported by another study conducted by Vestergaard and colleagues (225), who demonstrated that the fracture risk only was increased with use of oral glucocorticoids > 2.5 mg per day or inhaled glucocorticoids > 7.5 mg per day. Thus, the evidence suggests that it is safe to treat patients with glucocorticoids as long as the administered dose is relatively low. Since the main part of the prednisolone treated patients that participated in this study received relatively low doses of the drug, this could possibly also explain why these patients did not exhibit lower T-scores compared to the non-treated patients. Furthermore, the risk of developing osteoporosis depends on the administration route of the drug so that systemic glucocorticoids have a much larger impact on the skeleton compared to topical formulations (225). Hence, if many of the patients in the prednisolone group used topical formulations (e.g. dermal, rectal, inhalation and local administration into joints or eyes), their T-scores would be influenced to a lesser degree, thereby explaining why these patients did not show lower T-scores compared to the non-treated patients. RA patients are for example often treated with intra-articular injections of prednisolone in order to minimize the risk of developing systemic side effects such as osteoporosis (351).

In this study, the association between RA and T-score was investigated based on data obtained from the new questionnaire. It was hypothesized that patients with RA had an increased risk of developing osteoporosis, i.e. had lower T-scores, compared to patients without RA. Nevertheless, the RA patients had higher T-scores of the lumbar spine compared to non-RA patients, whereas the mean T-scores of the hip did not differ between the two groups. These results are in conflict with other studies (288-290) that have showed that RA patients exhibit increased bone loss and higher fracture risk compared to controls. This discrepancy may be explained by limitations associated with the present study, including the lack of information about disease duration and severity. If some of the included patients only had suffered from RA in a relatively short time period, they would not yet exhibit any signs of generalized bone loss, and thus they would have normal T-scores of the lumbar spine and the hip. This was confirmed in a study by Gough et al. (290), who showed that BMD measurements in RA patients, which were obtained 10.4 months after the onset of symptoms on average, were not significantly below the normal range after correction for age and sex, i.e. the RA patients exhibited normal Z-scores. They further demonstrated that BMD measurements varied with disease duration of RA so that BMD decreased with increasing disease duration.

In this study, BMD measurements were solely performed at the lumbar spine and the hip. However, the earliest manifestations of RA-associated bone loss occur locally in the vicinity of the affected joints (290). Thus, some of the included RA patients might have experienced peripheral bone damage, although this would not be evident from the central measurements of BMD performed in this study, which only provided information about generalized osteoporosis.

As mentioned above, the disease severity of RA was also not known in the present study. However, several studies (290, 352, 353) have demonstrated an association between disease severity and low BMD. Thus, the higher T-scores observed in RA patients compared to patients without RA in this study could possibly be explained by a relatively low disease activity and severity among the main part of these patients. In order to clarify this, an assessment of the disease severity of RA would have been necessary. There are several ways to assess this, including evaluation of morning stiffness duration, number of swollen joints, number of tender joints, pain score, functional level, radiological progression, RF titer, C-reactive protein, erythrocyte sedimentation rate as well as use of prednisolone and anti-rheumatic drugs. However, it would be impractical to incorporate these time consuming and resource demanding clinical assessments of RA disease severity in the database at Aalborg University Hospital. It would further be unethical to expose RA patients to invasive blood tests and time consuming clinical examinations.

As aforementioned, several studies have demonstrated that patients with RA have an increased risk of developing osteoporosis (288-290). Additionally, RA patients are often treated with glucocorticoids, which also lead to bone loss and increased fracture risk (225-227, 342, 343). Furthermore, the chronic inflammation observed in RA may ultimately result in joint destruction, thereby causing articular stiffness, chronic pain and reduced mobility. This may further contribute to the bone loss observed in RA, since immobility is associated with osteoporosis, whereas physical activity has a protective effect on the bones (354). Therefore, it can be difficult to determinate to

what degree osteoporosis in RA patients is a result of the disease per se, or whether it is caused by glucocorticoid treatment or immobility.

In the present study, multiple linear regression analyses were performed in order to identify significant predictors of hip and lumbar spine T-scores. The results obtained from the old and the new questionnaire varied slightly, which probably was related to discrepancies between the two questionnaires such as the inclusion of additional variables and the higher level of detail in the new questionnaire. However, both questionnaires found that lumbar spine T-score and weight were positive predictors of hip T-score, whereas age and smoking were negative predictors of hip T-score. The positive relation between weight and hip T-score is in agreement with a study conducted by Hannan et al. (344), which demonstrated that low body weight and weight loss were associated with BMD loss, whereas weight gain seemed to have a protective effect on bone. On the other hand, both old age and smoking are known risk factors of osteoporosis, which explain their negative impact on hip T-score (3, 355, 356). Several studies (235, 234, 357) have demonstrated that smoking especially affects the BMD of the hip, thereby increasing the risk of hip fractures. This might partly explain why both questionnaires identified smoking as a positive predictor of lumbar spine T-score, although the opposite result was expected. In addition to smoking, hip T-score, age and weight were identified as positive predictors of lumbar spine T-score by both questionnaires.
Moreover, the old questionnaire found that anti-osteoporotic therapy and previous fracture were significant negative predictors of lumbar spine T-score. The fact that anti-osteoporotic therapy was associated with a low T-score of the lumbar spine, although these drugs prevent bone loss, could be explained by the fact that anti-osteoporotic drugs usually are prescribed to patients with a poor BMD status in order to prevent further bone loss, whereas individuals with normal T-scores would not be advised to use this type of medication. Furthermore, the association between previous fracture and low T-score of the lumbar spine could be explained by the fact that patients with low T-scores are at higher risk of suffering osteoporotic fractures due to their more fragile bones (340). The old questionnaire further revealed that an alcohol consumption ≥ 3 units per day was associated with a high lumbar spine T-score, but a low hip T-score. This discrepancy could possibly be explained by the fact that a moderate intake of alcohol has a protective effect on the bones, whereas an excessive intake of alcohol leads to bone loss (358-360). Thus, if the subjects included in this study did not consume more than 3 units of alcohol daily, they would probably have higher T-scores, whereas subjects with an abuse of alcohol would exhibit lower T-scores (360). It is also possible that alcohol affects the BMD at different measuring sites to a various degree, thereby explaining the different effect of alcohol consumption on the lumbar spine T-score and hip T-score (358). Data from the old questionnaire further revealed that sun exposure has a positive effect on the hip T-score, because the ultraviolet radiation from the sun increases the cutaneous vitamin D production.
According to the new questionnaire, osteoporosis diagnosis was a significant negative predictor of T-score in the hip and the lumbar spine. This result was expected, since osteoporosis is diagnosed based on T-score measurements, thereby making osteoporosis diagnosis and T-score mutual dependent. Furthermore, based on the new questionnaire it was revealed that previous fracture and sex was negative predictors of hip T-score, whereas intake of cereal with milk, yogurt, A38 etc. was

a positive predictor of T-score. These results indicated that male gender was a risk factor of osteoporosis, which was opposed to the expected outcome, since osteoporosis is much more frequent in women than men (4, 185, 187). This might be associated with referral bias, because only men and women expected to suffer from low BMDs were referred to a DXA scan. Thus, the study population does not reflect the situation in the general population. Further, the intake of cereal with milk, yogurt, A38 etc. was considered a positive predictor of hip T-score, which suggests that a high dietary intake of calcium has a protective effect on bones. This was confirmed by Johnston et al. (361), who demonstrated that calcium supplementations enhanced the rate of increase in BMD in prepubertal children.

The new questionnaire further revealed that both prednisolone therapy and RA had a positive effect on lumbar spine T-score, which was opposed to the expected. This could possibly be explained by the abovementioned limitations of the study such as lack of information about treatment time of prednisolone as well as disease duration and severity of RA.

It is important to notice that the present study is a cross-sectional study, which only provides a snapshot of the bone status and the presence of risk factors in the study population. This study design is far from ideal regarding identification of a causal relationship between a potential risk factor and changes in bone mass, since only prospective, randomized clinical trials are capable of documenting causality. Thus, more valid results would be obtained if a prospective, randomized controlled study design was applied.

The data used in this study was obtained from a database. This was associated with several advantages such as easily accessible data as well as the possibility to include a large number of patients, thereby ensuring adequate statistical power and thus more valid results. Further, it is both time and resource saving compared to planning and conducting a clinical trial. On the other hand, the application of data from a database was also associated with several disadvantages and limitations. Firstly, nearly all the data were self-reported, except for T-scores of the lumbar spine and hip as well as height and weight. Thus, there was a considerable risk of recall bias, since some of the patients probably could not remember whether they have had a previous fracture, what dose of prednisolone they used or how long they had received anti-osteoporotic drugs. Further, many elderly women could not remember at what age they reached menopause. Additionally, some patients might not know if they received anti-osteoporotic drugs, if they suffered from RA or if one of their parents were diagnosed with osteoporosis. The questionnaires also included a question about severe disease, but it is individual whether a given disease is considered severe or not. Secondly, missing data were a common occurrence due to nonresponse, and it could influence the results of the study. Thirdly, the study was likely to include referral bias, because only patients expected to exhibit a low BMD were referred to a DXA scan. Thus, the results obtained from this study do not necessarily apply to the general population. Fourthly, the present study was limited by the information provided by the questionnaires. Therefore it was not possible to obtain information about treatment time of prednisolone or disease duration and severity of RA, although this could have helped clarifying the association between prednisolone therapy, RA and osteoporosis.

In this study, T-score was used to evaluate bone mass. However, the use of T-scores is associated with several limitations. Firstly, WHO's diagnostic classification ranges for T-score have only been validated in postmenopausal Caucasian women (341). Therefore, it is questionable whether they are applicable in men, premenopausal women and non-white individuals (341). Nevertheless, in Denmark a T-score $\leq$ -2.5 is recognized as diagnostic for osteoporosis in both men and women (363). Secondly, although T-scores are used to diagnose osteoporosis in both men and women, the fracture risk at a given T-score is generally lower in men compared to women, which makes it difficult to establish a limit value for pharmacological intervention among men (7). Thirdly, other independent significant predictors of fracture risk exist in addition to T-score such as age, previous fracture and chronic use of glucocorticoids, among others (185). Older age increases the risk of fragility fractures for which reason the 10-year fracture risk in a 70-year-old woman with a T-score of -2.5 is 22.8 %, whereas this risk is only 8.1 % in a 45-year old woman with the same T-score (7). The same tendency is valid for men, and a 70-year-old man with a T-score of -2.5 has a 10-year fracture risk of 13.1 %, whereas a 45-year-old man with similar T-score has a 10-year fracture risk of only 6.3 % (7). Thus, the diagnostic and prognostic value of T-scores is highly dependent on age, which is the reason why the WHO criteria cannot simply be extrapolated to younger men and women. Previous fracture is another important predictor of fracture risk, and a previous fracture predisposes to future fractures independent of T-score (15-18). Thus, an individual with a T-score consistent with osteopenia and a fragility fracture may have an actual increased fracture risk compared to an individual with a T-score consistent with WHO-defined osteoporosis without a fragility fracture (341). Furthermore, chronic treatment with glucocorticoids is a significant predictor of fracture risk, and patients receiving this type of therapy have a tendency to fracture at relatively higher T-scores (185, 341). Based on this evidence, it is important to assess the absolute fracture risk by evaluating multiple risk factors of low bone mass, including T-score, age, gender, weight, previous fracture, use of prednisolone, alcohol consumption, smoking etc. (341). All these factors are included in the questionnaires used at Aalborg University Hospital. Fourthly, T-score is a relative measure that is calculated based on data obtained from a reference population of young, healthy subjects of the same sex (333). In other words, T-score is dependent on the reference population, and therefore the use of a different reference population may cause a marked change in T-score, although the measurement are performed at the same skeletal measuring site using the same DXA device (333, 341). In this way, the choice of reference population has a significant impact on the diagnosis, and hence the management of a patient. Additionally, it is important to ensure that the used reference data are relevant to the population being studied with regard to sex, age, ethnicity etc. in order to obtain as valid results as possible (333). However, relevant reference data are not available for all subpopulations (341). Finally, the T-score is only used to quantify the bone mass, whereas it does not provide any information about the quality or strength of the bone tissue, although this also affects the fracture risk (3, 333, 362).

DXA is a well-established method that is used for the assessment of the bone mineral content in the hip and the spine with a view to diagnosing osteoporosis. However, though DXA is considered a simple, precise and safe method for BMD evaluation, the technique is also associated with certain disadvantages (332-334). Firstly, the DXA makes assumptions about the composition of the soft

tissue of the body, i.e. the ratio between lean and fat tissue, which it assumes is constant (332). However, the composition of soft tissue in the radiation area may differ from that of the adjacent soft tissue, which is used as a reference area (332-334). This could result in erroneous estimation of BMD, since the attenuation of X-rays varies between lean tissue and fat tissue (332, 333). Secondly, it is not possible to compare results from the same subject obtained from different DXA devices, because systematic differences exist between the devices (3, 196). For example is the evaluation of bone mineral content in the Hologic and Norland systems solely based on calcium hydroxyapatite, whereas the Lunar system takes the bone content of fat into account (334). Furthermore, the different manufactures use different reference populations, and therefore the normal range of BMD varies between the different devices (196, 333). Thus, in clinical studies it is important to perform all DXA measurements using the same device in order to obtain the most valid results (3, 334). This requirement was fulfilled in the present study, since all the included patients had their BMD measured by the Hologic Discovery QDR device, thereby minimizing the risk of systematic errors. Thirdly, several conditions can cause falsely increased BMD values, including metal implants, aortic calcifications, osteophytes, scoliosis and vertebral compression fractures (3, 332, 333). Additionally, variations in the position of the patient may cause erroneous BMD results and decreased reproducibility (3, 332, 333). In order to minimize potential sources of error, it is recommended that DXA scans are performed by trained personnel (3, 332). In this study, trained personnel at Aalborg University Hospital performed all the scans, thereby meeting this demand. On the other hand, all the DXA scans were not performed by the same person due to practical reasons, and this could lead to inter-observer variations that potentially could affect the results. However, a study by Haugeberg et al. (288) demonstrated that BMD measurements performed by three trained technicians in 30 RA patients only resulted in an inter-observer variation of 0.4-0.5 % in total hip and of 0.7-1.4 % in L2-L4. These inter-observer variations are fairly small, and therefore it could be argued that the results of the present study were not affected significantly by inter-observer variations.

6. Conclusion

In this study, it was investigated if patients suffering from RA or patients receiving prednisolone therapy had an increased risk of developing osteoporosis. In order to evaluate this, RA patients were compared to patients without RA and patients receiving prednisolone were compared to non-treated patients with regard to T-scores of the total hip and the lumbar spine. It was hypothesized that patients diagnosed with RA or patients treated with prednisolone had lower T-scores compared to patients without RA and patients receiving no prednisolone therapy, respectively. However, the results revealed that patients receiving prednisolone therapy had higher T-scores of both the total hip and the lumbar spine compared to non-treated patients. Additionally, no significant correlation between prednisolone dose and T-score was demonstrated. Furthermore, RA patients had higher lumbar spine T-scores compared to patients without RA, whereas the total hip T-scores were similar between the two groups. By means of stepwise multiple linear regression analysis it was further found that RA and prednisolone therapy were positive predictors of lumbar spine T-score, whereas they were not predictive of total hip T-score. These results were contrary to the hypothesis as well as the findings of other studies. This was thought to be related to limitations of the present study, including the lack of information regarding treatment time of prednisolone as well as disease duration and disease severity of RA.

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Appendix I: The Old Questionnaire

Osteoporoseklinikken, Endokrinologisk Afdeling, Aalborg Universitethospital

Din læge har henvist dig til knoglemineralmåling (DXA) i Osteoporoseklinikken ved Endokrinologisk Afdeling, Aalborg Universitetshospital.
Nedenfor er nogle spørgsmål med betydning for knoglernes styrke.
Du bedes besvare disse og medbringe det udfyldte skema ved undersøgelsen.

Spørgsmål du bedes besvare:

Min alder: _____ år

Jeg er Mand ☐ Kvinde ☐

Jeg har tidligere haft knoglebrud Nej ☐ Ja ☐

Hvis ja:	Ryg	Håndled	Lårbenshals	Andet
Årstal:				

Hvordan skete det? _____

Min mor eller far har haft osteoporose med en brækket hofte,

underarm eller sammenfald i ryggen Nej ☐ Ja ☐

Jeg ryger dagligt Nej ☐ Ja ☐

Jeg drikker dagligt mere end 2 øl / 2 glas vin / 4 cl spiritus Nej ☐ Ja ☐

Jeg får dagligt mere end 5 mg Prednisolon Nej ☐ Ja ☐

Jeg har en anden alvorlig sygdom Nej ☐ Ja ☐
Hvilken _____

Jeg får medicin for osteoporose (knogleskørhed) Nej ☐ Ja ☐

Hvilken _____

Hvor mange år har du fået den _____

Jeg tåler og får sol på huden: meget ☐ almindeligt ☐ lidt ☐

Jeg tåler og får mælk eller ost hver dag ☐ hver uge ☐ sjældent ☐

Jeg er gået i overgangsalder	Ja ☐	Nej ☐
Hvis ja: Alder ved overgangsalderen _____		
Jeg kan være gravid	Nej ☐	Ja ☐
Jeg er opereret i ryggen	Nej ☐	Ja ☐
Jeg har metal i ryggen	Nej ☐	Ja ☐
Jeg er opereret i hoften	Nej ☐	Ja ☐
Jeg har metal i hoften	Nej ☐	Ja ☐
Jeg har fået jodholdig kontrast / barium / radioaktive medicinske isotoper inden for de seneste 7 dage	Nej ☐	Ja ☐
Jeg har Pacemaker, metalimplantater eller kirurgiske hæfteklammer	Nej ☐	Ja ☐

Tak for hjælpen

Med venlig hilsen

Peter Vestergaard
Professor, overlæge, PhD, dr. med.

Peter Laurberg
Professor, overlæge, dr.med

Appendix II: The New Questionnaire

Osteoporoseklinikken, Endokrinologisk Afdeling, Aalborg Universitethospital

Din læge har henvist dig til knoglemineralmåling (DXA) i Osteoporoseklinikken ved Endokrinologisk Afdeling, Aalborg Universitetshospital.
Nedenfor er nogle spørgsmål med betydning for knoglernes styrke.
Du bedes besvare disse og medbringe det udfyldte skema ved undersøgelsen.

Spørgsmål du bedes besvare:

Min alder: _____ år

Jeg er Mand ( ) Kvinde ( )

Jeg har tidligere haft knoglebrud Nej ( ) Ja ( )

Hvis ja:	Ryg	Håndled	Lårbenshals	Andet
Årstal:				

Hvordan skete det? _____

Min mor eller far har haft brækket hoften Nej ( ) Ja ( )

Min mor eller far har haft osteoporose Nej ( ) Ja ( )

Min mor eller far har haft brækket

underarmen eller sammenfald i ryggen Nej ( ) Ja ( )

Jeg ryger dagligt Nej ( ) Ja ( )

Jeg drikker dagligt 3 eller flere genstande dagligt Nej ( ) Ja ( )

Jeg får dagligt Prednisolon Nej ( ) Ja ( )

Hvis ja – antal mg_____

Jeg har leddegigt (rheumatoid artrit – Ikke slidgigt) Nej ( ) Ja ( )

Jeg har en anden alvorlig sygdom Nej ( ) Ja ( )
Hvilken _____

Jeg får dagligt kalk og vitamin D tabletter Nej ( ) Ja ( )

Angiv venligst navn og antal_____

Jeg får medicin for osteoporose (knogleskørhed) Nej ( ) Ja ( )

Hvilken _____

Hvor mange år har du fået den _____

Jeg er gået i overgangsalder Nej ( ) Ja ( )

Hvis ja: Alder ved overgangsalderen _____

Hvis livmoderen er fjernet, skriv alder, da det skete _____

Hvor mange glas mælk drikker De om dagen ?_____

(Hvis det f.eks. er 3 glas per uge skrives 3/7, hvis det er 1 glas per dag skrives 1)

Hvor mange ostemadder (alle typer ost) spiser De om dagen ?_____

(Hvis det f.eks. er 3 skiver ost per uge skrives 3/7, hvis det er 1 per dag skrives 1)

Hvor mange portioner mælkemad (grød, surmælksprodukter mv.) spiser De om dagen ?_____

(Hvis det f.eks. er 3 portioner per uge skrives 3/7)

Jeg tåler og får sol på huden: meget ( ) almindeligt ( ) lidt ( )

Jeg kan være gravid Nej ( ) Ja ( )

Jeg er opereret i ryggen Nej ( ) Ja ( )

Jeg har metal i ryggen Nej ( ) Ja ( )

Jeg er opereret i hoften Nej ( ) Ja ( )

Jeg har metal i hoften Nej ( ) Ja ( )

Jeg har fået jodholdig kontrast / barium / radioaktive

medicinske isotoper inden for de seneste 7 dage Nej ( ) Ja ( )

Jeg har Pacemaker, metalimplantater eller kirurgiske klammer Nej ( ) Ja ( )

Tak for hjælpen

Med venlig hilsen

Peter Vestergaard Peter Laurberg

Professor, overlæge, PhD, dr. med. Professor, overlæge, dr.med.