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Dissertation in Sports Science

Bone quality, body composition and the influence of physical activity in people with haemophilia

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by

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1. Summary

This dissertation elaborates on people with haemophilia. This disease typically affects men, though is transmitted through females, carrying the affected gene. However, this investigation only involves male subjects. The data for this dissertation were gathered between 2019 and 2022 at the university hospital Bonn. The three publications are based on the same dataset of people with haemophilia.

The rare disease haemophilia is characterized by (spontaneous) bleedings into joints which consequently leads to degenerative joint changes known as haemophilic arthropathy. Evidence has shown that people with haemophilia are more frequently affected by reduced bone mineral density. Whether there is a direct causal relationship between haemophilia and reduced bone mineral density or an association due to the consequences of haemophilic arthropathy is to be investigated. The consequences of haemophilic arthropathy entail pain, reduced range of motion, contractures, muscle atrophy as well as reduced physical activity. Especially the level of physical activity and muscle mass play a significant role in bone metabolism. This dissertation aims to investigate the bone quality as well as the body composition in people with haemophilia with a subsequent analysis of influence of physical activity on both respectively. Hereby, one major focus is the differentiation between the haemophilia severity phenotypes, given the broad range of disease manifestations. The approach of this thesis is first, to depict the prevalence people with haemophilia suffering from reduced bone mineral density (**publication I**). With a representative sample size of 255 people with haemophilia, a dual x-ray absorptiometry was performed. Results showed that 63.1% of included subjects showed decreased bone mineral density values either in form of osteopenia, osteoporosis, or “below expected range for age”. However, bone quality is also represented by trabecular microarchitecture, which was normal in 88.1% of the subjects. This finding is of major interest, as a decreased trabecular bone structure represents a risk factor for osteoporotic fractures.

While the analysis of bone mineral density and trabecular microarchitecture provide fundamental insights into skeletal health, overall body composition is another key factor influencing musculoskeletal integrity and health in people with haemophilia. To further explore this aspect, a whole-body dual X-ray absorptiometry was

conducted to determine body composition parameters, which represents the focus of the second study (**publication II**). Parameters such as body fat percentage, lean mass and an estimation of visceral adipose tissue were determined. These data also can be used as reference values by clinicians and scientists in future times, since this is the first study which evaluates body composition in a representative sample size of people with haemophilia using dual X-ray absorptiometry, which is considered the gold standard for body composition analysis (**Publication II**). The main result of this study is that people with severe haemophilia show significantly less lean mass compared to people with moderate or mild haemophilia. This might be attributed to the increased prevalence of muscle atrophies in the severe phenotype. Furthermore, it has been shown that people with haemophilia of all severities have low body fat percentages, visceral adipose tissue and android/gynoid ratios compared to the general European population.

In a third step, physical activity has been evaluated objectively and subjectively for seven days to assess the subjects daily physical activity level (**publication III**). Based on this overarching approach, the influence of physical activity on both bone quality and body composition was examined. Results show that the majority of people with haemophilia, regardless of the severity phenotype, engaged in low-impact physical activity such as walking or cycling. These activities may not provide enough stress to stimulate bone formation, explaining why there were no significant correlations between physical activity and bone mineral density in the correlation analysis. However, positive correlations between lean mass and bone mineral density, as well as between step activity and trabecular bone score were found. Therefore, it is crucial to focus on increasing or maintaining good trabecular microarchitecture through activities like brisk walking to reduce fracture risk, while also enhancing lean mass through strength training, for example.

Based on the results obtained in the three presented studies it can be concluded that haemophilia is a risk factor for reduced BMD, which both clinicians and patients should recognize. Further, it can be seen that a worse joint situation leads to changes in body composition, such as muscle atrophy. Despite this, physical activity should be promoted into daily life to improve bone quality and body composition, enhancing overall quality of life.

2. Theoretical background

2.1 Haemophilia

Haemophilia is a rare, inherited bleeding disorder characterized by a deficiency or absence of clotting factors [1]. The term “haemophilia” derivates from ancient Greek as “haîma” means “blood” and “philia” can be translated with “friend”, reflecting an affinity or a connection to blood [2]. In people with haemophilia (PwH), there is a deficiency or absence of clotting factor, so that the coagulation cascade is disrupted within secondary haemostasis [3]. Hereby, either clotting factor VIII (haemophilia A) or factor IX (haemophilia B) can be affected [1]. Having a closer look on the coagulation cascade, the activated factors VIIIa and IXa together activate factor X to Xa as the intrinsic tenase complex. Together with factor Va, this complex activates prothrombin to thrombin as the prothrombinase complex, which in turn cleaves fibrinogen to fibrin. Thus, a deficiency of factor VIII or factor IX results in a delay in coagulation and (spontaneous) bleedings [3].

The world-wide prevalence of haemophilia A is 17.6 per 100.000 men while 3.8 cases per 100.000 men are affected by haemophilia B [4]. Therefore, haemophilia is classified as a rare disease. Haemophilia is inherited recessively via the X-chromosome, so that mainly men are affected by the disease. Women, having a second X-chromosome, can compensate this gene mutation [5]. Still, it needs to be highlighted that also females can suffer from symptoms as in increased menorrhea but the main consequence of carrying a mutated X-chromosome is the transfer of this gene to their sons [5]. The affected women are known as carriers. Currently, there is effort to highlight females with hemophilia, ensuring they are no longer regarded solely as carriers. Evidence states that in female patients, haemophilia manifests different compared to male patients, which frequently causes a delayed diagnosis. The main concern of female patients is heavy menstrual bleeding, while data on joint degeneration is scarce [5].

Next to the type of haemophilia, the disease is further classified into three phenotypes based on the residual activity of the deficient clotting factor in the blood: severe haemophilia (factor level <1%), moderate haemophilia (factor level from 1% to 5%), and mild haemophilia (factor level between 5-40%) [1]. Persons with clotting factor activity of >50% are regarded as standard values [6]. There is no distinct

classification of subjects between 40% and 50% of factor activity levels though are frequently referred to as subhaemophilic [6, 7].

Symptoms of haemophilia and haemophilic arthropathy

The symptoms of the factor deficiency of either factor VIII or factor IX are bleedings into muscles and joints [1]. Muscle bleeds are characterized by swelling, pain, and limited range of motion in the affected muscle. Untreated or recurrent muscle bleeds can result in muscle fibrosis, leading to chronic pain and reduced functionality of the affected muscle [1]. However, the most frequent types of bleeds are joint bleeds, known as haemarthroses. The latter can occur spontaneously in people with severe haemophilia or by trauma in people with mild or moderate haemophilia [8]. Typical symptoms are characterized by sudden joint pain, swelling, warmth, and decreased range of motion [9]. Hereby, the most affected joints are ankles, knees and elbows [10, 11]. However, the major consequence is that repeated joint bleeds can lead to chronic inflammation of synovia (synovitis), including hypertrophy and neoangiogenesis causing progressive joint damage. This phenomenon is known as hemophilic arthropathy (see figure 1). In detail, the presence of blood, especially the deposition of hemosiderin within the joint triggers an inflammatory response [8, 12]. This leads to swelling of the joint and increased amount of fluid within the joint space. Further, the inflammatory cells release enzymes, which contribute to the degradation of cartilage through inhibiting the proteoglycan synthesis, reducing the cartilage proliferation and leading to chondrocyte apoptosis. As the articular cartilage erodes, the space between the bones in the joint narrows. Progressing, the bone starts to build osteophytes, which contribute to a decreased range of motion. In turn, decreased joint function directly affects the ability to perform different physical activities [11, 13]. Especially people with severe haemophilia suffer from synovial, cartilage and bone damage. Also, it is known that haemophilic arthropathy can have a progressive course even without further bleedings, though there is a correlation between bleeding events and joint degeneration [12]. Haemophilic arthropathy is frequently compared to other joint diseases such as osteoarthritis or rheumatoid arthritis [14]. These three conditions belong to chronic inflammatory arthritis and motivate the same molecular pathways, though the initial etiologies

clearly differ [15]. While the underlying cause of haemophilic arthropathy are the (recurrent) bleedings due to the genetic disorder, osteoarthritis is induced by the disbalance of load and load-bearing capacity of the cartilage. Causes, in addition to the idiopathic form, can include genetics, deformities due to pre-existing conditions, misalignments as well as both overuse and underuse of the joint. Hereby, cartilage breaks down leading to subchondral bone degeneration. Osteoarthritis commonly affects knees and hips [16]. Rheumatoid arthritis, however, is an autoimmune disease, leading to chronic systemic inflammation. This pathology is frequently seen in smalls joints such as fingers or toes [15].

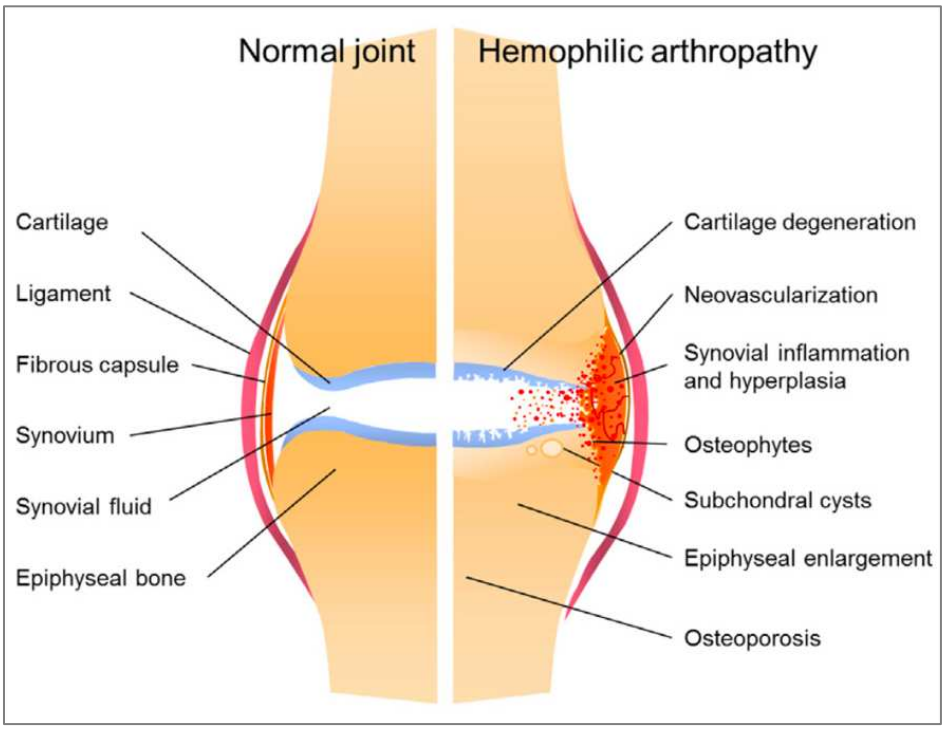

Figure 1: Schematic representation of haemophilic joint according to Pulles et al. (2017)

Patients affected by haemophilic arthropathy often experience muscle atrophies due to the adoption to a relieving posture and decreased use of the joint. Going along, PwH frequently experience instability while walking on uneven ground for instance, lose strength and suffer from joint stiffness [8, 13]. As mentioned before, many PwH especially those with severe haemophilia suffer from pain [9, 11]. Joint pain can originate from bleedings, synovitis and/ or through haemophilic arthropathy, leading

to chronic conditions [17]. Chronic pain is present in about 40% of PwH, which is considerably higher compared to the general European population (27%) [18]. The prevalence rate for acute pain is even higher (about 75%), which originate either from acute bleedings or flare ups [19]. Sudden flare ups of arthropathy, i.e. inflammation of an arthropathic joint caused by overuse, are shown to be prevalent in PwH, which in turn lead to high intensities of joint pain [17, 20]. Research has revealed that the ankle followed by knee and elbow is the most subjectively affected joint, though the majority of PwH have more than one affected joint [10, 11]. But not also joint pain affects PwH, but also back pain was found to be present in 43% of PwH [10].

Treatment of haemophilia and haemophilic arthropathy

The primary treatment for bleeds in haemophilia involves replacing the deficient clotting factor. This can be done on-demand (in response to a bleed) or as prophylaxis to prevent bleeds [1]. In Germany, the majority of people with severe haemophilia receive prophylactic treatment, yet it is legitimate that people with moderate or mild haemophilia can substitute on-demand [21]. The medical treatment varies between factor replacement (standard half-life or extended half-life), which is injected intravenously or non-factor replacement, which is injected subcutaneously [22]. The latter implies a bispecific antibody, which binds to factor IX and X, so that the function of factor VII is imitated [23]. There is a lot of progress in haemophilia treatment, highlighting a further approach of gene therapy, which aims to provide long-term treatment. Through a virus, a functional copy of the gene is delivered targeting liver cells, which enables the production of the deficient clotting factor [24]. However, gene therapy in haemophilia is still a matter of research. Without getting into too much detail, the main difference between the treatment regime is the peak and trough factor level. Nowadays, it is aimed to reach a trough factor level of 1-3% in people with severe haemophilia, though the multiple treatment options imply different trough levels [1]. The treatment regime is in agreement with specialized haemophilic care centers, physicians and weighted up intraindividually with special regard to dose and frequency of injections [22]. It needs to be emphasized that the treatment varies across countries based on the health care

system, since factor replacement therapy is very expensive [25]. In Germany, the treatment options are quite advanced, emphasizing a recent release of extended half-life factor, which allows to reduce the frequency of injections while maintaining effective factor levels [26]. Next to a higher trough level, the burden of frequent injections is reduced, which in turn increases the quality of life of the individual.

As mentioned, the primary treatment and prophylaxis is replacing or imitating the deficient or missing clotting factor. However, haemophilia is an internal condition with musculoskeletal consequences. Thus, a multidisciplinary team for appropriate treatment is needed. Because of the high prevalence rate of haemophilic arthropathy, orthopedic joint monitoring is of major interest. Different clinical tests are used to determine joint status in PwH. Nowadays, the most popular clinical diagnostic tool is the Haemophilia Joint Health Score (HJHS), which examines elbows, knees and ankles with regard to range of motion, crepitus and swelling among others [27]. Based on the joint status and symptoms, orthopedic surgeries are conducted. The main focus is either on increasing the joint function or to decrease pain [28]. Hereby, the joint debridement, arthrodesis or total arthroplasty play a major role [28]. To target chronic synovitis, the minimal invasive method of radiosynoviorthesis is recommended, which reduces not only the inflammation, but also lowers the bleeding tendency, through injecting radioactive isotopes [29]. Furthermore, physiotherapy can help maintaining joint flexibility, increase joint function and range of motion as well as muscle strength, while wearables such as orthoses increase joint stability, especially during PA [30]. Of course, pain management has become a critical component of therapy in comprehensive haemophilia care [31]. This implies pharmacologic interventions such as the intake of COX-2 inhibitors as well as non-pharmacologic interventions, e.g. physio- and sports therapy [31].

Comorbidities in haemophilia

During the 1980s a scandal regarding the factor replacement therapy attained the PwH. Contaminated factor concentrates led to the transmission of HIV and/or Hepatitis B and/or C or both, which still represents an additional burden for the affected patients [32]. Of course, these comorbidities needed further antiviral

treatment, which in turn cause negative side effects [33, 34]. Patients suffering from both diseases are prone to a progression to end-stage liver diseases [35]. However, over the past four decades, PwH were not exposed to contaminated factor products and treatment options considerably improved [36]. Therefore, life expectancy of PwH rises and the aspect of “aging PwH” gained more interest [35, 37]. Evidence states that PwH have a life expectancy of about 77 years (in the Netherlands), which is close to the general Dutch male population of 83 years [38]. The increased life expectancy is accompanied by increased incidences of age-related comorbidities such as cardiovascular and metabolic disease or obesity [36, 39]. Although it is known, that PwH are less prone to mortality due to coronary artery disease, the prevalence of atherosclerosis is similar compared to the non-haemophilic population [40]. Especially the antithrombotic treatment is challenging given the risks of bleedings in PwH [40]. Regarding obesity, literature highlights the increased prevalence within the whole population as well as in PwH [41, 42]. It needs to be underlined that most research is done in the United States of America or western-European countries, where obesity in general is more prevalent compared to African or Asian countries, subsequently the results are not transferable for the overall haemophilic population [43].

Clearly, suffering from a chronic condition, such as haemophilia can cause mental distress. It has been well studied that the presence of a chronic condition as well as haemophilic arthropathy has a significant impact on the individual's quality of life and that PwH are affected by lower quality of life [8, 44]. An additional comorbidity, such as HIV, hepatitis or osteoporosis demands even more adjustments of lifestyle and does not only represent a physiological but also an increased psychological burden [45]. Previous research confirmed that psychological comorbidities, including depression and anxiety are also increased in PwH compared to the non-haemophilic population [46].

Next to the mentioned age-related comorbidities, osteoporosis plays a major role in haemophilia. Already in 1994, a first study revealing increased prevalence of osteoporosis in PwH was conducted [47]. Ever since, the discussion is ongoing whether there is a direct causal relationship of factor deficiency and osteoporosis or if the bone mineral density (BMD) is reduced due to consequences of haemophilic arthropathy [48, 49]. This will be focused on in the chapter “bone and haemophilia”.

2.2 The bone

Bones can have multiple critical functions, such as protection of internal organs, mineral storage and blood cell production [50]. There are different types of bones, i.e. long bone, short bone, irregular bone, flat bone and sesamoid bone. Though this thesis will focus on long bones (e.g. femur) as well as irregular bones (vertebral bodies). The anatomy of a typical long bone can be divided in macroscopic and microscopic anatomy. On the macroscopic level, it is differentiated between the epiphysis, which build the round ends of a long bone and the diaphysis, which represents the central part of the long bone and embeds the medullary cavity, which is responsible for blood cell production and fat storage [51]. The surface of the bone is covered by the periosteum. Diving deeper into the microscopic level, the bone is composed of cortical bone, known as compacta and spongy bone, i.e. trabecular bone [51]. An insight into the structure of a long bone is presented in figure 2 [52].

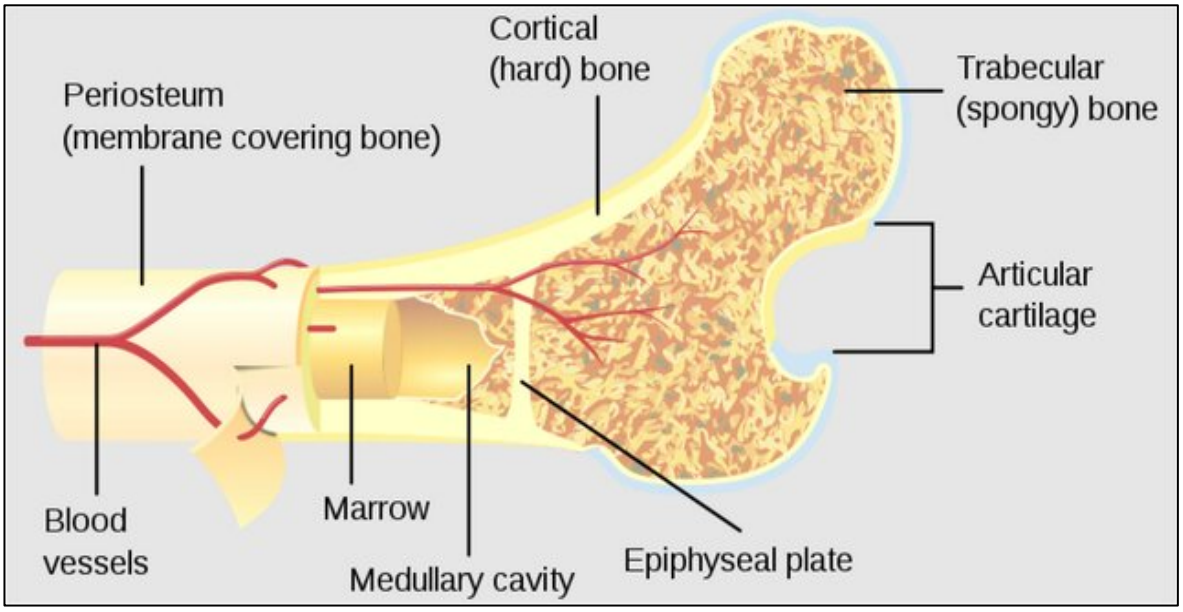

Figure 2: Insight in bone structures according to Barekar et al. (2024)

Bone remodeling

Bone remodeling is a physiological process involving different bone cells, which are in charge of bone resorption and bone formation [53]. Hereby, osteoclasts are responsible of bone resorption of old bone while osteoblasts deposit new bone. This balance is regulated by different stages of interaction between cells and cytokines

[53, 54]. Figure 3 shows a schema of the complex bone remodeling process, including the activation, resorption, reversal, formation and mineralization.

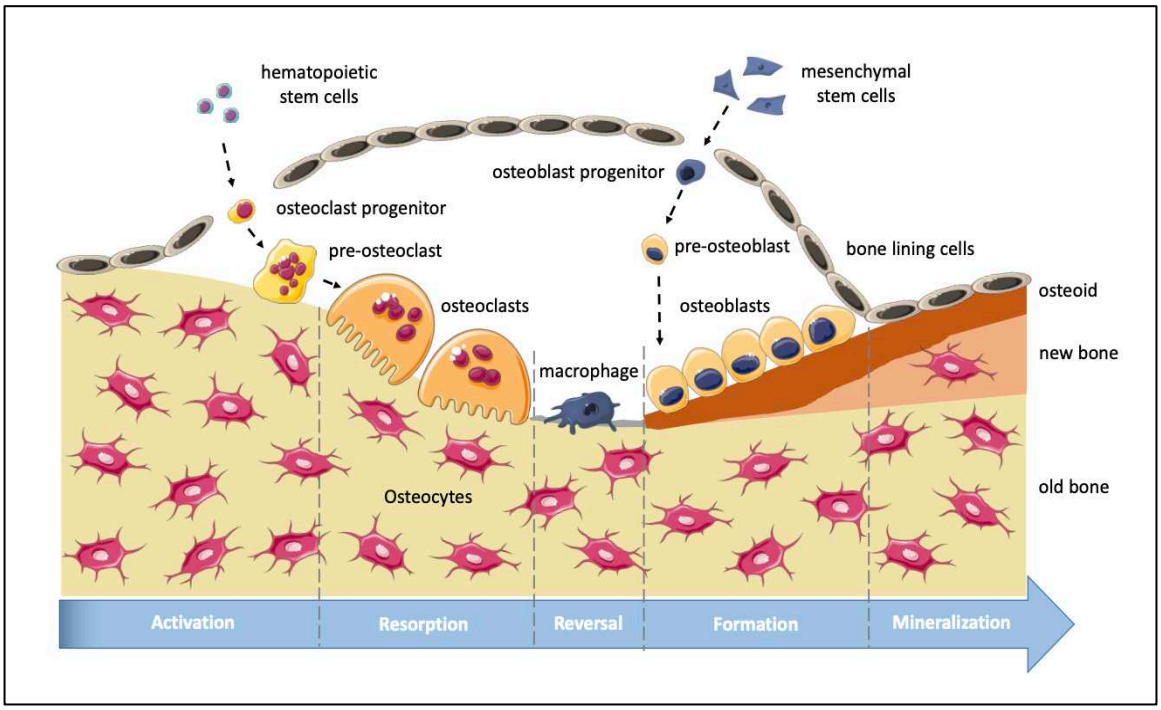

Figure 3: Schematic diagram of bone remodeling process according to Treusdell, et al. (2019)

Furthermore, the receptor activator of nuclear factor κB (RANK) as well as the receptor activator of nuclear factor κB ligand (RANKL) play an essential role in the bone remodeling pathway [55]. The mechanism of action is the differentiation of osteoclasts and activation. Among others, osteoblasts and stromal cells express RANKL, which in turn binds to the RANK on osteoclasts precursors. This binding causes a signaling cascade, promoting the differentiation of the precursors into mature osteoclasts. The activated mature osteoclasts are in charge of the resorption of the bone. This process can be influenced by osteoprotegerin (OPG) [56]. Through preventing RANKL to bind to RANK, bone resorption can be decreased. These three players (RANK, RANKL and OPG) maintain the balance between bone formation and resorption [55, 57]. This mechanism is displayed in figure 4. Additionally, sclerostin is part of the bone haemostasis. Sclerostin is a protein expressed by osteocytes, which inhibits bone formation [58]. Further, vitamin D and calcium play a pivotal role in bone remodeling as well as sex hormones (i.e. estrogen and testosterone) through stimulating osteoblasts and osteoprotegerin. [59]. Because of

the different composition of sex hormones, the exact bone remodeling processes differs between the sexes.

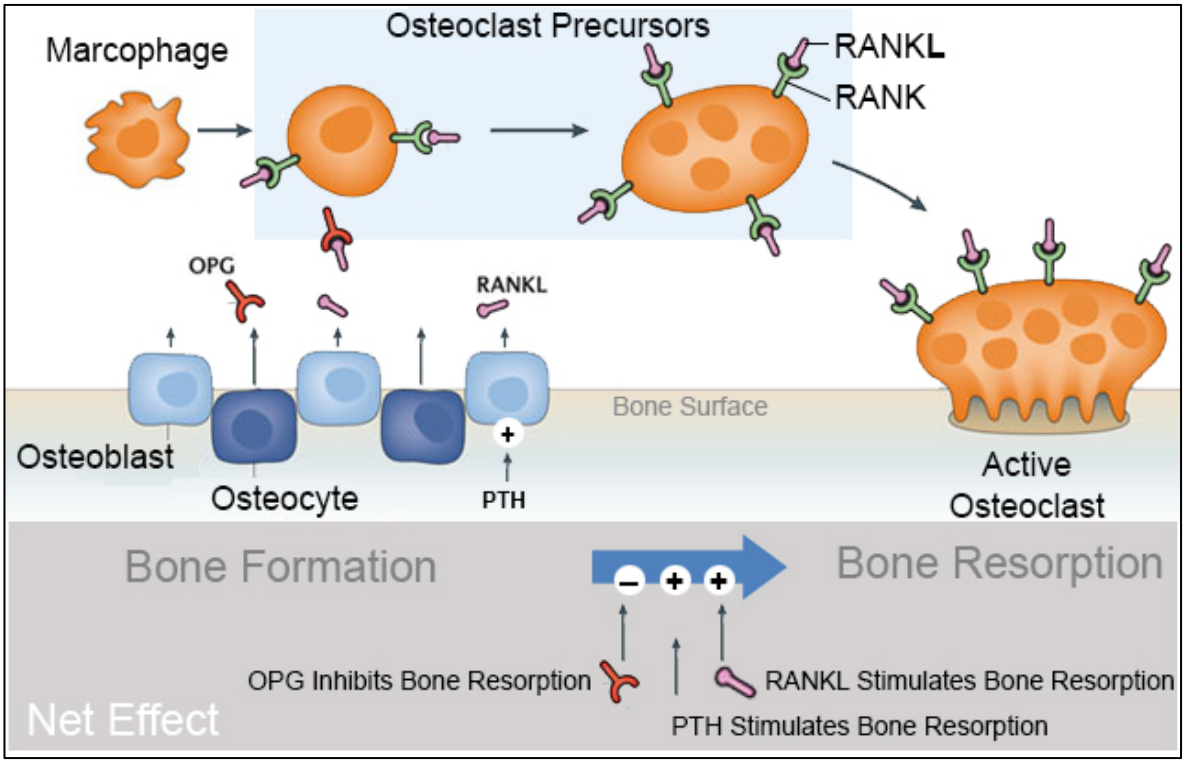

Figure 4: Mechanisms of RANK and RANKL according to Cao (2018)

Moreover, mechanical stress and microdamage repair contribute to bone remodeling. Osteocytes can detect the mechanical stress, such as weight-bearing exercises and enable a signaling process, which promotes bone formation [60]. Evidence states that the bone adapts linear to the applied stress. Lack of mechanical load causes bone resorption. Osteocytes are further responsible for detecting microdamage, which can occur during normal activities [60]. After detection, RANKL is released so that bone resorption of the damaged bone is initiated followed by the reversal phase of bone remodeling, implying the bone formation of the specific damaged area [61]. These processes happen on a regular basis [60, 61].

Bone Quality

The quality of a bone is described by bone mass, characterized by the BMD as well as the bone's microarchitecture [62]. BMD is expressed as grams of the present

minerals in the bone, such as calcium or phosphate, per area (g/cm^2). BMD is influenced by multiple modifiable and non-modifiable criteria such as age, sex, body weight, genetics as well as PA, nutrition and presence of diseases or intake of medication (see figure 5) [59, 63, 64].

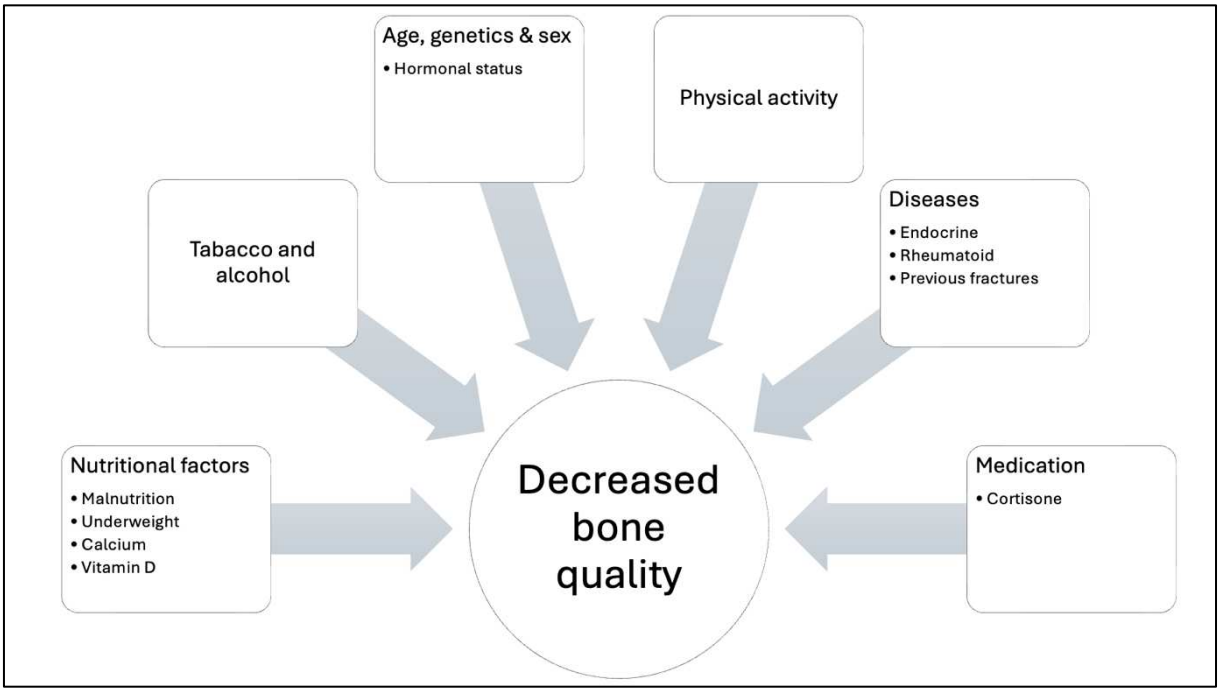

Figure 5: Factors influencing the quality of the bone (author's own illustration)

The bone mass increases during childhood and adolescence [65, 66]. It reaches its peak around age 30 and decreases with age (see figure 6) [67]. Peak bone mass is considered the maximal bone strength achieved in life and serves as key determinant of osteoporosis and fragility fractures. In females, the menopause has a crucial impact on BMD. Due to the decline of estrogen, osteoclasts activity is increased leading to enhanced bone resorption.

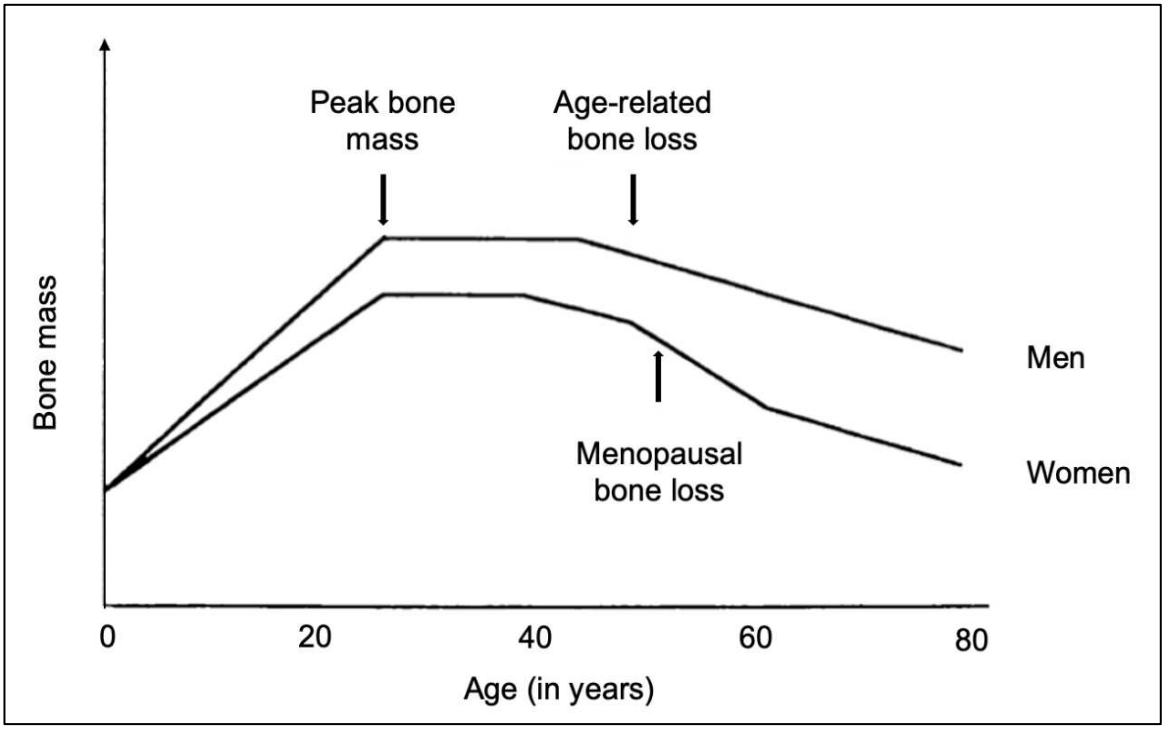

Figure 6: Age-related changes in bone mass based on McDonnell et al. (2007)

Literature pointed to the fact that the BMD is limited to determine fracture risk, as the latter is highly dependent on the microscopic architecture of the bone, i.e. the structure of trabeculae [62, 68]. This describes the spongy part of the bone tissue, which form a network that provides structural support to the bone [68]. The trabecular bone absorbs and distributes mechanical load based on diverging axial arrangement of the trabeculae, so that the stress on the cortical bone structure is reduced. A dense trabecular microarchitecture therefore decreases the risk of fractures [69]. Next to load distribution, the spongy bone stores minerals, such as calcium and phosphate and contributes to blood cell production [51]. Moreover, the exerted strain on the bone is essential for bone formation. The so-called “Wolff’s Law” suggest that repetitive loading of the bone leads to adaptive responses in bone structure [70]. These mechanical loads can either be compression or tension forces, which activates osteocytes and osteoblasts and directly impact the trabecular trajectories [71]. Osteocytes sense mechanical signals and mediate the activity of osteoclasts, triggering a process, which entails the realignment of trabecular bone along primary stress pathways to enhance mechanical efficiency. This procedure leads to the optimal structured framework of different trabecular axes (see figure 7) [72, 73].

One area of relative weakness is present at the so-called Wards triangle, where the axes of the trabeculae to not cross and density of trabecular is low [71]. Therefore, this area is prone to femoral neck fractures, making it a common site for fracture incidences [74].

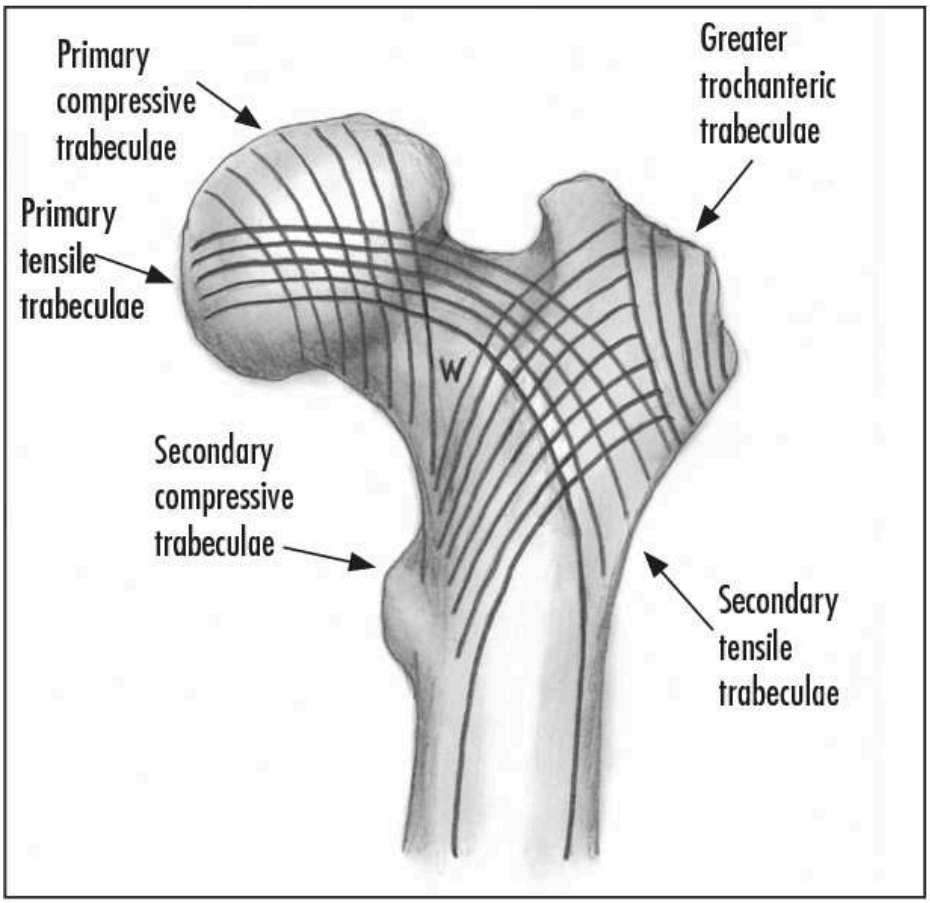

Figure 7: Pattern of trabecular bone in femoral neck according to Shivi et al. (2015)

Explanation: W = wards triangle, an area of relative weakness

Nevertheless, it is suggested that strain rate as well as strain magnitude influence the adaptive process of the bone cells. Previous studies show that jump training including drop jumps involving large strain rates, promotes periosteal as well as endocortical bone formation [75]. Still, general weight bearing exercises including running, strength training but also walking have shown to produce sufficient strain on the bone and therefore positively affects the bone quality at the femoral neck [76, 77].

Osteoporosis

Osteoporosis is defined as a “systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue” [64]. Osteoporosis is a complex disease with multiple influencing factors [59]. There are different types of osteoporosis, which can be classified as primary or secondary osteoporosis. The senile or postmenopausal osteoporosis are considered as primary osteoporosis, which are based on hormonal changes [59]. Secondary osteoporosis involves several underlying conditions, such as hypogonadism, hyperthyroidism, rheumatic diseases or medical drug intake (e.g., cortisone), leading to a disbalance of bone remodeling [59]. The primary osteoporosis frequently affects postmenopausal women, though also men are affected by osteoporosis: in Europe, the average male population shows a prevalence rate of 9.7% (confidence interval 4.4-18.5) [78]. In many cases, osteoporosis remains asymptomatic and is detected by (spontaneous) fractures only. It needs to be underlined, that a persons’ mortality is increased due to osteoporosis based on fall risk associated fractures. Other symptoms of osteoporosis include, kyphosis of the thoracic spine, reduction of height, and pain [59, 64]. The main and most serious consequence of osteoporosis is the risk of fractures, which most frequently affect the vertebrae, femoral neck or the wrist [59, 64]. They can occur after falls but in severe stadiums, fractures of the ribs result even following sneezes or intense coughing. Also, in osteoporotic fractures, healing can take longer compared to non-osteoporotic fractures [79].

Dual X-ray absorptiometry for densitometry

DXA is an established assessment tool for densitometry. It is used in various clinical settings, e.g., for individuals with genetic or eating disorders. Regarding the determination of BMD, DXA is considered the gold standard [80]. An example of a DXA evaluation is presented in figure 8. This imaging technique relies on low-dose X-rays with two different energy levels, allowing the software can differentiate between bone and soft tissue and enables the measurement of BMD [81]. To diagnose osteoporosis, a clinical assessment including anamnesis, physical examination is conducted. Also, various blood parameters (a.o. calcium, parathyroid hormone, sex hormones) are determined to exclude secondary osteoporosis is

conducted. According to the German guidelines for osteoporosis, DXA-screening of the spine (vertebral bodies 1-4) and the left femoral neck should be conducted as a second step. If there is a limitation to measure one of the landmarks, it is stated to use the lower arm to examine BMD [64]. To interpret BMD a T-score is calculated, which reflects the standard deviation of a healthy adult of same sex and ethnicity at the age of 30 years. The World Health Organization stated, that the lowest T-score of either femoral neck or spine (sum of vertebral bodies 1-4) should be used to classify the subject into one of the following categories: normal (T-score ≥-1.0), osteopenia (T-score range -1.0 to -2.4) or osteoporosis (T-score ≤-2.5) [64, 82]. These classifications are valid for subjects aged 50 years or older. For subjects younger than 50 years, the Z-score should be used. The Z-score compares the BMD results with an average BMD of people at same age. Subjects having a Z-score <-2.0 are considered as “below expected range for age” [83].

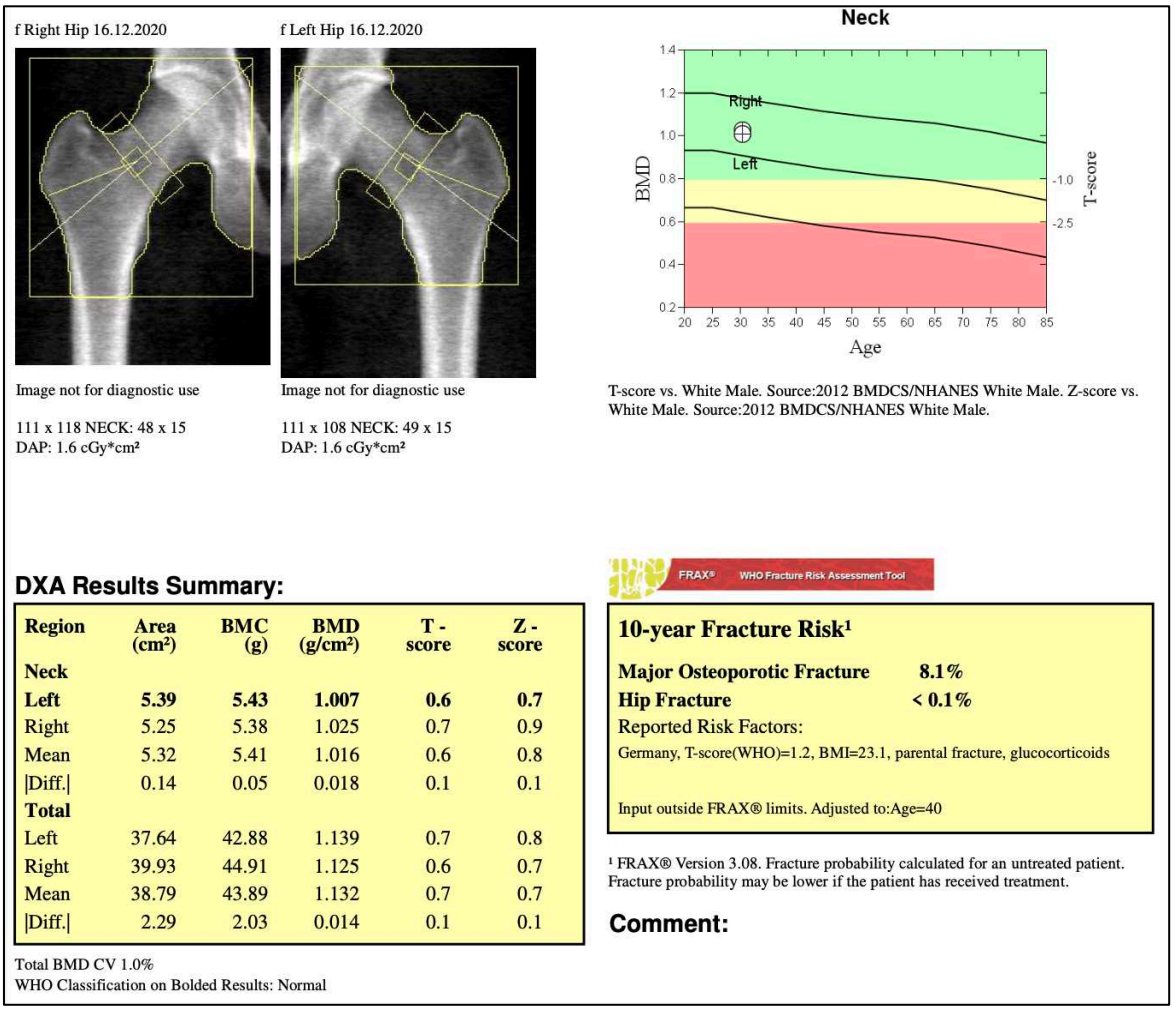

Figure 8: Example of evaluation of bone mineral density and the left and right hip including 10-year fracture risk using Hologic.

Along with the BMD, a 10-year probability of experiencing fractures is calculated. This algorithm, known as FRAX®, has been validated in subjects aged ≥ 40 years [84]. The FRAX® includes the results of the BMD measurement as well as relevant data on medical and family history, previously experienced fractures and other risk factors such as smoking or alcohol intake. The examiner is in charge of gathering the respective data and to complete the questionnaire before assessment.

With an additional software (TBS iNsight® (V. 3.1.2. Medi Maps; Switzerland)) the trabecular bone score (TBS), which is a parameter for determining the quality of bone microarchitecture, can be used complement the assessment of fracture risk [68]. It analyses gray-scale variation of the vertebral bodies L1-4 [85]. The TBS can be examined based on the DXA of the lumbar spine, so that no additional measurement is needed. The TBS is measured in score points, which are classified as “normal” (TBS ≥ 1.31), “partially degraded” (TBS between 1.30 and 1.24), and “degraded” (TBS ≤ 1.23). The following figure 9 shows a healthy vertebral compared to one with poor trabecular structure. A total analysis of a haemophilic subjects’ TBS is attached as Appendix 1.

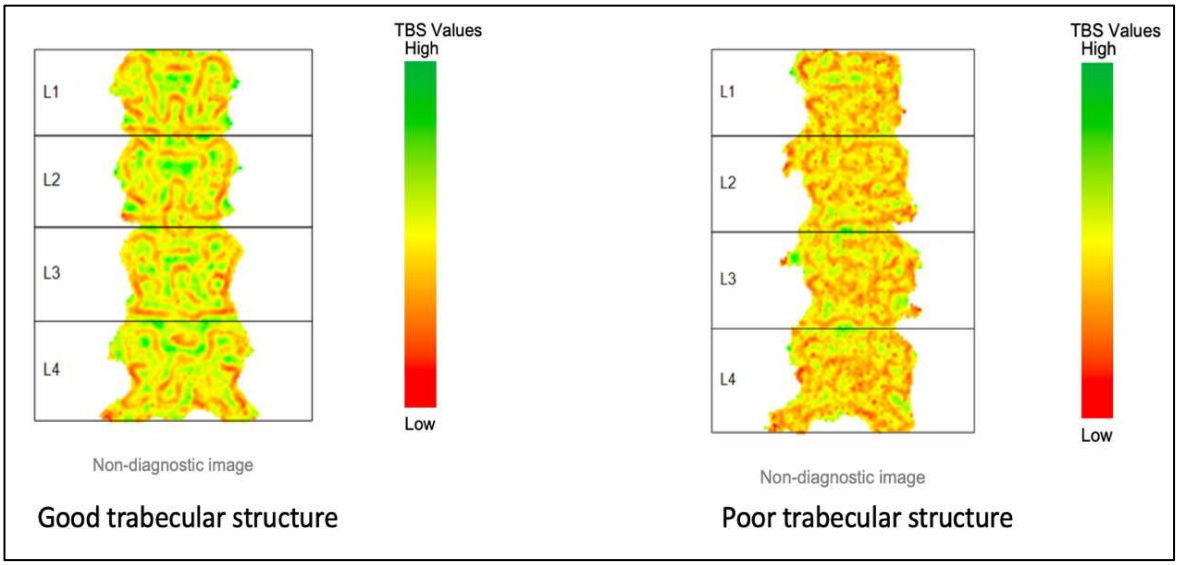

Figure 9: Example of a healthy vertebral body and one with poor trabecular structure

To conclude, the BMD measures the mineral density of the bone and is the standardized and most fundamental test for osteoporosis diagnosis. However, the TBS is a complementary method, which together provides a comprehensive

evaluation of the bone quality. Additionally, determining the FRAX® allows a comprehensive risk assessment beyond bone density.

Treatment of osteoporosis

The ultimate treatment objective is to lower the individuals fracture risk. According to the German guidelines of osteoporosis, it is differentiated between basic and specific medication therapy. The basic therapy includes the intake and sufficient supply of calcium and/or vitamin D as well as general high-protein nutritional recommendations, avoiding underweight (BMI >20) and PA. Lifestyle adaptations, including balance and strength training as well as home safety adjustments to reduce risk of falling should to be considered [64]. Exercises promoting enough stimulus on the bone to reach bone remodeling should be executed. This will be focused on in chapter 2.4 (physical activity and bone quality). These aspects are relevant for both prophylaxis and treatment of osteoporosis.

The specific medication therapy varies between the sexes as the origin of osteoporosis deviates. In Germany, several pharmacological agents are approved for the treatment of osteoporosis in males, including bisphosphonates, denosumab, and teriparatide. These medications target different aspects of bone metabolism and work through various mechanisms to either inhibit bone resorption or stimulate bone formation. An established treatment are bisphosphonates which inhibit osteoclasts, so that bone resorption is decelerated [55, 64]. Denosumab is a RANKL-antibody, which in turn leads to a decrease of osteoclast activity [55]. Another approach are teriparatides as an anabolic therapy, which inhibits the promotion of sclerostin [58]. Bearing in mind, that the treatment of primary and secondary osteoporosis is distinct, since these two forms have different underlying causes. As mentioned earlier, secondary osteoporosis originates from either another disease (e.g. hypogonadism, hyperthyroidism) or other extrinsic factors (e.g. intake of corticoids), these will be treated accordingly. For instance, hypogonadism is treated using testosterone substitution, which might be sufficient to also treat osteoporosis.

Bone and haemophilia

The disease haemophilia itself is not directly seen as a risk factor for reduced bone quality. However, several prior investigations point towards the association between (severe) haemophilia and an increased prevalence of osteoporosis [86, 87]. Recent literature has shown that there is a significant decrease of BMD in PwH compared to healthy males. It is stated that the risk for low BMD was about four times higher compared to control subjects [49]. Results of a recent meta-analyses is presented in figure 10 [49, 87].

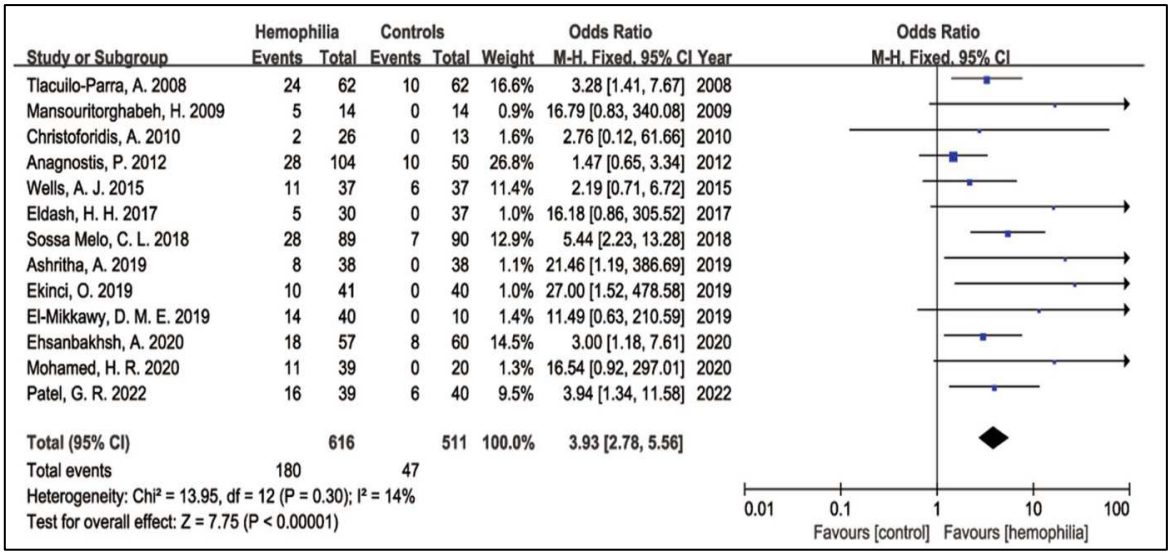

Figure 10: Forest plot of the risk of low BMD in PwH compared with non-haemophilic controls according to Zhou et al. (2024)

However, the evidence of the influence of severity phenotypes and other potential influencing factors is limited. When discussion potential influencing factors of haemophilia on bone quality, four different major parameters need to be considered of which an overview is displayed in figure 11.

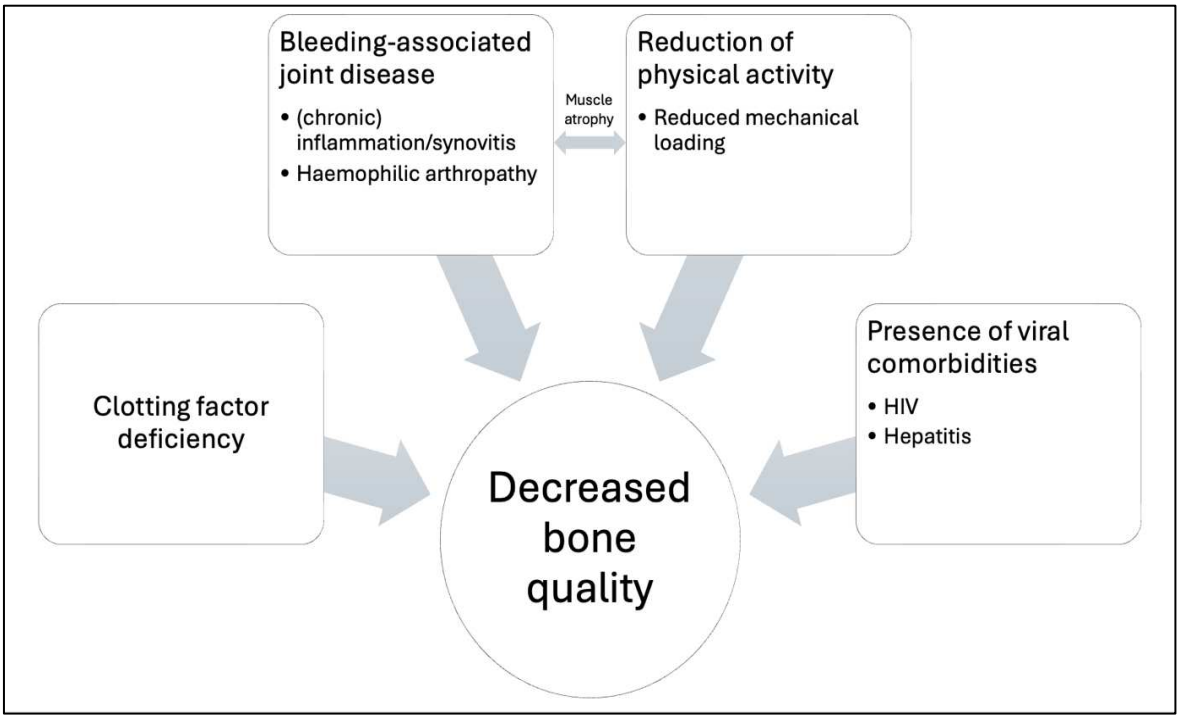

Figure 11: Potential haemophilia-associated factors influencing the quality of the bone (author's own illustration)

First, the clotting factor deficiency itself might lead to a lower bone quality including both cortical and spongy bone [88]. Mice models showed that the coagulation cascade plays a role in bone development, which gets disrupted due to the deficient factor [88, 89]. It is suggested that factor deficiency therefore impairs bone health directly by decreasing thrombin production, an enzyme which is known to stimulate osteoblasts [90]. Consequently, a decreased thrombin level causes a decreased osteoblasts level [90, 91]. In addition to this direct influencing mechanism, factor deficiency may also impair bone quality indirectly: A recent systematic review, evaluating murine as well as in vitro studies points to the fact that FVIII can inhibit the activity of osteoclasts through reducing the ability of RANKL to promote osteoclastogenesis. Furthermore, it has been emphasized that factor VIII was shown to increase the effects of OPG (see figure 12) [92].

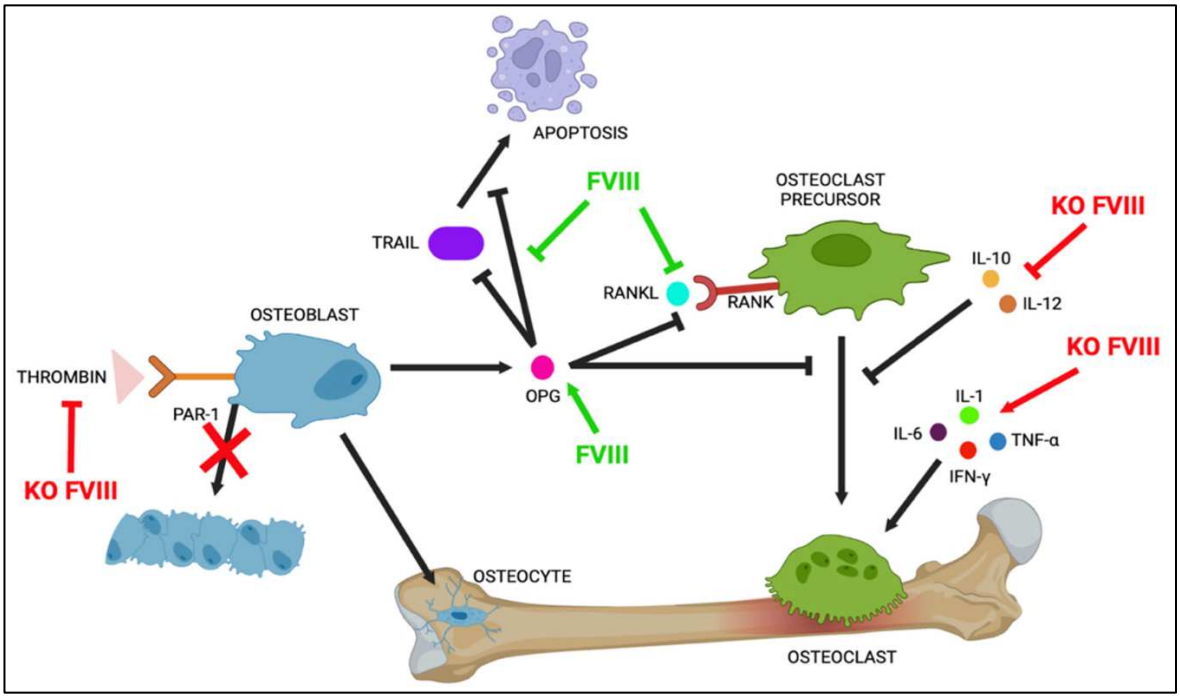

Figure 12: Role of factor VIII in bone turnover according to Berni et al. (2025)

Explanation: FVIII = factor VIII, OPG = osteoprotegerin, RANK = receptor activator of nuclear factor κB, RANKL = ligand of RANK, TRAIL = TNF-related apoptosis-inducing ligand, PAR-1 = protease-activated receptors 1, IL-10 = cytokine interleukin-10, IL-12 = cytokine interleukin-12, IL-1 = cytokine interleukin-1, IL-6 = cytokine interleukin-6, IFNα = interferon gamma, TNF-α = tumor necrosis factor, KO FVIII = knockout of factor VIII.

Previous murine studies showed that peak bone mass was not reached in six-month old mice with haemophilia A due to the lack of bone formation compared to mice without haemophilia [93]. Most likely, there is a multifactorial underlying pathogenic mechanism, which originates in both inside and outside the coagulation cascade [94]. It has been highlighted stat there are many discrepancies of data also being limited through various factors such as transfer of animal models to humans or role of genetics, which lead to difficulties in interpretation [94]. It needs to be emphasized that the available research on the role of clotting factor deficiency, especially of clotting factor IX, on bone quality is limited, and the precise microbiological processes require further investigation. This knowledge gap should be addressed also to question non-factor treatment in regard to bone health [94]. In non-factor treatments, the factor itself is not replaced but functionally mimicked only [22]. Thus,

this treatment option may not replicate protective bone effects compared to factor replacement therapies.

Second, there is evidence that haemophilic arthropathy and the prevalence of local synovitis influence bone metabolism [86, 95, 96]. Already in year 2007, it was pointed out a worse joint situation is associated with a lower BMD in PwH (see figure 13) [86].

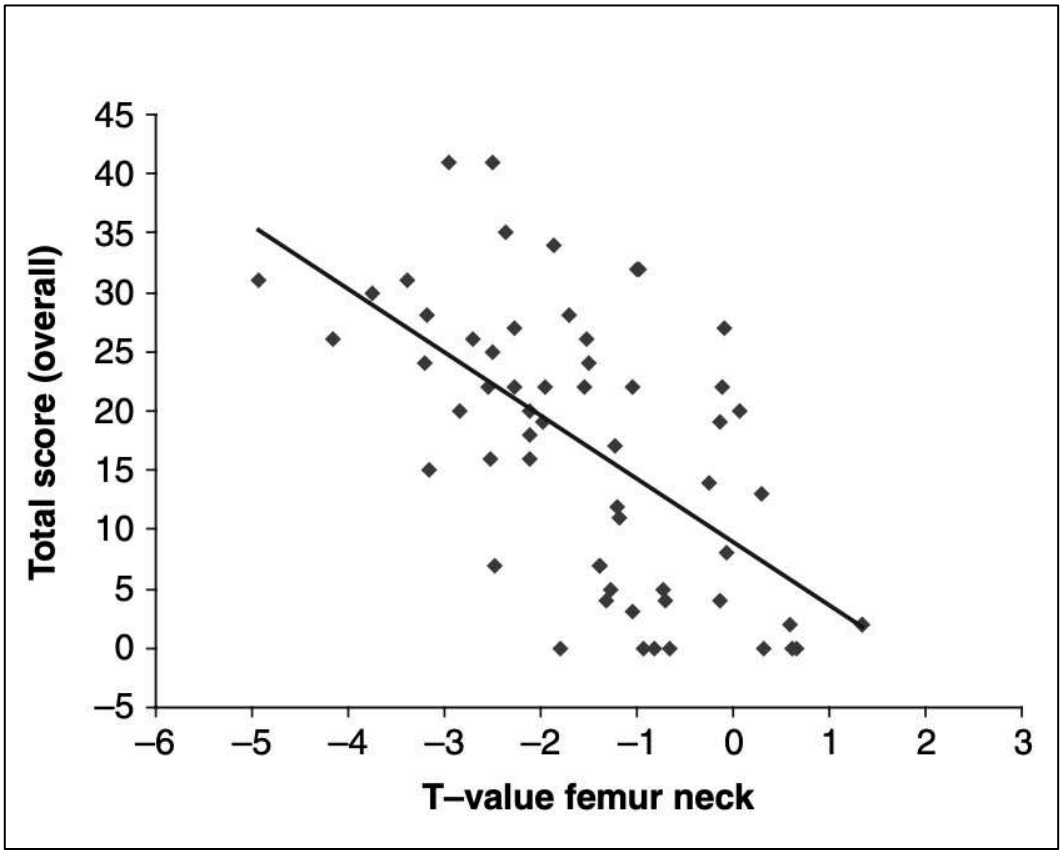

Figure 13: Correlation of t-score of femoral neck and total joint score according to Wallny et al. (2007)

As mentioned before, recurrent bleedings lead to chronic synovitis, frequently leading to a worse joint status in PwH. The process of synovitis includes the expression of pro-inflammatory cytokines (e.g. TNF-α, IL-6), which also cause an expression of RANK and RANKL [96]. Osteoprotegerin has been shown to be decreased in PwH with arthropathic joints (see figure 14). This imbalance of the RANK/RANKL/osteoprotegerin pathway promotes bone resorption and subchondral damage [97].

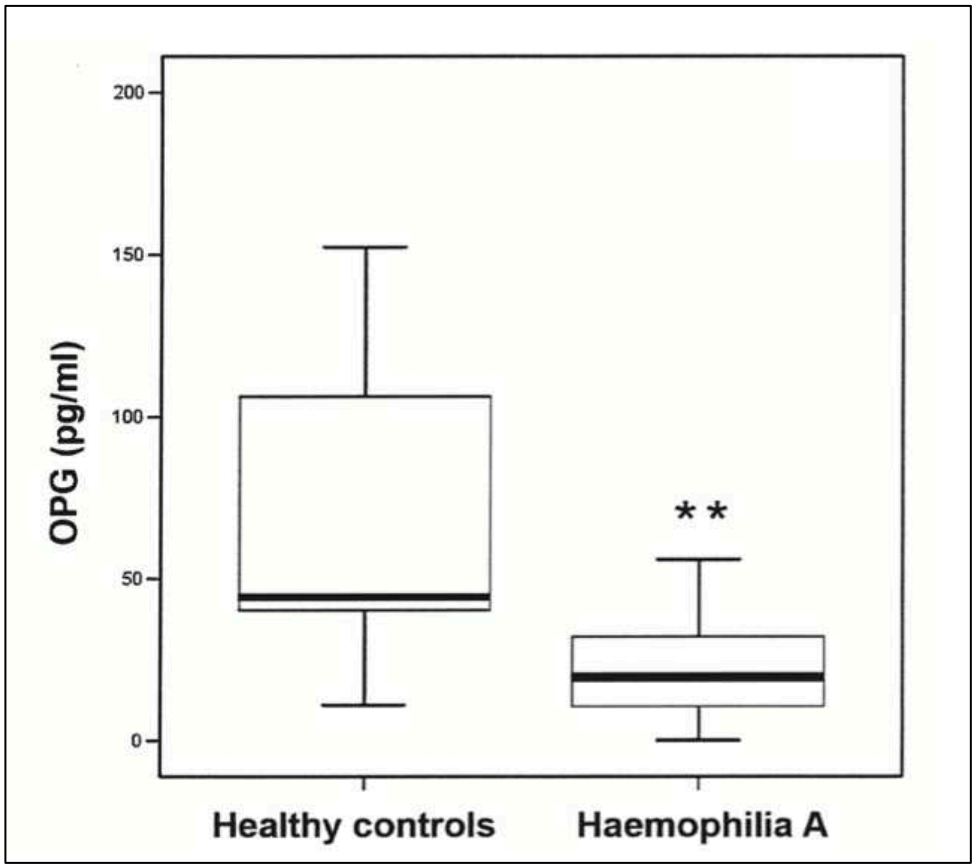

Figure 14: Serum concentration of osteoprotegerin in 67 PwH and 30 healthy controls according to Melchiorre et al. (2012)

*Explanation: Boxes show 25th and 75th percentiles. Lines within boxes represent the median. Whisker represents 10th and 90th percentile. **indicates significant difference of p<0.001*

Lower osteoprotegerin was detected in both, serum and synovialis samples, though increased RANK/RANKL only in synovialis samples. This observation is comparable to patients with rheumatoid arthritis, especially those with active synovitis, as lower OPG levels are seen here as well [98]. Moreover, a previous blood analysis of 51 boys (aged 5-19 years) with haemophilia revealed a dysregulated bone metabolism of lower osteocalcin and increased urine deoxypyridinoline/creatinine, which both correlated with lower BMD [99]. The authors suggest an uncoupling of bone turnover with increased bone resorption and decreased bone formation to take place because of previous joint bleeds and the accompanied altered OPG pathway [99]. However, the question remains whether this process is only present on a local level,

meaning that bone quality might only reduce around the affected joint or systemically, which would then indicate general reduction of bone quality.

Third, a closer look on the relationship between the bone and PA as well as haemophilia and PA is necessary. It is widely known that PA is a direct influencing factor for bone quality as impact on the bone through PA is essential for bone formation and bone remodeling. Two aspects need to be considered in the haemophilic context. First, prior investigations highlight the lower levels of PA in PwH compared to healthy control subjects. There are several reasons why PwH might engage less in PA, though previous studies also highlight a narrowing of the activity levels between PwH and the non-haemophilic population [100]. These aspects will further be elaborated on in the chapter “physical activity and haemophilia” on page 30. Secondly, it is not recommended to perform high impact PA when being affected by a coagulation disease because of the accompanied bleeding risk or to avoid joint pain. Thus, PwH less often perform weight-bearing PA and chose to conduct light impact PA only [101]. Therefore, the risk of insufficient strain on the bone of the PwH is meaningly high. This aspect is already of relevance during childhood, as the development of peak bone mass is limited [102, 103]. However, as a matter of fact, many PwH are affected by muscle atrophy as a consequence of haemophilic arthropathy. Literature suggests an association between muscle atrophies with lower bone quality since muscle weakness limits the potential exerted mechanical load [104].

The fourth aspect influencing bone metabolism is the presence of haemophilia-associated comorbidities, including Hepatitis and HIV as well as their treatment [86, 95, 96]. It is suggested that the RANK/RANKL/osteoprotegerin pathway is altered through viral coinfections, indicating increased RANKL- and suppressed OPG expression [105, 106]. Viral coinfections can lead to an increase of the production of proinflammatory cytokines, such as TNF-α, IL-6 [105]. Furthermore, antiviral treatments such as protease inhibitors and ribavirin have been linked to increased bone resorption markers and heightened osteoclastic activity [107, 108]. Still, there is contradictory research, as it has also been stated that there is no association between the presence of viral comorbidities and reduced bone quality [95]. The evidence supporting these mechanisms remains limited, and further research is needed to clarify their precise role in bone metabolism.

Considering the classification of primary and secondary osteoporosis, it should be discussed whether osteoporosis in hemophilia should be categorized as secondary osteoporosis, given its multifactorial origin. The mentioned influencing factors on the bone quality are directly associated with hemophilia. Recognizing osteoporosis in PwH as a secondary condition highlights the need for early diagnosis and targeted interventions, focusing on adequate factor replacement, treatment of haemophilic arthropathy and promoting PA. This perspective may help improve bone health management in hemophilia patients and reduce the risk of fractures and long-term disability.

To sum up, there is an increased risk for PwH to develop lower bone quality. Generally, a lower bone quality is accompanied by an increased risk for fractures. Having a closer look on the incidence on fractures in PwH, evidence is inconclusive, and prevalence rates range from 4% to 37% [109]. A recent Taiwanese study showed that even though the risk for fractures might be increased, there was no significant difference in the risk of fractures between PwH and the general Taiwanese population [110]. Another study revealed contradictory results showing an overall increased incidence of fractures, especially in people with severe haemophilia [111]. This monocentric study further investigated the influence of inhibitors as well as the difference between haemophilia A and B (see figure 15).

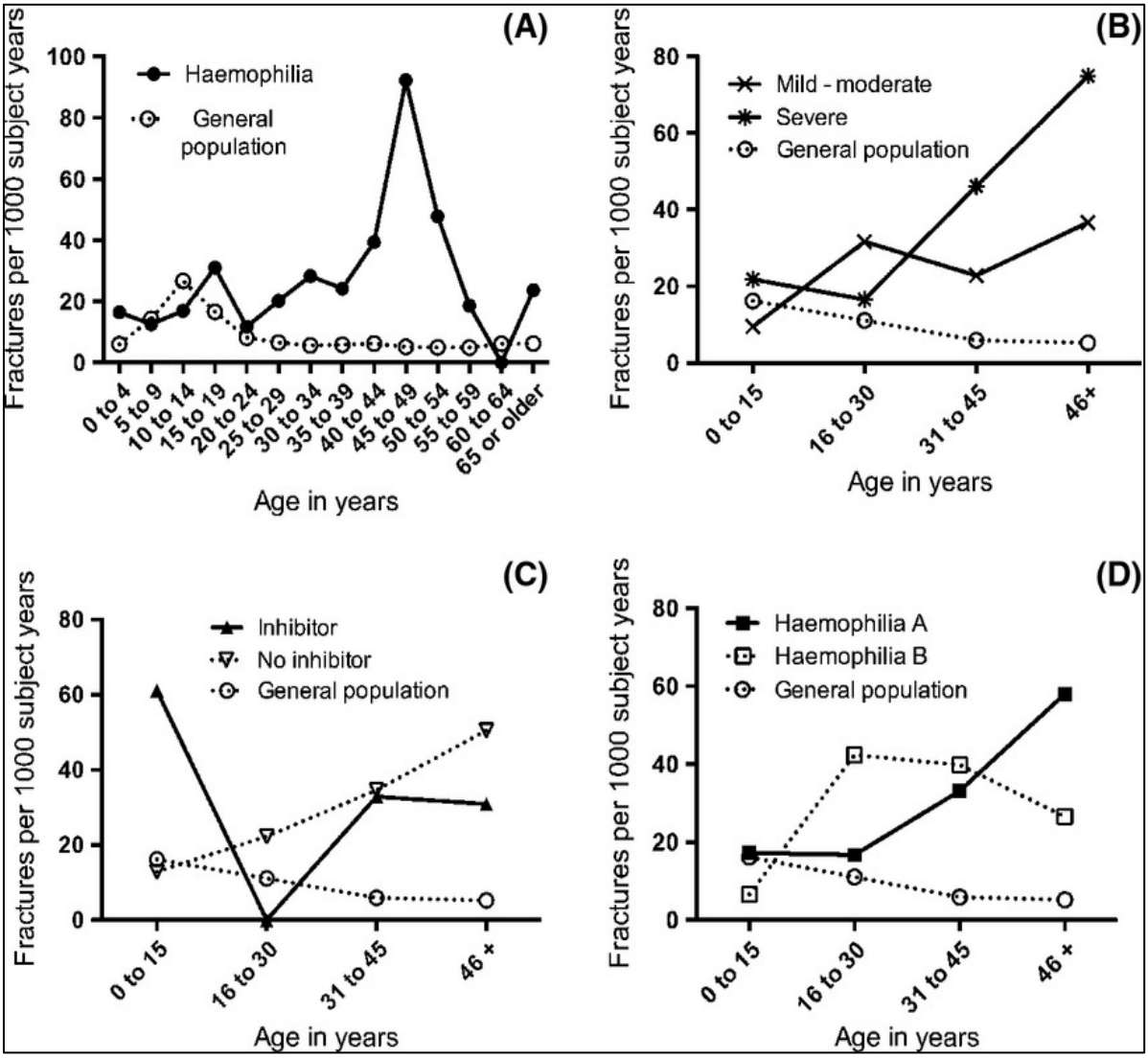

Figure 15: Incidence of fractures in people with haemophilia according to Gay et al. (2015)

Explanation: (A) People with haemophilia had an increased rate of fracture ($p < 0.0001$) compared with the control population. (B) people with severe haemophilia were found to have increased rate fracture ($p = 0.0384$) compared to people with milder disease. (C) Haemophilia subjects with inhibitors had no significant increase in rate of fracture ($p = 0.1168$) compared to Haemophilia subjects with no inhibitors. (D) There was no difference in fracture rates between people with haemophilia A or B ($p = 0.9535$).

Literature on fracture risk and incidences of fractures is inconclusive, though it seems essential to consider individual factors, such as severity phenotype, PA-level and joint status of the subject.

2.3 Body composition

Body composition refers to the proportion of different compartment that make up the human body [112]. The most commonly used ratio, which helps getting a quick overview on a person's body composition is the body mass index (BMI). This index divides the weight by height * height. This is a simple and standardized tool to classify individuals as either underweight (BMI <18.5), normal weight (BMI between 18.5 - 24.9), overweight (BMI 25 - 29.9) or obese (obesity class I: BMI 30 - 34.9, obesity class II: BMI 35 - 39.9, obesity class III: BMI ≥ 40). The determination of body composition is more detailed including different parameters of fat tissue and fat-free mass [112]. The assessment of body composition is done for both, physiological and pathological rationales. Body composition analysis reveals risks of chronic diseases through revealing the amount of (abdominal) body fat, can monitor athletic performance, and can help to tailor medical treatment e.g., in cancer patients undergoing therapies affecting the body weight [113]. There are different options to measure body composition parameters, such as bioimpedance analysis or calipometry, though the gold standard is DXA [80]. The DXA evaluates the absorption of two energy levels to differentiate between fat mass, lean tissue, and bone mineral content of the whole body as well as of the extremities, core and head [114]. An example of a total body composition analysis is presented in figure 16. This examination further provides a ratio between android and gynoid fat, estimates visceral adipose tissue (VAT) and determines indices of fat and lean mass in relation to height [115].

As body composition parameters vary between the sexes as well as ethnicities, it needs to be highlighted that the following section of this dissertation refers to Caucasian males only [116].

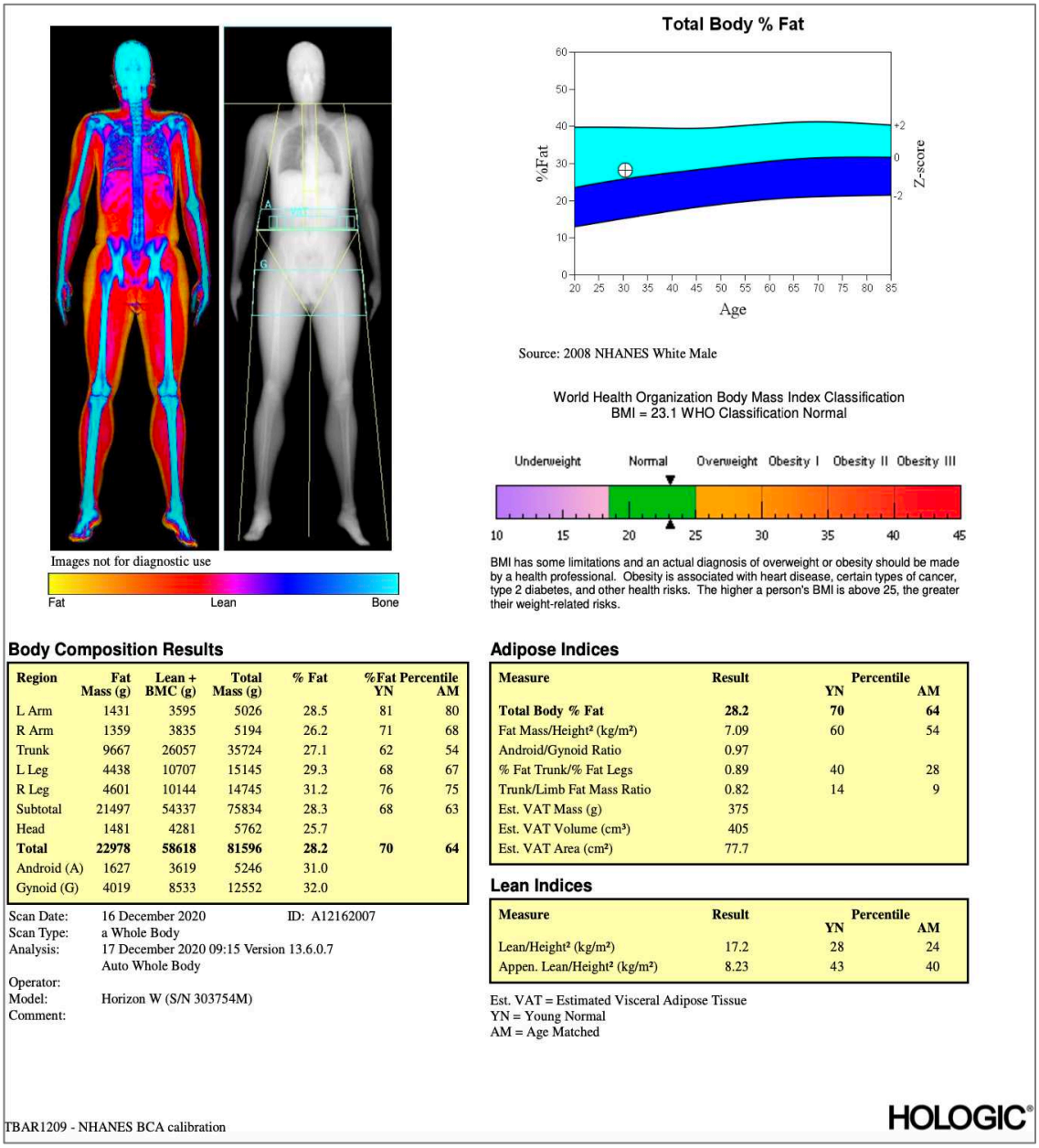

Figure 16: Example of body composition analysis

In detail, body fat percentage is expressed in lipids, which ranges from 10-20% in healthy male populations [117]. It is further differentiated between overall body fat and VAT. The latter surrounds internal organs such as the liver or pancreas. There is no recommendation of VAT levels, though it is known that to high amount of VAT is positively correlated with an increase of metabolic and cardiovascular diseases [114, 118]. The lean mass refers to the amount of skeletal muscle tissue in the body. However, it needs to be differentiated between lean mass and fat-free mass. Lean

mass includes muscle mass only while fat-free mass involves bone, organs and water. Within this thesis, the focus lies on lean mass as the bone content or organs are not of special interest. A decreased amount of lean mass is assumed in PwH, as many PwH are affected by muscle atrophy [119]. Muscle atrophies originate by muscle disuse as a result of impaired joint function as well as fear of movement because of pain and bleeding risks. The inactivity of the muscle increases oxidative stress and release of TNF-α, which can enhance inflammatory responses and therefore might lead to a RANK/RANKL-imbalance [120]. Moreover, a second body composition parameter – VAT – is associated with inflammation processes: increased VAT levels are linked to elevated TNF-α production and chronic IL-6 expression. Generally, it is known that fat mass increases, while lean mass decreases with age (see table 1) [121].

Table 1: Reference data for body composition parameters of non-haemophilic European men based on Ofenheimer et al. (2020)

Variable	**Age 18 < 82** (n=5147)	**Age 18-29** (n=1233)	**Age 30-39** (n=875)	**Age 40-49** (n=830)	**Age 50-59** (n=839)	**Age 60-69** (n=784)	**Age 70- <82** (n=576)
BMI (kg/m²) M±SD	26.6±4.3	24.2±3.8	25.9±4.0*	27.0±4.2*	27.8±4.1*	28.2±4.0	28.1±3.7
Fat/height² (kg/m²) M±SD	7.7±3.0	6.0±2.7	7.1±2.8*	8.0±2.9*	8.6±2.8*	9.0±2.7*	9.3±2.7
Lean/height² (kg/m²) M±SD	17.8±1.8	17.3±1.9	17.8±1.9*	18.1±1.8*	18.2±1.8	18.1±1.7	17.8±1.5*
Body fat percentage (%) M±SD	29.3±7.3	24.8±7.4	27.7±6.9*	29.8±6.5*	31.3±6.2*	32.7±5.7*	33.6±5.9*
Est. VAT mass (g) M±SD	1218±939	424±385	767±543*	1243±755*	1612±894*	1904±914*	2037±880*
Android/ Gynoid Ratio M±SD	0.7±0.2	0.4±0.1	0.5±0.2*	0.7±0.2*	0.8±02*	0.8±0.2*	0.9±0.2*

*Explanation: Age in years; * indicates significant age effects on mean vs. previous age group p<0.01; T-test for independent samples for parametric data and Wilcoxon Rank test for not normally distributed data was used.*

Further literature points to an increased level of obesity in the general population, which is comparable in the haemophilic population, though precise data on VAT levels do not exist, emphasizing the need for investigating body composition parameters in PwH [41].

2.4 Physical activity

It is well-known that PA has significant beneficial health effects for body and mind. The world health organization defines PA as “any bodily movement produced by skeletal muscles that requires energy expenditure” [122]. It is further stated that conducting PA on a regular basis can prevent noncommunicable diseases such as diabetes mellitus type II or coronary heart disease. However, the question arises to what extent should people be physically active? The World Health Organization recommends conducting at least 150-300 minutes of moderate-intensity aerobic PA or at least 75-150 minutes of vigorous-intensity aerobic PA throughout the week. The same is recommended for persons with disabilities. Nevertheless, the World Health Organization indicates that more than 25% of the world`s adult population is insufficiently active, and this ratio increases especially in high-income countries. It is important to differentiate between PA and exercising: while PA compromises every bodily movement in daily life, exercising is a subcategory referring to structured intentional PA, focusing on the improvement or maintenance of physical health [123].

To measure PA, different tools can be used. For instance, electronic activity tracking is a validated and well-established method for longitudinal data acquisition especially regarding step activity [124, 125]. Moreover, handgrip strength is an overall indicator for fitness levels [126]. Especially for cross sectional investigations, measuring handgrip strength is a quick and feasible way to objectively determine fitness levels. It is an applied method in clinical and research settings determining the fitness levels in e.g. patients suffering from sarcopenia or rheumatoid arthritis [127]. Increased handgrip strength indicates enhanced fitness level. Further, subjective data on activity levels can be gathered through questionnaires and diaries, which surely lack in precision, though it is a reasonable additional tool to complement objective data [128].

Physical activity and haemophilia

A physically active lifestyle was not recommended for PwH until the 1970 because of the increased bleeding risk. Nowadays, PA in PwH is indispensable [129, 130]. Depending on the severity and amount of arthropathic joints, PwH have a variable degree of restrictions. Literature has shown that PwH are often limited in their PA levels compared to the non-haemophilic population [131]. This can be due to arthropathic related pain, restricted range of motion or kinesiophobia [44, 132]. Fortunately, recent data showed that in line with adherent prophylactic treatment, there is no increase of bleeding risk while exercising, which might lead to a decline of kinesiophobia in future time [101]. Hence, enabled by better treatment options and the overall risen awareness of beneficial effects of exercising on the individual health, haemophilia care takers encourage the PwH to be more physically active [133]. Scientific data revealed increased quality of life and enhanced well-being in PwH who perform PA on a regular basis [130, 134]. Next to positive cardiovascular effects, PA directly impacts haemophilic arthropathy. Previous research indicates an increased range of motion, stabilized joints due to strengthened muscle and a reduced risk of injury [135]. Both resistance and aerobic training are recommended for better overall health in PwH, emphasizing an adequate supervision and safety measures [129, 136]. However, evidence suggests lower PA levels including lower handgrip strength in PwH compared to non-haemophilic subjects [137, 138]. The past few years pointed towards a trend showing that PA levels in PwH become more and more alike non-haemophilic subjects, emphasizing that possible differences between severity phenotypes have not been investigated so far [100].

Physical activity and bone quality

The relationship of PA and bone quality is complex and involves multiple physiological mechanisms [76]. Weight-bearing exercises, such as running or jumping are particularly effective in promoting bone formation because they generate dynamic stress including muscle contraction as well as gravitational force on the bone, which in turn triggers osteoblastic activity [139]. Moreover, PA influences the expression of various hormones, such as growth hormone that regulate bone remodeling [140] (see figure 17).

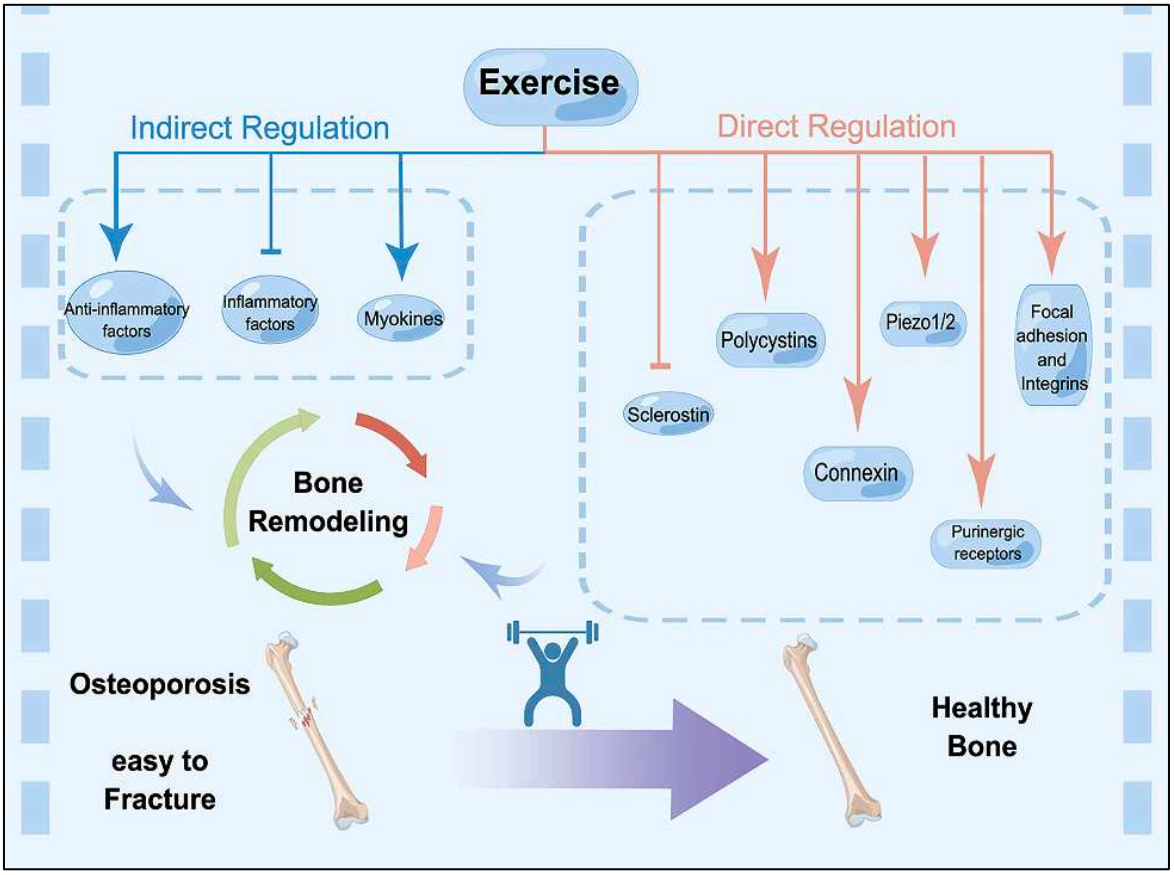

Figure 17: Direct and indirect regulation mechanisms of exercise for osteoporosis according to Chang et al. (2022)

Explanation: Cell surface receptors are the starting point for understanding the regulatory effects of mechanical loading, which include focal adhesions, integrins, purinergic receptors, connexin polycystins, Piezos, Wnt signaling and sclerostin. In addition, exercise-induced regulation of bone environment also occurs through cytokines, including inflammatory factors and myokines, such as irisin. These factors collectively affect bone remodeling.

Additionally, PA increases the production of estrogen in women and testosterone in men, which both are essential for bone health [141, 142]. However, it needs to be emphasized that the desired result of PA on bone quality varies depending on the age. During childhood and adolescence, PA is strongly recommended to achieve a high peak bone mass. In adults, PA is needed to maintain BMD and to prevent osteoporosis [139]. It is of note that significant results of PA on bone metabolism can vary based on individual conditions. Evidence based recommendations vary between 6-12 month of PA to achieve a significant increase of BMD [143, 144].

However, most research is done in postmenopausal women, so that for male subjects evidence is lacking.

Of course, excessive duration and intensity are to be considered as this can have negative effects on bone health, leading to stress fractures or overuse injuries [145]. Though as a matter of fact, these issues are present in elite athletes with high training volumes and can be neglected with regard to persons with lower training volume [145].

Physical activity and body composition

The different body composition parameters are directly linked to PA, though this is a complex interplay of metabolic and hormonal processes [146, 147]. It is well-known that regular PA causes muscle gain [148]. The latter is caused by muscle fiber damage, triggering repair and growth processes [149, 150]. Of course, the type of PA plays an essential role: evidence shows that the most efficient option to increase of lean mass is regular resistance training [150]. Another major impact of PA is fat loss, though increased caloric expenditure [151]. Fat loss mainly occurs after engaging in aerobic exercises such as jogging, cycling or swimming because of the calory burn [151]. A high amount of lean mass raises the metabolic rate, which enhances the efficacy of fat loss [151]. Thus, it is recommended to perform exercises which engage multiple muscle groups in order to increase overall lean mass [147]. However, energy metabolism, aerobic capacity and muscle gain processes are negatively influenced by age [152, 153]. Being aware of the fact that the individual nutrition is a major coefficient which certainly needs to be considered, though this will not be focused on in this thesis.

3. Research questions and hypotheses

This investigation had multiple objectives; however, the major focus was to depict the influence of severity of haemophilia on BMD and fracture risk. Through examining 255 PwH via DXA-screening, a prevalence rate for osteoporosis and osteopenia within the different severity phenotypes could be displayed and data of the trabecular bone as well as the fracture risk were gathered and evaluated **(Publication I)**. In a second step, the data of the body composition parameters were used in order to provide reference data of PwH for both overall and appendicular lean and fat mass **(Publication II).** Hereby, it was further differentiated between severity phenotypes and age. The third aim was to evaluate on the PA levels within the different haemophilia severities and the interplay of PA and bone quality **(Publication III).** The following section will present the individual research questions and hypotheses.

Publication I

1) *Research question:* What is the prevalence of reduced bone quality (bone mineral density and trabecular bone score) in people with haemophilia?

   Due to the explorative nature of this investigation, no hypothesis can be postulated.

2) *Research question:* Does the bone quality differ between mild, moderate or severe haemophilia?

   Hypothesis: People with severe haemophilia are more often affected by reduced bone quality compared to people with moderate or mild haemophilia.

3) *Research question:* How high is the risk of fractures in a representative haemophilic cohort based on bone quality results?

   Due to the explorative nature of this investigation, no hypothesis can be postulated.

Publication II

4) *Research question:* Is the body composition in German people with haemophilia altered compared to non-haemophilic Europeans with regard to body fat percentage, estimated visceral adipose fat and lean mass?

   Hypothesis: German people with haemophilia exert increased body fat percentage, increased visceral adipose tissue and decreased lean mass compared to non-haemophilic Europeans.

5) *Research question:* Does the body composition with regard to body fat percentage, estimated visceral adipose tissue and lean mass deviate within the haemophilia severity phenotypes?

   Hypothesis: People with severe haemophilia have increased levels of body fat percentage, visceral adipose tissue and show decreased lean mass compared to people with moderate or mild haemophilia. There is a linearity given within the severity phenotypes.

Publication III

6) *Research question*: Do PA levels deviate between the haemophilia severity phenotypes?

   Hypothesis: People with severe haemophilia are less physically active compared with people with moderate or mild haemophilia.

7) *Research question*: How is PA associated with bone quality (bone mineral density and trabecular bone score)?

   Hypothesis: PA positively affects bone quality and lean mass. The higher the activity level, the better the bone quality and the more the lean mass.

8) *Research question*: Does handgrip strength correlate with PA levels, lean mass, and bone quality (bone mineral density and trabecular bone score)?

   Hypothesis: Handgrip strength positively correlates with PA levels, lean mass, and bone quality. A high handgrip strength is seen in people with high PA levels, who show better the bone quality and increased lean mass.

4. Publications

In the following, three publications which have been published in peer-reviewed journals will be presented. The data for **publications I-III** were gathered within one large single-center cohort study, which took place between August 2020 and October 2023. A total of 255 PwH were recruited for this study and analyzed within **publication I.** Though because of compliance and missing data, the sample sizes investigated in **publication II** and **III** were n= 201 and n=223, respectively.

This investigation was named *Osteoporosis and Haemophilia* (Haem-Osteo-Study) and was registered at clinicaltrials.gov (ID: NCT04524481). The primary aim of this study was to depict the prevalence rate of reduced BMD within PwH of different severity phenotypes. Subsequently a comprehensive analysis of musculoskeletal health was aimed via investigating different body composition parameters and the influence of PA on both.

The workplace of this investigation was the orthopedic department in cooperation with the haemophilic care center of the university hospital Bonn. The methodological approach is displayed in Figure 18. The clinical examination in addition to the DXA screening was conducted by three researchers, who received a standardized training in advance. One examination took about 90 minutes.

This study was conducted in accordance with the principles of good clinical and ethical practice and was approved by the local ethic committee (339/19). The Bayer Vital GmbH supported the study financially in the context of an initiator investigated trial. PD. Dr. Andreas Strauss was the initiator and principal investigator of this study.

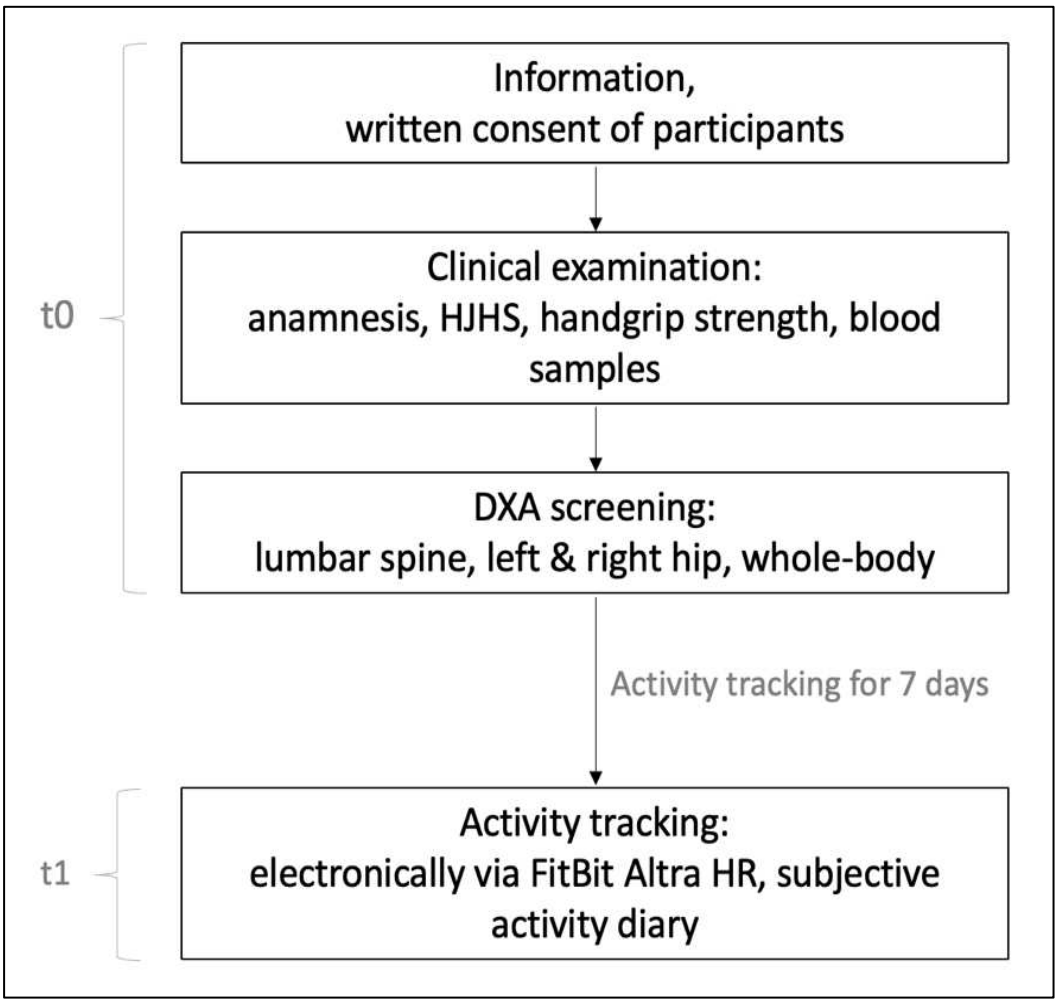

Figure 18: Methodological approach of the study "Osteoporosis screening in adult people with haemophilia and the influence of physical activity on the prevalence of osteoporosis" (author's own illustration)

Explanation: HJHS = haemophilia joint health score (version 2.1), DXA = dual x-ray absorptiometry; DXA-analysis was done using Horizon™ (Hologic, United States of America), t0 = initial clinical examination day, t1 = timepoint after 7 days of activity tracking, when electronic activity tracker and subjective activity diary were returned

In the following section, the three peer-reviewed publications that form the foundation of this dissertation will be presented in detail. Each publication contributes to a comprehensive understanding of bone quality, body composition, or the impact of PA in PwH, highlighting different aspects of the study's overarching research objectives.

Publication I - The influence of severity of haemophilia on bone mineral density and fracture risk

Ransmann P, Hmida J, Brühl M, Schildberg FA, Goldmann G, Oldenburg J, Jaenisch M, Tomschi F, Hilberg T, Strauss AC. (2024) *The influence of severity of haemophilia on bone mineral density and fracture risk.* Research and Practice in Thrombosis and Haemostasis. doi: 10.1016/j.rpth.2024.102624.

Preliminary Remark

Publication I was published in the journal "Research and Practice in Thrombosis and Haemostasis" on November 12th, 2024. The impact factor of the journal "Research and Practice in Thrombosis and Haemostasis" was 3.4 in year 2024.

Original data can be found on the enclosed CD.

Publication I investigated the primary outcome parameter of the large cohort study. Evidence has stated that PwH are frequently affected by low BMD, though data assessing the relationship between severity of haemophilia and occurrence of osteoporosis is lacking. The aim of **publication I** was to depict a prevalence rate of reduced BMD within a large sample size of 255 PwH and to assess the impact of haemophilia severity on BMD as well as to investigate TBS and fracture risk.

In this prospective cohort study, PwH A or B between 18 and 80 years old were included. Based on the analysis of the hip and/or spine, BMD levels were determined. Based on the resulting T- and Z-scores, the subjects were classified into the following categories: osteoporosis (T-score ≤-2.5), osteopenia (T-score ≤-1.0 to -2.4), normal (T-score >-1.0). Subjects younger than 50 years with a Z-score ≤-2.0 were considered below the expected range for age, while above -2.0 PwH are considered as normal. During data analysis, it was observed that some PwH (n=3) had very bright vertebral bodies, potentially due to factors such as sclerosis or vertebral calcification. In such cases, only the hip data was used for further analysis. Both, BMD and FRAX are automatically determined via the Horizon software. To examine the TBS, an additional software (TBS iNsight® (V. 3.1.2. Medi-Maps; Switzerland)) was used.

The key result of **publication I** is that BMD either in form of osteoporosis or osteopenic values is decreased in 63.1% of PwH also depending on the severity of haemophilia. People with severe haemophilia show lower BMD than people with moderate or mild haemophilia. In contrast TBS is predominantly normal within all three severity phenotypes, which suggest that the microarchitecture of the bone does not seem to be affected. The largely healthy status of TBS also affects the risk of fractures: In this study cohort, the FRAX® score is relatively low at 4.4%, which decreases to 2.8% after the TBS-adjustment respectively.

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ORIGINAL ARTICLE

The influence of severity of hemophilia on bone mineral density and fracture risk

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Abstract

Background: Evidence states that persons with hemophilia are frequently affected by low bone mineral density (BMD). Data assessing the relationship between severity of hemophilia and occurrence of osteoporosis are lacking.

Objectives: This prospective cohort study aimed to assess the impact of hemophilia severity on BMD and to investigate trabecular bone score (TBS) and fracture risk (FRAX).

Methods: This prospective cohort study evaluated the BMD, TBS, and FRAX in 255 persons with hemophilia using dual x-ray absorptiometry. The International Society for Clinical Densitometry guidelines were used for classification: osteoporosis (T-score <−2.5), osteopenia (T-score <−1.0), normal (T-score >−1.0). Patients younger than 50 years of age with a Z-score of <−2.0 were considered below the expected range for age.

Results: Of 255 persons with hemophilia (mild: n = 52, moderate: n = 53, severe: n = 150) aged 43 ± 15 years (mean ± SD), 63.1% showed reduced BMD. Even 11.9% of persons with hemophilia aged <50 years were classified as below the expected range for age. Neck BMD decreased linearly with severity (mild: 0.907 ± 0.229, moderate: 0.867 ± 0.131, severe: 0.799 ± 0.143; P = .01). TBS was classified as "normal" in n = 178 (81.3%) with a mean value of 1.403 ± 0.136, and there were no differences between severity levels (P = .54). The FRAX was 4.4% ± 3.0%. After adjustment of TBS, it was 2.8% ± 3.7%.

Conclusion: The present study shows that BMD is decreased in 63.1% of persons with hemophilia also depending on the severity of hemophilia. However, the largely normal TBS implies that the microarchitecture of the bone does not seem to be affected. It is recommended to include osteoporosis screening, including TBS analysis, in the comprehensive diagnostic work-up of persons with hemophilia, especially as they age.

KEYWORDS
bone mineral density, fracture risk, prevalence, severity phenotype, trabecular bone score

Essentials

- Data on the link between hemophilia severity and reduced BMD are lacking.
- Examination including dual x-ray absorptiometry was performed at the University Hospital Bonn.
- This study shows that 63.1% of persons with hemophilia have decreased BMD, linked to hemophilia severity.
- Osteoporosis screening, including the evaluation of trabecular bone, is recommended.

1 | INTRODUCTION

Persons with hemophilia have either a lack of factor (F)VIII (hemophilia A) or FIX (hemophilia B), which can both cause spontaneous bleeding into muscles and joints. Repetitive hemarthroses lead to arthropathy, which is associated with restricted joint function [1]. However, already in 1994, it has been emphasized that next to hemophilic arthropathy, the comorbidity osteoporosis occurs frequently in hemophilic populations [2–4]. Prior literature has shown that coagulation factors FVIII and FIX can also affect the patient's bone mineral density (BMD) [5,6]. Findings of animal research highlighted that mice lacking FVIII show a higher prevalence of decreased BMD [5]. It has further been emphasized that the presence of severe hemarthroses and arthropathy trigger local molecular processes, which have been shown to impact bone metabolism [7,8]. Additionally, persons with hemophilia are often affected by viral comorbidities (HIV, hepatitis), which are suspected to be potential risk factors for the development of osteoporosis [3,9,10]. Although the reduced BMD is often seen in persons with hemophilia, no direct causal relationship, especially considering the severity of hemophilia has been drawn so far [11].

Generally, osteoporosis is a result of an imbalance between bone formation and bone resorption, which causes a reduced BMD. Hereby, aging is a major risk factor for developing osteoporosis. It is a common phenomenon in postmenopausal women due to a deficiency of estrogen [12]. In Europe, about 19.8% of women are diagnosed with osteoporosis, while only 9.7% of men are affected [13]. In the male population, the deficiency of testosterone represents a further key factor in developing osteoporosis [9,14]. The disease is characterized by an increased incidence of fractures as well as deformity of vertebral bodies. However, there are several more factors, such as physical activity, vitamin D status, smoking, or organic comorbidities that need to be considered for investigating osteoporosis [9,15].

To diagnose osteoporosis, the BMD is determined, using dual x-ray absorptiometry (DXA) of the lumbar spine and hip, including risk of fractures based on the Fracture Risk Assessment Tool (FRAX). Moreover, the microarchitecture of the skeletal bones can be measured using trabecular bone score (TBS), predicting fracture risk independently of the BMD and providing information on the overall bone quality. It has been found that patients with lower TBS have an increased risk for fractures [16]. However, only little research has been done investigating TBS and hemophilia [17].

In general, different studies and meta-analyses describe an association, especially between severe hemophilia and low bone mineral density [2,3,11,18,19]. Still, data assessing the relationship between severity of hemophilia and occurrence of osteoporosis is lacking [20,21]. Thus, this study aimed to depict the prevalence of osteoporosis as well as FRAX and TBS in a representative sample size of 250 patients with either mild (n = 50), moderate (n = 50), or severe (n = 150) hemophilia A or B.

2 | METHODS

2.1 | Study design and participants

This study was conducted as a single-center prospective cohort study at the University Hospital Bonn. Adult male patients suffering from mild, moderate, or severe hemophilia A or B were included. Patients who experienced joint bleeding in the past 2 weeks were excluded from this investigation as the Haemophilia Joint Health Score (HJHS) manual demands [22]. This study was conducted in accordance with the principles of good clinical and ethical practice and after approval by the local ethics committee (339/19). Along with the Declaration of Helsinki, all participants gave written informed consent after being informed about the study's protocol. The study was registered at clinicaltrials.gov (ID: NCT04524481).

2.2 | Data acquisition

In accordance with the German guidelines for osteoporosis, all patients underwent densitometry using Horizon (Hologic) of the spine (vertebrae bodies 1-4) and both femoral necks [15]. Both exact values of BMD (g/cm^2) and T-scores (standard deviation of average BMD of healthy young (aged 30 years) population of the same ethnicity) were gathered and automatically calculated by the Horizon software. According to the World Health Organization, the lowest T-score of either femoral neck or spine (sum of vertebrae bodies 1-4), assessed with DXA, was used for classification into the following categories: normal (T-score, ≥−1.0), osteopenia (T-score range, −1.0 to −2.4) or osteoporosis (T-score ≤−2.5) [15,23]. Patients younger than 50 years, having a Z-score <−2.0 are considered as "below expected range for age" [24]. The Z-score

compares the BMD results with the average BMD of people of the same age. In addition, based on BMD results and individual data on medical and family history, the FRAX is calculated by the Horizon software. This algorithm depicts a 10-year probability of experiencing fractures in patients aged ≥30 years. However, the score has been validated in patients aged >40 years [25].

TBS was calculated using TBS iNsight (V. 3.1.2. Medi-Maps). This score expresses the homogeneity bone microarchitecture of the lumbar spine and can, in combination with BMD results, provide an additional and more sensitive fracture risk estimate [26,27]. It is recommended to use the score for subjects with a body mass index of <37 kg/m^2 [28,29]. Next to a densitometry, relevant blood parameters (vitamin D, calcium, testosterone) were examined to both capture risk factors for osteoporosis and to differentiate from secondary osteoporosis or further morbidities (ie, osteomalacia or malignancies) [15,30]. The serum blood samples were determined in the central laboratory of the University Hospital Bonn.

Supplementary anamnesis including medical history and any pharmacological treatment regime was collected. To objectively evaluate patients' daily physical activity level, the patients were given an electronic activity tracker (Fitbit Alta Hr, Fitbit Inc) that was worn at the wrist for 7 consecutive days after clinical assessment. The total number of steps taken within a week was used for further analysis.

Orthopedic joint status was assessed using the HJHS (version 2.1; maximum score 124 points, 20 points × 6 joints, plus 4 points assigned to global gait), which assesses the elbows, knees, and ankles in regard to swelling, muscle atrophy, crepitation, stability, and range of motion. High values indicate a worse clinical joint status. The recruitment of persons with hemophilia and their examination was conducted by 3 research fellows (J.H., M.B., P.R.) between September 2019 and October 2022.

2.3 | Statistics

Statistical evaluation was performed to analyze the prevalence of osteopenia and osteoporosis within the different hemophilia severities. The IBM Statistical Package for the Social Sciences 29 (IBM) for Mac was used for calculations.

Given nonparametric data, a Kruskal–Wallis test compared the means between the hemophilia severities. In case of significant differences, Bonferroni correction was used to account for multiple comparisons for post-hoc testing. Multiple linear regression analysis was calculated to examine potential predictors (severity phenotype, age, presence of viral comorbidities, smoking, physical activity, HJHS) of BMD (left femoral neck). A general significance level of $P = .05$ (95% CI) was established.

3 | RESULTS

In total, 260 patients were recruited. Data from 255 patients, aged 43.4 ± 15.4 (mean ± SD) years (median = 42, range: 18-79) with either mild ($n = 52$), moderate ($n = 53$) or severe ($n = 155$) hemophilia A or B was analyzed (Table 1). Five patients with severe hemophilia dropped out due to unusable data of the DXA scan. Patients were either treated on-demand (35.7%) or with prophylactic treatment (64.3%). Details on the treatment regimen, of the patients treated on prophylaxis are shown in Table 2.

The data of the BMD values of the entire study group and for the different hemophilia severity grades separately shown in Table 3. Comparing BMD values within the 3 severities, the Kruskal–Wallis test revealed that in both, left and right femoral neck, a significant difference was found between patients with mild and severe hemophilia. Bonferroni post-hoc analysis showed that patients with severe hemophilia have lower BMD values in the femoral necks than patients with mild hemophilia (left: $P = .008$, right: $P = .004$). There was no significance between moderate and mild or moderate and severe hemophilia. Although a tendency of descending BMD scores within the severities is seen, BMD values at the spine do not differ significantly between the severities ($P = .22$). Considering the T/Z-scores, a significant difference between the severity phenotypes can be seen revealing that patients with severe hemophilia have a lower score than patients with mild hemophilia (T-score: $P = .04$, Z-score: $P = .008$).

Since the T-score is used in patients aged >50 years only, the sample size was divided into 2 groups. Persons with hemophilia aged >50 years ($N = 104$), who are therefore categorized as "normal," "osteopenia," or "osteoporosis," out of which 19.2% ($n = 20/104$) were diagnosed with osteoporosis and 53.8% ($n = 56/104$) with osteopenia. And $n = 151$ persons with hemophilia, younger than 50 years who are classified as either "normal" or "below expected range for age" (Figure 1). Here, 11.9% ($n = 18/151$) of patients were classified as "below expected range for age." Within the whole study cohort, 36.9% were classified as "normal" based on the Z/T-score analyses.

As seen in Table 2, 14 patients are currently affected by inhibitors. Regarding their BMD values, it can be seen that, 1 showed is classified as "normal", while 10 are considered "osteopenic" and 3 "osteoporotic."

Furthermore, 10% ($n = 25/255$) of the cohort showed a calcium-deficiency (<2.2 mmol/L), while 19.2% ($n = 49/255$) had a vitamin D deficiency (<12 ng/mL). There was no significant difference of calcium or vitamin D levels between the severities. Moreover, 5.1% ($n = 13$) of patients were suspected to be affected by secondary osteoporosis given the intake of cortisone, antiepileptics, or the presence of diseases, such as hypogonadism.

A multiple linear regression was conducted to examine the influence of the following parameter on BMD: severity phenotype, age, HJHS, HIV, hepatitis C, smoking, and physical activity. Linear regression analysis reveals that age (B= −0.004; 95% CI: −0.005, −0.002; $P < .001$) predicts the BMD (left femoral neck) best, followed by severity phenotype (B= −0.048; 95% CI: −0.08, −0.02; $P = .001$) and HJHS (B= −0.002; 95% CI: −0.004, −0.001; $P = .02$). The presence of viral comorbidities (HIV, hepatitis C), smoking, or physical activity does not seem to predict the BMD ($P > .05$; see Supplementary Table).

Emphasizing the influence of age, it needs to be highlighted that 32 patients younger than 25 years old were included in this study.

TABLE 1 Anthropometric and descriptive data of persons with hemophilia included in this study.

Variables	Severe (*n* = 150)	Moderate (*n* = 53)	Mild (*n* = 52)	Total (*n* = 255)	*P* value
Age (y)					.16
Mean ± SD	41.7 ± 14.6	46.3 ± 15.2	45.3 ± 17.5	43.4 ± 15.4	
Median (IQ1, IQ3)	40 (29.0, 54.0)	49.0 (31.5, 58.5)	47.5 (29.2, 58.0)	42.0 (30.0, 56.0)	
Weight (kg)					.15
Mean ± SD	84.9 ± 18.5	89.3 ± 17.6	86.4 ± 13.9	86.1 ± 17.5	
Median (IQ1, IQ3)	81.0 (73.7, 91.0)	86.0 (78.0, 95.0)	83.5 (78.0, 93.7)	83.0 (75.0, 93.0)	
Height (m)					.34
Mean ± SD	1.80 ± 0.1	1.82 ± 0.1	1.79 ± 0.1	1.80 ± 0.1	
Median (IQ1, IQ3)	1.80 (1.75, 1.84)	1.80 (1.76, 1.89)	1.80 (1.76, 1.84)	1.80 (1.76, 1.85)	
BMI (kg/m^2)					.34
Mean ± SD	26.2 ± 5.2	26.7 ± 5.0	26.8 ± 4.4	26.4 ± 5.0	
Median (IQ1, IQ3)	25.2 (23.4, 27.8)	25.8 (23.4, 28.2)	26.0 (23.9, 28.9)	25.5 (23.5, 28.0)	
Caucasian ethnicity, *n* (%)	150 (100)	53 (100)	52 (100)	255 (100)	n/a
Hemophilia A, *n* (%)	134 (89.3)	42 (79.2)	45 (86.5)	221 (86.7)	n/a
Hemophilia B, *n* (%)	16 (10.7)	11 (20.8)	7 (13.7)	34 (13.3)	n/a
HIV, *n* (%)	32 (21.3)	3 (5.7)	3 (5.8)	38 (14.9)	n/a
Hepatitis C, *n* (%)	17 (11.3)	2 (3.8)	1 (2.0)	20 (4.6)	n/a
HJHS (score points)					<.001[a]
Mean ± SD	22.7 ± 18.7[a]	10.0 ± 8.2[a]	9.9 ± 9.4[a]	17.5 ± 16.6	
Median (IQ1, IQ3)	18.5 (6.0, 35.2)	7.0 (5.0, 13.5)	7.0 (4.2, 13.0)	11.0 (5.0, 28.0)	
Steps per day[b]					.16
Mean ± SD	7509 ± 3851	8653 ± 4246	8446 ± 3764	7934 ± 3935	
Median (IQ1, IQ3)	7095 (4757, 9801)	7049 (5530, 11472)	8466 (5219, 10785)	7392 (4981, 10579)	
Vitamin D level (ng/mL)					.55
Mean ± SD	24.2 ± 13.8	22.0 ± 9.7	22.0 ± 11.3	23.3 ± 12.6	
Median (IQ1, IQ3)	23.6 (13.4, 31.6)	22.1 (13.1, 29.5)	19.9 (14.6, 28.2)	23.0 (13.7, 30.4)	
Calcium level (mmol/L)					.08
Mean ± SD	2.32 ± 0.11	2.29 ± 0.08	2.30 ± 0.08	2.31 ± 0.10	
Median (IQ1, IQ3)	2.33 (2.26, 2.37)	2.28 (2.24, 2.43)	2.29 (2.23, 2.37)	2.31 (2.25, 2.37)	
Smoking daily, *n* (%)	45 (31.0)	11 (20.8)	12 (23.1)	68 (27.2)	n/a
Alcohol intake,[c] *n* (%)	28 (18.7)	11 (20.8)	16 (30.8)	55 (22.0)	n/a

BMI, body mass index; HJHS, Haemophilia Joint Health Score (v2.1); n/a, not applicable.
[a]Indicates significant difference at HJHS: severe-mild: $P < .001$, severe-moderate: $P < .001$.
[b]Steps per week, steps taken in a week measured by an electronic activity tracker (FitBit Altra HR) for 7 days.
[c]Alcohol intake indicates regular alcohol intake.

A Z-score <−2.0 was present in 18.8% (*n* = 6/32) of young persons with hemophilia out of which 5 patients had severe and one from moderate hemophilia.

Moreover, the TBS was analyzed in persons with hemophilia with a body mass index of <37 kg/m^2 (*n* = 219). *N* = 178 showed normal values (TBS >1.31; Figure 2). Mean TBS was 1.403 ± 0.136. TBS decreased significantly with age ($P < .001$), although no significant difference was observed between the hemophilia severities ($P = .54$).

Along with BMD analysis, the FRAX was calculated in patients aged ≥30 years (*n* = 186). The mean risk of having a fracture in the next 10 years was 4.4% ± 3.0%. After TBS adjustment, the FRAX decreased to 2.8 ± 3.7%. As FRAX is validated in patients aged >40

TABLE 2 Details of replacement treatment regimen of included subjects.

Variables		Severe (*n* = 150)	Moderate (*n* = 53)	Mild (*n* = 52)	Total (*n* = 255)
Treatment regimen	**Prophylaxis,** *n* (%)	146 (97.3)	17 (32.1)	1 (1.9)	164 (64.3)
	On-demand, *n* (%)	4 (2.7)	36 (67.9)	51 (98.1)	91 (35.7)
Development of inhibitor	**Current presence of inhibitor,** *n* (%)	11 (7.3)	3 (5.7)	0 (0.0)	14 (5.5)
	Inhibitors successfully treated, *n* (%)	25 (16.7)	1 (1.9)	2 (3.8)	28 (11.0)
	Never history of inhibitors, *n* (%)	114 (76.0)	49 (92.4)	50 (96.2)	213 (83.5)
Patients on prophylactic treatment	**Factor consumption per week (IU),** mean ± SD	7234 ± 4670	5906 ± 3688	12000	7130 ± 4589
	median (IQ1, IQ3)	6000 (4000, 9000)	6000 (2125, 8750)		6000 (4000, 9000)
	Extended half-life, *n* (%)	108 (77.7)	17 (100)	1 (100)	126 (80.3)
	Standard half-life, *n* (%)	24 (17.3)	–	–	24 (15.3)
	Nonfactor replacement, *n* (%)	7 (5.0)	–	–	7 (4.5)

years, a subanalysis differentiating in persons with hemophilia aged between 30 and 40 years (*n* = 69) and persons with hemophilia older than 40 years (*n* = 117), was performed. In patients aged >40 years, the FRAX was 4.8% ± 3.4% and decreased to 3.6% ± 4.2% after TBS adjustment. In patients aged between 30 and 40 years FRAX was 3.7% ± 2.0% and after TBS adjustment 1.5% ± 2.1%.

4 | DISCUSSION

This is the first study to exploratively analyze a representative sample of 255 people with hemophilia of all severities with regard to the prevalence of osteoporosis: 19.2% (95% CI: 12.4-27.0) of persons with hemophilia aged >50 years had osteoporosis and 11.9% (95% CI: 7.1-

TABLE 3 Overview of bone mineral density within the hemophilia severities.

Variables	Severe (*n* = 150)	Moderate (*n* = 53)	Mild (*n* = 52)	Total (*n* = 255)	*P* value
BMD femoral neck left (g/cm^2)					.01[a]
Mean ± SD	0.799 ± 0.143[a]	0.826 ± 0.131	0.907 ± 0.229[a]	0.828 ± 0.167	
median (IQ1, IQ3)	0.779 (0.696, 0.896)	0.807 (0.727, 0.933)	0.871 (0.748, 1.002)	0.807 (0.711, 0.929)	
BMD femoral neck right (g/cm^2)					.005[a]
Mean ± SD	0.807 ± 0.148[a]	0.830 ± 0.147	0.906 ± 0.180[a]	0.831 ± 0.159	
Median (IQ1, IQ3)	0.802 (0.703, 0.896)	0.826 (0.735, 0.916)	0.863 (0.756, 1.038)	0.821 (0.720, 0.923)	
BMD lumbar spine (g/cm^2)					.22
Mean ± SD	0.986 ± 0.126	1.013 ± 0.146	1.021 ± 0.150	0.999 ± 0.136	
Median (IQ1, IQ3)	0.990 (0.904, 1.07)	1.009 (0.913, 1.100)	1.040 (0.931, 1.086)	1.005 (0.905, 1.077)	
Lowest T-score					.04[a]
Mean ± SD	−1.8 ± 0.9[a]	−1.4 ± 1.0	−1.0 ± 1.2[a]	−1.5 ± 1.0	
Median (IQ1, IQ3)	−1.8 (−2.4,−1.1)	−1.5 (−2.1, −0.4)	−1.2 (−2.0, −0.4)	−1.6 (−2.2, −0.9)	
Lowest Z-score					.006[a]
Mean ± SD	−0.9 ± 0.8[a]	−0.8 ± 1.0	−0.5 ± 1.1[a]	0.8 ± 0.9	
Median (IQ1, IQ3)	−0.9 (−1.6, −0.4)	−0.7 (−1.3, −0.5)	−0.5 (−1.2, −0.1)	−0.9 (−1.5, −0.2)	

Mean and standard deviation displayed; Kruskal–Wallis test was conducted to assess mean differences between the severities. Sample sizes differ based on age groups (T-score [persons with hemophilia aged >50 years]: severe, *n* = 54; moderate, *n* = 24; mild, *n* = 26; total, *n* = 104; Z-score [persons with hemophilia aged <50 years]: severe, *n* = 96; moderate, *n* = 29; mild, *n* = 26; total, *n* = 151).

BMD, bone mineral density.

[a]Indicates significant difference at BMD neck left: severe-mild, *P* = .008; BMD neck right: severe-mild, *P* = .004; lowest T-score: severe-mild, *P* = .04; lowest Z-score: severe-mild, *P* = .008.

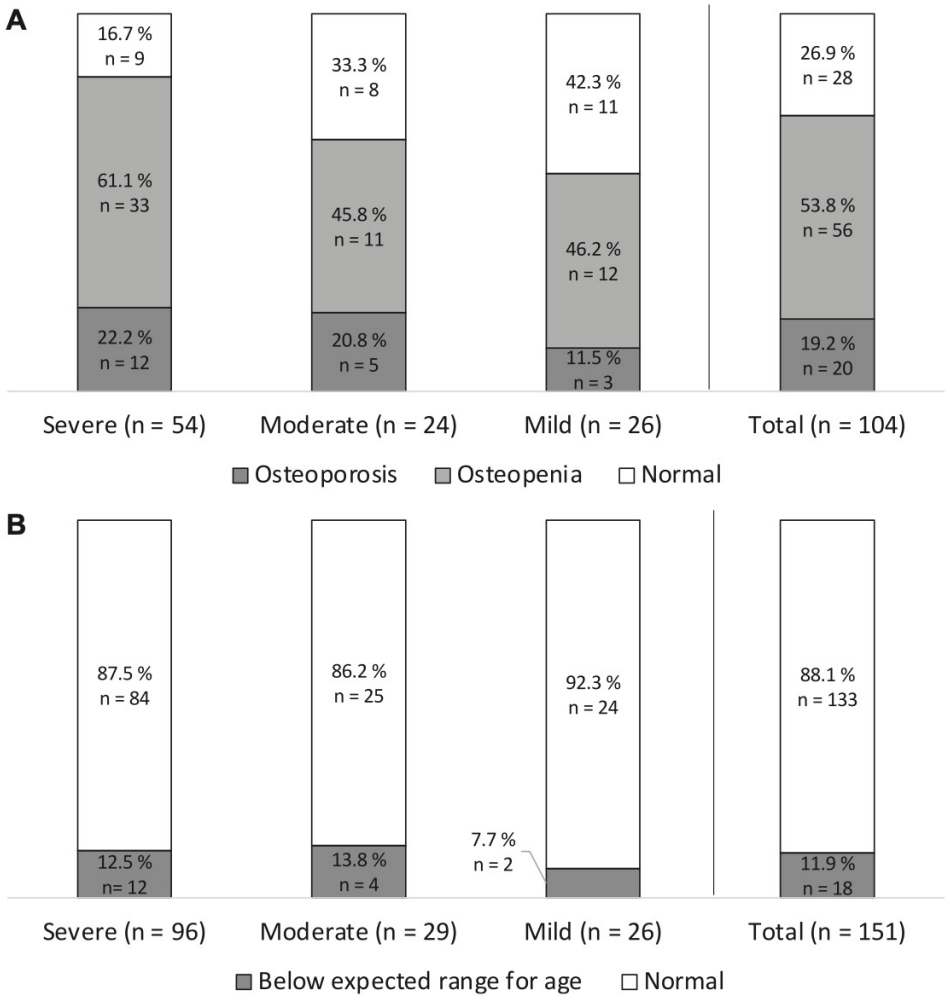

FIGURE 1 Classification of osteoporosis within the different severities of hemophilia.

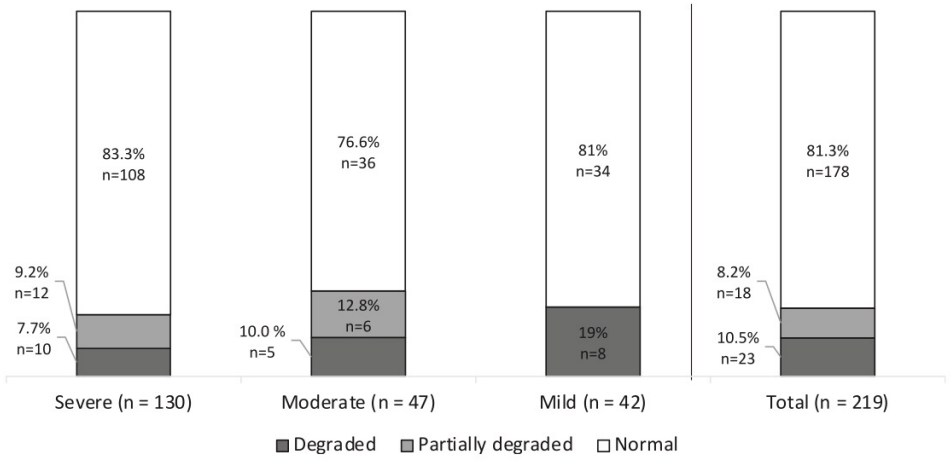

FIGURE 2 Trabecular bone scores within different severities of hemophilia.

17.1) of persons with hemophilia aged <50 years have a BMD below the expected range for their age. Literature provides prevalence rates of the average male population in Europe of 9.7% (95% CI: 4.4-18.5), further specified 2.4% (no 95% CI given) in men aged 50 to 60 years, although no data are present for men aged <50 years [13,15].

Low BMD was observed in both femoral necks and the lumbar spine, with the femoral necks being more affected than the lumbar spine. These results and a general discrepancy of BMD in these 2 body areas are consistent with previous research in persons with and without hemophilia [17,31,32]. In this study the lumbar spine shows a tendency to have higher BMD values. One reason could be due to methodological measurement errors of the DXA such as falsely high t-values due to degenerative sclerosis of lumbar vertebral bodies.

The present data show significant differences between the prevalence of osteoporosis in dependence with hemophilia severity. Patients with mild hemophilia seem to be less affected by reduction of BMD. Within literature several approaches deal with explaining this observation. Recent animal research revealed a correlation of FVIII or FIX deficiency and reduction of BMD [5,6]. This finding can be supported by the present observation of an increased prevalence of patients with osteopenia within the subgroup of patients with current positive inhibitor status. However, the sample size of 14 persons with hemophilia within the present study cohort is too small to draw further conclusions. The impact of inhibitors and history of inhibitors should be investigated further to examine the influence of FVIII and FIX on bone health. Moreover, not only inhibitor status but also the factor level, considering precise pharmacokinetic data of the individual should be focused on in future studies.

Previous literature highlighted the relation of a poor joint status and low BMD in persons with hemophilia [17,19,33] and these findings can be confirmed by the present results. Generally, patients with mild hemophilia show a better joint status and less affection of hemophilic arthropathy than patients with moderate or severe hemophilia. Through synovial tissue, it has been shown, that persons with hemophilic arthropathy show higher RANK and RANKL and decreased osteoprotegerin than healthy controls [7]. These molecular markers control bone turnover and cause an increase of osteoclastic activation, leading to a reduction in BMD [7,34]. Even in young persons with hemophilia, who are affected by reduction of BMD, increased serum levels of RANKL and decreased osteoprotegerin were overserved [20,35]. Within the present study cohort, 18.8% (n = 6/32) of young patients (aged <25 years) are "below expected range for age." Previous studies that investigated the relationship of young persons with hemophilia and their fracture incidence, pointed toward an increased fracture incidence compared with healthy control subjects [36].

Noteworthy, although BMD scores are reduced in persons with hemophilia, TBS is largely normal and thus the microarchitecture of the bones does not seem to be affected. Compared with the German population, which shows an average FRAX of 7.2% in males aged ≥50 years, this study cohort shows rather low FRAX of 4.4% and a TBS-adjusted FRAX of 2.8%, respectively [37]. Meaning that despite the prevalence of osteoporosis was increased in this cohort of persons with hemophilia, yet FRAX was lower than in the normal population. This is in line with observations from hemophilia care centers, which report that persons with hemophilia rarely experience fractures [38]. It appears that, aside from low BMD, persons with hemophilia do not present many other risk factors for fractures. However, given the innovative nature of TBS analysis, future research is needed in study cohorts of both persons with and without hemophilia to prove the significance and generalizability of the TBS.

Multiple linear regression analysis revealed further predictors for BMD (left femoral neck). It was shown that the presence of viral comorbidities does not seem to impact the BMD. It needs to be emphasized that the sample size of the investigated persons with hemophilia affected by HIV (n = 34) or hepatitis C (n = 20) is rather small. Hence, the evidence of this analysis is limited. Previous research revealed a correlation between viral comorbidities and reduced BMD, although there is no consensus in literature [2,39,40]. Despite the decline of viral comorbidities in persons with hemophilia, clinicians should be aware of the possible correlation to control for osteoporosis in patients who have viral comorbidities. No significant correlations were found between physical activity and BMD. With use of an objective activity tracker, an insight into daily physical activity level over a period of 7 days was gathered. It needs to be emphasized that the strain triggered by walking on the bone might not be enough to influence bone metabolism [41]. Nonetheless, an active lifestyle can prevent the reduction of BMD. Emphasizing that previous research found that the duration and intensity of physical activity only play a minor role, although the type of activity is decisive [42].

Moreover, this study revealed a vitamin D deficiency in 19.2% of persons with hemophilia. In comparison, the male adult German population shows a vitamin D deficiency prevalence of 30.8% [30]. A limitation of this study is that subjective information on vitamin D supplementation based on the anamnesis questionnaire lacks in details. Hence, the expressiveness of the information on the level of vitamin D is limited and is assumed to be lower than the present results reveal. Examining vitamin D status and a respective supplementation is recommended [9].

5 | CONCLUSION

The present study found that 63.1% of persons with hemophilia have decreased BMD, either in the form of osteoporosis, osteopenia, or "below expected range for age," depending on the severity of hemophilia. The orthopedic joint status is directly associated with lower BMD. However, the largely normal TBS indicates that the microarchitecture of the bone does not appear to be affected. Accordingly, a TBS adjustment reduces FRAX by a delta of 1.6%. These findings should be considered in clinical routine to ensure best care and to protect persons with hemophilia from the consequences of undiagnosed osteoporosis, such as fracture risk, reduced mobility, and spine misalignments. In particular, persons with hemophilia are at higher risk of developing osteoporosis, especially with increasing age, calling for action to include screening for osteoporosis in clinical routine at age 30 and older, including vitamin D supplementation if necessary.

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AUTHOR CONTRIBUTIONS

P.R., J.H., and M.B. generated and analyzed the data. F.S., M.J., and F.T. contributed scientifically to the study. G.G. and J.O. supported with the participant recruitment process. T.H. supervised the project. A.S. supervised and developed the project.

RELATIONSHIP DISCLOSURE

P.R. received speakers' fees and travel support from Takeda Pharmaceuticals as well as travel support from Swedish Orphan Biovitrum GmbH. M.B. revived travel support from Takeda Pharmaceuticals as well as travel support from Swedish Orphan Biovitrum GmbH. F.T. received speaker's fees and travel support from Takeda and received an educational grant from Swedish Orphan Biovitrum GmbH. M.J. received speakers fee from Implantcast GmbH and a research grant from Peter Brehm GmbH. G.G. has received funding for lectures by Swedish Orphan Biovitrum GmbH, Takeda, Bayer, Octapharma, Novo Nordisk, Biotest, Roche, BPL, LFB, and further support for attending meetings by Swedish Orphan Biovitrum GmbH, Novo Nordisk, and Biotest. T.H. received research funding from Biotest, Chugai, CSL Behring, Intersero, Roche, Swedish Orphan Biovitrum, and Takeda. Travel expenses, speaker or scientific advisory board honoraria from Bayer, Biotest, CSL Behring, Chugai, NovoNordisk, Pfizer, Roche, Sanofi, Swedish Orphan Biovitrum, and Takeda. J.O. has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphao Biovitrum, and Takeda. Consultancy, speaker's bureau, honoraria, scientific advisory board, and/or travel expenses from Bayer, Biogen Idee, BioMarin, Biotest, Chugai, CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. A.S. has received research funding from Bayer, Swedish Orphan Biovitrum, and Takeda and has received consultancy, speaker's bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biotest, CSL Behring, Novo Nordisk, Swedish Orphan Biovitrum, and Takeda. J.H. and F.S. have no competing interests to declare.

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SUPPLEMENTARY MATERIAL

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Publication II - Determination of body composition by dual x-ray absorptiometry in persons with haemophilia

Ransmann P, Brühl M, Hmida J, Goldmann G, Oldenburg J, Strauss AC, Hagedorn T, Schildberg FA, Hilberg T, Strauss AC. (2024) *Determination of body composition by dual x-ray absorptiometry in persons with haemophilia*. Haemophilia. doi: 10.1111/hae.15091

Preliminary Remark

Publication II was published in the journal “Haemophilia” on August 13th, 2024. The impact factor of the journal “Haemophilia” was 3.0 in year 2023.

Original data can be found on the enclosed CD.

As previously stated, research lacks on investigating body composition in PwH, though there is some evidence that describes an increased body fat distribution and decreased lean mass in PwH compared to healthy controls using bioimpedance analysis. Using DXA, **publication II** aimed to postulate reference data for body composition parameters within haemophilia severity phenotypes and age groups. The included PwH underwent whole body DXA screening using Horizon™. Body fat percentage, VAT, appendicular fat and lean mass, and lean and fat mass in relation to body height were assessed. A further distinction between the three haemophilia severity phenotypes and five age groups (18-29, 30-39, 40-49, 50-59, 60-80 years) was conducted.

Due to technical or personal reasons, not all subjects, who were recruited and examined for osteoporosis-screening in **publication I** (n=255) underwent the whole-body DXA-screening. Hence, in **publication II** a total of 201 persons with mild (n=44), moderate (n=41), or severe (n=116) haemophilia A/B (median age 40 [28-55; 1.IQ-3.IQ] years) were analyzed. The included people were all Caucasian men, so that data cannot be transferred to other ethnicities since body composition diverges between nations [116].

Overall, this study provides valuable reference data for body composition parameters in PwH. One key result is that people with severe haemophilia show

significantly less lean mass compared to people with moderate or mild haemophilia. In contrast, data on body fat percentage and VAT did not differ between severity phenotypes but increased with age. Compared to the general Caucasian population, these fat-related parameters are meaningly lower in PwH.

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ORIGINAL ARTICLE

Determination of body composition by dual x-ray absorptiometry in persons with haemophilia

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Abstract

Background: There is limited research on body composition in persons with haemophilia (PwH). The literature describes an increased body fat distribution and decreased lean mass in PwH compared to healthy controls using bioimpedance analysis. Using dual x-ray absorptiometry (DXA), which is known to be the most accurate method, this investigation aims to postulate reference data for body composition parameters within haemophilia severity phenotypes and age groups.

Methods: Persons underwent whole body DXA screening using Horizon. Body fat percentage, estimated visceral adipose tissue (VAT), appendicular fat and lean mass, and lean and fat mass in relation to body height were assessed. Haemophilia severity and five age groups were distinguished.

Results: Two hundred and one persons with mild ($n = 44$), moderate ($n = 41$), or severe ($n = 116$) haemophilia A/B (median age 40 [28–55; 1.IQ–3.IQ] years) were analysed. The median body fat percentage was 28.7% [25.5%–33.9%] and median estimated VAT was 657 g [403–954 g] with no significant difference between severity phenotypes ($p = .474$; $p = .781$). Persons with severe haemophilia had less lean mass compared to moderate and mild haemophilia ($p = .013$; $p = .034$). Total and appendicular fat is increased in older PwH (aged ≥40 years) compared to younger PwH (aged ≤29 years; $p < .05$). Lean mass did not differ between age groups.

Conclusion: This study provides valuable reference data for body composition parameters in PwH. Persons with severe haemophilia show significantly less lean mass compared to persons with moderate or mild haemophilia. Body fat percentage and VAT did not differ between severity phenotypes, but increased with age.

KEYWORDS
BMI, body composition, dual x-ray absorptiometry, haemophilia, severity phenotype

1 | INTRODUCTION

Persons with haemophilia (PwH) are known to be affected by arthropathy due to joint bleeding, which is more common in persons with severe haemophilia than in persons with moderate or mild hemophilia.[1–3] Many studies evaluated arthropathy in haemophilia since this is the persons' main restriction going along with pain, loss of function and decrease of quality of life.[4–6] These symptoms frequently add up to a reduction of physical activity, which in turn has a negative impact on, among others, the cardiometabolic system.[7,8] Due to the reduction of joint function, relieving postures or intramuscular haemorrhage, arthropathy frequently goes along with muscle atrophy.[9] Therefore, it is assumed that PwH, especially those affected by severe haemophilia show altered body composition compared to subjects without bleeding disorders. There are only few studies dealing with body composition in PwH using bioimpedance analysis, revealing that PwH show increased fat and decreased leg muscle mass compared to healthy controls.[8,10–13] It has been shown, that the overall prevalence of obesity is increased in haemophilic populations. However, this depends to a certain extent on the country of origin and is not transferable to the general population.[7,14,15] Both obesity and reduced muscle mass are linked to increased risk for cardiometabolic diseases or insulin resistance for instance.[16] The risk profile is further dependent on other contributing body composition parameters as in trunk accentuated (android) fat distribution as well as body fat percentage in relation to height.[17] To get an insight into these parameters different tools can be used, such as calipometry, bioimpedance, infrared analysis or dual x-ray absorptiometry (DXA), of which DXA is considered the gold standard regarding body composition analysis.[18,19] In general, body composition analysis is used for both physiological rationales, for example, to examine elite athletes but also to investigate pathological objectives in different patient cohorts. Among others, it provides estimated values for an individual's body fat percentage, visceral adipose tissue (VAT), or lean mass as surrogate measure of skeletal muscle.[20] Literature provides parameters for both the whole body and different subregions, that is, extremities and trunk, which are yet to be described in PwH.[21–23]

This explorative study aims to provide severity- and age-related reference data of body composition parameters for PwH. By using DXA, data of body fat and lean mass for both overall body and subregions are provided, which might enhance patient care as comprehensive understanding of body composition can help tailoring treatment plans, nutritional advice, and physical therapy programs.

2 | METHODS

2.1 | Study design and participants

This investigation was part of a large prospective single-centre cohort study examining the relation between haemophilia and osteoporosis (registered at clinicaltrials.gov (ID: NCT04524481)). Data were gathered between 2019 and 2022 at the University Hospital Bonn. Adult Caucasian male persons with either mild, moderate, or severe haemophilia A or B were included. Persons with other bleeding disorders or younger than 18 years were excluded from this investigation. This study was conducted in accordance with the principles of good clinical and ethical practice and was approved by the local ethic committee (339/19). Along with the Declaration of Helsinki, all participants gave written informed consent.

2.2 | Data acquisition

Persons underwent a 7-min whole body DXA screening. This assessment was conducted and analysed using Horizon (Hologic, USA; Apex Software; Auto Whole-Body V. 13.6.7). Fat and lean mass in relation to body size (kg/m^2) as well as body fat percentage (percentage of lipids) were measured for the whole body. the software analysis estimated VAT in grams and evaluated android and gynoid fat distribution.[24] Android fat is considered as trunk accentuated fat, while gynoid fat accumulates around the hips, breasts and thighs. It also calculated the android/gynoid ratio. Additionally, the four extremities are also examined regarding the total amount fat and lean mass, displayed in grams. The BMI was calculated (kg/m^2) and classified according to the World Health Organization.[25] Joint status was examined using the Haemophilia Joint Health Score (HJHS, Version 2.1; maximum score 124 points, 20 points × 6 joints, plus 4 points assigned to global gait; one joint can reach 0–20 points; higher values indicating worse joint status). Anamnesis including medical history and pharmacological treatment regime was assessed.

2.3 | Statistics

The IBM© Statistical Package for Social Sciences 29 (Armonk, NY, USA) was used for calculations. A significance level of $p \leq .05$ (95% confidence interval (95%-CI)) was established. A descriptive analysis of the body composition data was conducted. The body composition data were analysed by severity and independently by age group (18–29, 30–39, 40–49, 50–59 and 60–80). Data were not normally distributed so that the dependent variables were compared within the three different severities as well as within the five age groups using the Kruskal–Wallis-test. In case of significant differences, Bonferroni correction was used for post hoc testing to account for multiple comparisons.

3 | RESULTS

Two hundred and ten participants were recruited for this study. Due to technical issues or personal reasons, data of 201 persons with either mild ($n = 44$), moderate ($n = 41$) or severe ($n = 116$) haemophilia A or B with a median age of 40[28–55] years were analysed. Persons with severe haemophilia had a significantly worse joint status with a mean HJHS of 21.6 ± 18.6 compared to persons with moderate (9.6 ± 8.8, $p = .001$) or mild (10.0 ± 10.2, $p < .001$) haemophilia. The most affected

TABLE 1 Characteristics of included persons with haemophilia.

Variables		Total ($n = 201$)	Severe ($n = 116$)	Moderate ($n = 41$)	Mild ($n = 44$)	p-value
Age (years) M ± SD Median [IQ25–75]		43.2 ± 15.5 40 [28–55]	40.3 ± 14.4 37 [27–52]	43.9 ± 15.1 45 [29–57]	44.7 ± 18.3 45 [28–60]	.298
Weight (kg) M ± SD Median [IQ25–75]		85.3 ± 15.9 83 [76–92]	84.6 ± 17.6 81 [75–90]	87.7 ± 15.5 86 [77–95]	85.0 ± 10.3 83 [78–90]	.304
Height (m) M ± SD Median [IQ25–75]		1.80 ± 0.06 1.80 [1.76–1.85]	1.80 ± 0.06 1.80 [1.76–1.85]	1.81 ± 0.07 1.80 [1.76–1.87]	1.79 ± 0.06 1.80 [1.76–1.84]	.829
Haemophilia A (n[%])		175 (87.1)	104 (89.7)	33 (80.5)	38 (86.4)	n/a
Haemophilia B (n[%])		26 (12.9)	12 (10.3)	8 (19.5)	6 (13.6)	n/a
Treatment regime	Prophylaxis (n[%])	122 (60.7)	109 (94.0)	12 (29.3)	1 (2.3)	n/a
	On-demand (n[%])	68 (33.8)	3 (2.6)	25 (61.0)	40 (93.0)	
	Missing (n[%])	11 (5.5)	4 (3.4)	4 (9.8)	3 (6.8)	
HIV (n[%])		27 (13.4)	22 (19.0)	2 (5.1)	3 (7.0)	n/a
Hepatitis C (n[%])		20 (10.0)	15 (12.9)	4 (10.3)	1 (2.3)	n/a
HJHS (score) M ± SD Median [IQ25–75]		16.7 ± 16.5 10.0 [5.0–27.5]	21.6 ± 18.6[a] 18 [5.0–33.0]	9.6 ± 8.8[a] 7.0 [4.0–11.0]	10.0 ± 10.2[a] 6.0 [4.0–13.0]	.001[b]

Abbreviations: BMI, body mass index; HJHS, haemophilia joint health score (2.1); n/a, not applicable.
[a]Indicates significant Bonferroni post-hoc analysis (severe-moderate: $p = .001$, severe-mild: $p < .001$).
[b]Indicates significant difference.

joints were ankles (5.3 ± 5.4) followed by elbows (4.7 ± 4.8) and knees (3.2 ± 4.7). An overview of demographic and haemophilia-specific data are provided in Table 1.

Table 2 provides an overview of BMI and raw data of body composition parameters of the included PwH and further differentiates within the three haemophilia severities. Of 201 PwH, 85 (42.3%) had normal weight (BMI between 18.5 and 24.9) while three persons (1.5%) were considered underweight (BMI < 18.5). Eighty-four (41.8%) PwH were overweight showing BMI scores between 25.0 and 29.9. Twenty-two (11.0%) persons were categorized into obesity class I corresponding to a BMI between 30.0 and 34.9. Furthermore, three (1.5%) PwH had a BMI between 35.0 and 39.9, being classified as obesity class II and four (1.9%) PwH were classified as obesity class III having a BMI of >40.0.[26] The mean BMI value did not differ between the severity phenotypes ($p = .308$).

Overall, PwH showed a median body fat percentage of 28.7% [25.5–33.9]. Regarding the VAT, a median of 657 g [403–954] was found. The analysis revealed no significant difference in overall body composition parameters between the severity phenotypes, except for the lean mass index (lean/height^2; $p = .033$). Bonferroni post hoc analysis revealed a significant difference between persons with severe and mild haemophilia ($p = .037$).

Regarding the lean mass, the analysis of the subregions (four extremities) revealed, that there is a significant difference between the severity phenotypes in the legs and the right arm (see Table 3). Bonferroni post hoc analyses revealed a significant difference considering the right arm between persons with severe compared to persons with mild haemophilia ($p = .035$) as well as persons with moderate haemophilia ($p = .020$). There was no significant difference considering the left arm ($p = .058$). Appendicular fat mass is not significantly different between severity phenotypes ($p > .05$).

To provide age-related reference data, the cohort was divided into five different age groups (18–29 [$n = 50$], 30–39 [$n = 47$], 40–49 [$n = 26$], 50–59 [$n = 43$], 60–80 [$n = 35$]). It was found that fat-related parameters such as fat/height^2, body fat percentage, VAT, android fat and android/gynoid ratio, respectively, significantly differ across the age groups (Table 4). Hereby, the difference is mainly observed between the youngest age group (18–29 years) compared to persons aged 40–49, 50–59 and 60–80 years, respectively. However, between the three older age groups only trends can be observed that indicate higher fat content with increasing age.

Going more into detail, lean and fat mass of the legs do not differ between the different age groups (see Table 5). But fat mass of the arms differs significantly between the youngest age group (18–29) and the groups of persons aged 30 and older, indicating a linear increase of arm fat with increased age. However, post hoc analysis showed that there is no significant difference within age groups 40–49 to 50–59 or 60–80.

4 | DISCUSSION

This is the first study investigating a whole body DXA analysis in PwH. The focus was to differentiate between the haemophilia severities. It has been hypothesized that persons with severe haemophilia

TABLE 2 Overall body composition determined with single x-ray parameters of different severity phenotypes.

Variable	Total ($n = 201$)	Severe ($n = 116$)	Moderate ($n = 41$)	Mild ($n = 44$)	p-value
BMI (kg/m^2) M ± SD Median [IQ25–75]	26.1 ± 4.5 25.3 [23.4–28.0]	25.8 ± 4.9 25.2 [23.3–27.7]	26.5 ± 4.4 25.8 [23.6–27.9]	26.3 ± 4.4 25.8 [23.9–29.5]	.414
Fat/height^2 (kg/m^2) M ± SD Median [IQ25–75]	7.9 ± 3.9 7.2 [5.9–9.1]	8.1 ± 4.7 7.0 [5.8–9.3]	7.7 ± 2.9 7.2 [6.0–8.7]	7.5 ± 2.6 7.4 [5.6–9.0]	.987
Lean/height^2 (kg/m^2) M ± SD Median [IQ25–75]	17.5 ± 4.9 17.0 [15.6–18.3]	17.4 ± 6.2[a] 16.7 [15.2–18.2]	17.5 ± 2.4 17.1 [15.8–18.8]	17.6 ± 1.8[a] 17.8 [16.2–18.9]	.033
Body fat percentage (%) M ± SD Median [IQ25–75]	29.2 ± 6.2 28.7 [25.5–33.9]	29.7 ± 6.5 29.3 [25.5–34.8]	29.0 ± 5.7 27.7 [25.7–32.5]	28.2 ± 5.7 28.9 [24.2–32.5]	.474
Est. VAT mass (g) M ± SD Median [IQ25–75]	737 ± 431 657 [403–954]	731 ± 442 602 [403–953]	754 ± 384 681 [427–1002]	736 ± 453 646 [382–945]	.781
Android fat (g) M ± SD Median [IQ25–75]	2476 ± 1370 2216 [1533–3106]	2491 ± 1502 2210 [1473–3222]	2500 ± 1243 2404 [1695–2897]	2413 ± 1120 2273 [1707–3074]	.970
Gynoid fat (g) M ± SD Median [IQ25–75]	3851 ± 1539 3661 [2884–4311]	3894 ± 1646 3670 [2831–4359]	3898 ± 1531 3703 [2949–4130]	3694 ± 1245 3548 [2726–4243]	.862
Android/Gynoid ratio M ± SD Median [IQ25–75]	0.6 ± 0.2 0.6 [0.4–0.7]	0.6 ± 0.2 0.6 [0.5–0.7]	0.6 ± 0.2 0.6 [0.5–0.7]	0.7 ± 0.2 0.6 [0.4–0.8]	.621

Note: Data displayed as mean ± SD and median [Interquartile range 25–75].
Kruskal–Wallis Test was used.
Abbreviations: Est., estimated; VAT, Visceral adipose tissue.
[a]Indicates significant Bonferroni post-hoc analysis in lean/height^2 between persons with severe and mild haemophilia ($p = .037$).

show altered body composition parameters, especially considering the lean mass, compared to persons with mild or moderate haemophilia. Primary results displayed in Tables 2 and 3, reveal that body fat distribution, estimated VAT, appendicular fat mass, and android/gynoid ratio do not differ between severity phenotypes. Emphasizing, the lean mass diverges over the severities, that is, persons with severe haemophilia show less lean mass compared to persons with moderate or mild haemophilia. Going more into detail, a differentiation between appendicular lean mass was conducted as especially persons with severe haemophilia are frequently affected by muscular atrophies due to haemarthroses and impaired joint functionality. Muscle atrophies imply a loss of lean mass, therefore it is understandable that persons with severe haemophilia suffer from a higher reduction of both appendicular lean mass and lean/height^2, although there was only a tendency in this respect on the left arm, which could be due to the fact that the frequency of bleeds is increased in the dominant (i.e., right) elbow. It is further assumed that significant difference is missed, given a high number of right-handed subjects, leading to a systemic bias. However, the presence of muscular atrophy as a frequent comorbidity might be a key relevant factor for lower levels of lean mass. The whole muscle surrounding the affected joint, for example, musculus gastrocnemius, quadriceps muscle, the biceps brachii or triceps brachii degrade due to a deficiency of activation when trying to avoid pain.[27,28] It is known that overall contracting muscle mass mediates among others the myokine IL-6 for instance, which positively affects metabolic aspects, as it increases insulin sensitivity and fat oxidation.[29] Research further showed that immobilization causes a reduction of the volume of the musculus quadriceps as well as a decrease of whole-body insulin sensitivity.[30] However, it is still a matter of research to what extent local atrophies, which are present in PwH, play a role on the metabolism, though it can be assumed that there are the above-mentioned alterations in PwH affected by atrophies.

It needs to be emphasized that reference data for body composition parameters of the general European population are limited.[31] There are two recent studies from Norway and Austria, respectively, providing reference data using the GE Lunar device, which can be used for comparison.[32,33] Considering the BMI, the present data show an increased BMI (>25) in 56.2% of PwH, which corresponds to the male European prevalence of 54.4%,[34] highlighting that both values are notably high. Prior research suggests that the presence of muscle atrophy is associated with lower BMI.[35] This indicates that the BMI can be biased in PwH and might even be higher, respectively, to the high prevalence of atrophies within this study cohort.[8,35] Considering the lean mass, the general Caucasian male population shows

TABLE 3 Lean- and fat-mass of subregions within different severity phenotypes.

Variable	Total ($n = 201$)	Severe ($n = 116$)	Moderate ($n = 41$)	Mild ($n = 44$)	p-value
Arm left fat (g) M ± SD Median [IQ25–75]	1535 ± 613 1431 [1120–1857]	1566 ± 674 1467 [1081–1892]	1515 ± 572 1401 [1140–1790]	1428 ± 475 1375 [1050–1832]	.737
Arm left lean (g) M ± SD Median [IQ25–75]	3355 ± 638 3305 [2932–3775]	3273 ± 660 3167 [2854–3748]	3475 ± 596 3415 [3106–3861]	3460 ± 594 3419 [3011–3774]	.058
Arm right fat (g) M ± SD Median [IQ25–75]	1548 ± 580 1443 [1148–1826]	1556 ± 639 1409 [1092–1945]	1523 ± 527 1420 [1123–1709]	1515 ± 471 1484 [1151–1865]	.995
Arm right lean (g) M ± SD Median [IQ25–75]	3596 ± 621 3562 [3169–4006]	3479 ± 636 3409 [3102–3844]	3757 ± 573[a] 3731 [3459–4119]	3755 ± 568[a] 3786 [3267–4072]	.004
Leg left fat (g) M ± SD Median [IQ25–75]	3929 ± 1606 3703 [2845–4445]	3992 ± 1752 3786 [2775–4625]	3857 ± 1496 3661 [2901–4308]	3717 ± 1297 3619 [2791–4357]	.752
Leg left lean (g) M ± SD Median [IQ25–75]	9053 ± 1691 8951 [7959–10114]	8836 ± 1907 8651 [7503–10029]	9407 ± 1326 9286 [8311–10081]	9295 ± 1275 9149 [8493–10415]	.028
Leg right fat (g) M ± SD Median [IQ25–75]	3988 ± 1613 3804 [2947–4498]	4025 ± 1718 3816 [2922–4551]	4008 ± 1607 3740 [2994–4474]	3750 ± 1343 3581 [2762–4407]	.705
Leg right lean (g) M ± SD Median [IQ25–75]	9054 ± 1605 8966 [8028–10029]	8861 ± 1802 8645 [7501–10036]	9288 ± 1368 9163 [8437–9976]	9251 ± 1144 9116 [8441–10171]	.034

Note: Data displayed as mean ± SD and median [Interquartile range 25–75].
[a]Indicates significant difference (Bonferroni corrected) to severe haemophilia: Arm right lean: severe-mild: $p = .035$, severe-moderate: $p = .020$.

a lean/height^2 of 17.8 ± 1.8 kg/m^2, increasing with age.[33] A gradual increase of lean mass with age is also seen in the presented study cohort, though overall lean mass is lower, especially in young PwH (lean/height^2 of PwH aged 18–29: 16.6 ± 1.9 kg/m^2; reference data 17.3 ± 1.8 kg/m^2).[33]

Regarding body fat distribution, reference data reveal a mean body fat percentage of 29.3 ± 7.3]% within the European male population, which equals the mean body fat percentage of the analysed PwH of 29.2 ± 6.2%.[33] Taking a closer look at VAT, there is no consensus in literature for the respective reference values of the European population, but VAT strongly increases with age.[33,36] Reference data suggest a mean VAT of 424 ± 385 g in healthy subjects aged 18–29, which is similar, though even higher compared to the data of PwH (404 ± 150 g). In healthy subjects aged 40–80 (65.9 ± 9.1) years the mean VAT is 1660 ± 687 g, which is higher than in PwH (see Table 4).[32,33] This difference might partially be explained by the fact that especially the older age groups of the reference data showed an increased BMI, leading to a systemic bias and a high standard deviation.[33,36] The low proportion of VAT in haemophilia should be investigated further.

In spite of that, the mean android/gynoid ratio of 0.6 ± 0.2 is similar compared to non-haemophilic European reference data (ratio 0.7 ± 0.2). To the best of our knowledge, there are no previous studies on android/gynoid ratio in PwH, though data might be comparable to waist-to-hip-ratio. Kennedy et al. revealed that in 66% (n = 35/53) of the PwH analysed, the waist-to-hip-ratio is increased.[8] However, android fat is known to be an indicator for cardiovascular risk, while in contrast gynoid fat might be rather protective.[33,37] With regard to fat-related parameters, the presented findings do not show any significant differences between the haemophilia severities, which is in in line previous findings.[8] Either way, in persons with high VAT and android fat, it is recommended to monitor individual vascular health in persons with high VAT and android fat. Physical activity in PwH should be promoted to oppose reduced lean mass, increased (android) fat distribution and the associated risks. The training schedule should therefore entail both, the training of isolated muscle groups persons with atrophies as well as aerobic exercising to reduce (android) fat distribution.[38]

Moreover, the body composition parameters were also displayed and analysed in regard to different age groups. Here, it can be seen that fat parameters, such as fat/height^2, total body fat percentage, VAT, android fat, and the android/gynoid ratio, respectively, increase with age. It has further been revealed, that the absolute amount of appendicular fat varies across age groups. There is less fat of the arms in young PwH compared to older PwH, which is consistent with previous findings.[39] The fat mass of the legs does not differ between age groups in PwH. It was found to be highest in persons aged 40–59 and decreases again with advanced age. These findings are not consistent with previous literature but might be explained through high prevalence of atrophies in older PwH and the concomitant altered limb composition.[39]

TABLE 4 Overall body composition parameters within different age groups.

Variable	Age group 18–29 ($n = 50$)	Age group 30–39 ($n = 47$)	Age group 40–49 ($n = 27$)	Age group 50–59 ($n = 42$)	Age group 60–80 ($n = 35$)	p-value
BMI (kg/m^2) M ± SD Median [IQ25–75]	24.0 ± 2.9 24.0 [22.2–26.0]	26.9 ± 6.7 24.9 [23.4–28.6]	26.6 ± 4.1 26.8 [23.8–28.4]	27.1 ± 3.9[a] 26.9 [24.8–30.2]	26.6 ± 3.1[a] 26.1 [24.7–27.7]	<.001
Fat/height^2 (kg/m^2) M ± SD Median [IQ25–75]	6.2 ± 2.0 6.1 [4.4–7.3]	8.1 ± 4.3 6.9 [5.8–9.3]	8.5 ± 2.8[a] 8.5 [6.0–9.8]	8.8 ± 6.0[a] 7.8 [6.2–9.8]	8.5 ± 2.2[a] 8.2 [7.1–9.0]	<.001
Lean/ height^2 (kg/m^2) M ± SD Median [IQ25–75]	16.6 ± 1.9 16.6 [14.8–18.2]	17.2 ± 2.9 16.8 [15.6–18.3]	17.2 ± 1.9 17.2 [16.0–18.3]	19.2 ± 9.7 17.9 [16.3–19.2]	17.0 ± 1.6 16.9 [15.6–18.1]	.106
Body fat percentage (%) M ± SD Median [IQ25–75]	25.9 ± 6.0 26.7 [21.1–29.1]	29.5 ± 7.1 28.6 [25.5–33.1]	31.2 ± 5.3[a] 31.5 [26.3–35.2]	29.5 ± 5.1 29.7 [26.1–34.1]	31.9 ± 5.0[a] 31.1 [27.5–35.6]	<.001
Est. VAT mass (g) M ± SD Median [IQ25–75]	404 ± 150 370 [286–477]	667 ± 495 480 [375–830]	808 ± 317[a] 729 [578–955]	868 ± 370[b,a] 769 [608–1140]	1092 ± 405[b,a] 1034 [797–1255]	<.001
Android fat (g) M ± SD Median [IQ25–75]	1615 ± 775 1450 [1020–1946]	2505 ± 1932 1860 [1508–3074]	2896 ± 1246[b,a] 2806 [1949–3516]	2761 ± 1078[a] 2744 [2022–3441]	3001 ± 962[b,a] 2814 [2404–3296]	<.001
Gynoid fat (g) M ± SD Median [IQ25–75]	3614 ± 1312 3538 [2641–4166]	4297 ± 2157 3852 [3249–4800]	4084 ± 1709 3617 [2870–4840]	3752 ± 1127 3860 [3021–4207]	3530 ± 935 3360 [2918–3964]	.280
Android/ Gynoid Ratio M ± SD Median [IQ25–75]	0.4 ± 0.1 0.4 [0.3–0.5]	0.5 ± 0.1[a] 0.5 [0.4–0.6]	0.7 ± 0.2[b,a] 0.7 [0.6–0.8]	0.7 ± 0.2[b,a] 0.7 [0.6–0.8]	0.8 ± 0.2[b,a] 0.8 [0.7–1.0]	<.001

Note: Data displayed as mean ± SD and median [Interquartile range 25–75].
Abbreviation: Est., estimated; VAT, Visceral adipose tissue.
[a]Indicates significant difference (Bonferroni corrected) to age group 18–29.
[b]Indicates significant difference (Bonferroni corrected) to age group 30–39; Kruskal–Wallis Test was used: BMI: 18–29 to 50–59: $p = .001$; 18–29 to 60–80: $p = .010$; Fat/height^2: 18–29 to 40–49: $p = .005$, 18–29 to 50–59: $p = .001$, 18–29 to 60–80: $p < .001$; Body fat percentage: 18–29 to 40–49: $p = .008$, 18–29 to 60–80 $p < .001$; Est. VAT: 18–29 to 30–39: $p = .010$, 18–29 to 40–49: $p < .001$, 18–29 to 50–59: $p < .001$, 18–29 to 60–80 $p < .001$, 30–39 to 50–59: $p = .014$; 30–39 to 60–80: $p < .001$; Android fat: 18–29 to 40–49: $p < .001$, 18–29 to 50–59: $p < .001$, 18–29 to 60–80 $p < .001$, 30–39 to 60–80: $p = .010$, Android/gynoid ratio: 18–29 to 30–39: $p = .027$ 18–29 to 40–49: $p < .001$, 18–29 to 50–59: $p < .001$, 18–29 to 60–80 $p < .001$, 30–39 to 40–49: $p = .004$, 30–39 to 50–59: $p < .001$; 30–39 to 60–80: $p < .001$.

However, an increase of body fat and VAT is physiologically linked to age, which has been stated in previous literature.[33] Thus, the present findings are unsurprising, though it needs to be highlighted that the life expectancy of the haemophilic population has fortunately increased as treatment options have improved.[28] Hence, a higher awareness on age-related comorbidities implying the consequences of higher fat distribution is required, and should be considered with regard to individual cardiometabolic risk assessment.

4.1 | Strengths and limitations

One major strength of this study is the methodology since DXA is considered as gold standard for body composition analyses. Therefore, this investigation provides reliable results, extending prior literature on body composition in PwH, and can be used as reference data on PwH in both future research and clinical routine. the sample size and age range of this study is large ($n = 201$, 18–79 years). Nevertheless, this study is not able to provide reference data for age groups differentiated by severities, which is because the subgroups are too small. As body composition diverges within ethnicities, it needs to be highlighted that this study cohort is of Caucasian ethnicity and can therefore only apply as reference in Caucasian populations.[40] Being aware of the fact that the present data were gathered using Hologic DXA system, the transferability to other DXA systems might be limited.[33] The difference within these systems further limits the degree of comparing the present PwH to European reference data. Moreover, effects of nutrition have not been investigated in this study. As nutrition has a major impact on body composition it should be investigated in future research.

5 | CONCLUSION

This study provides reference data for body composition parameters in PwH determined by DXA in a representative sample. persons with

TABLE 5 Lean- and fat mass of subregions within different age groups.

Variable	Age group 18–29 ($n = 50$)	Age group 30–39 ($n = 47$)	Age group 40–49 ($n = 27$)	Age group 50–59 ($n = 42$)	Age group 60–80 ($n = 35$)	p-value
Arm left fat (g) M ± SD Median [IQ25–75]	1188 ± 425 1065 [860–1500]	1606 ± 880 1335 [1126–1890]	1655 ± 498[a] 1693 [1266–1878]	1590 ± 473[a] 1588 [1228–1875]	1750 ± 466[a] 1720 [1401–2126]	<.001
Arm left lean (g) M ± SD Median [IQ25–75]	3352 ± 606 3273 [2895–3797]	3426 ± 659 3392 [3017–3864]	3331 ± 615 3275 [2987–3640]	3502 ± 622 3433 [3007–3835]	3106 ± 651 3118 [2686–3611]	.169
Arm right fat (g) M ± SD Median [IQ25–75]	1289 ± 468 1231 [918–1533]	1590 ± 851 1359 [1088–1774]	1658 ± 465[a] 1595 [1280–2029]	1585 ± 435[a] 1596 [1226–1939]	1716 ± 397[a] 1677 [1443–2017]	<.001
Arm right lean (g) M ± SD Median [IQ25–75]	3641 ± 624 3490 [3224–4030]	3681 ± 557 3603 [3287–4101]	3693 ± 631 3623 [3158–4208]	3670 ± 546 3644 [3229–3894]	3256 ± 693 3152 [2565–3855]	.052
Leg left fat (g) M ± SD Median [IQ25–75]	3747 ± 1436 3610 [2646–4288]	4347 ± 2140 3945 [3122–4939]	4127 ± 1836 3723 [2583–4824]	3815 ± 1253 3946 [3096–4341]	3503 ± 976 3454 [2686–3981]	.379
Leg left lean (g) M ± SD Median [IQ25–75]	9257 ± 1531 9349 [7834–10429]	9292 ± 1900 9061 [8078–10113]	9148 ± 1524 9321 [8029–10031]	9052 ± 1561 8979 [8151–10235]	8363 ± 1790 8510 [7284–9946]	.164
Leg right fat (g) M ± SD Median [IQ25–75]	3799 ± 1488 3433 [2784–4392]	4435 ± 2119 4055 [3174–5092]	4222 ± 1832 3835 [2969–5106]	3780 ± 1216 3817 [3098–4300]	3562 ± 1013 3565 [2741–4336]	.296
Leg right lean (g) M ± SD Median [IQ25–75]	9232 ± 1429 9318 [8211–10435]	9239 ± 1858 9006 [8299–9678]	9041 ± 1727 9033 [7792–10280]	9182 ± 1357 8948 [8193–10115]	8407 ± 1576 8062 [7447–9674]	.100

Note: Data displayed as mean ± SD and median [Interquartile range 25–75].
Kruskal–Wallis Test was used.
Abbreviations: Est., estimated; VAT, Visceral adipose tissue.
[a]Indicates significant difference (Bonferroni corrected) to age group 18–29: Arm left fat: 18–29 to 40–49: $p = .001$, 18–29 to 50–59: $p = .002$, 18–29 to 60–80 $p < .001$; Arm right fat: 18–29 to 40–49: $p = .008$, 18–29 to 50–59: $p = .020$, 18–29 to 60–80 $p < .001$.

severe haemophilia have significantly less lean mass than persons with moderate or mild haemophilia. This can be attributed mainly to a higher presence of muscle atrophy. PwH show low VAT mass, average body fat percentage, and android/gynoid ratio compared to Caucasian individuals without bleeding disorders. These fat-related body composition parameters do not differ between the severity phenotypes. Age is directly linked to increased fat in PwH, but does not affect the amount of lean mass. As the haemophilic population ages due to better pharmacologic treatment options, awareness of age-related comorbidities, including fat-related comorbidities, should rise in haemophilia management.

AUTHOR CONTRIBUTIONS

P. Ransmann, M. Brühl and J. Hmida performed the data collection and analysis. G. Goldmann and J. Oldenburg supported with the recruitment process. T. Hagedorn, T. Hilberg, A.C. Strauss[2], A.C. Strauss[4] and F. A. Schildberg contributed scientifically to the manuscript. T. Hilberg and A.C. Strauss[2] designed and supervised the project.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

This study was conducted in accordance with the principles of good clinical and ethical practice and was approved by the local ethic committee (339/19). Along with the Declaration of Helsinki, all participants gave written informed consent.

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Publication III - Physical activity and handgrip strength in patients with mild, moderate and severe haemophilia: Impacts on bone quality and lean mass

Ransmann P, Brühl M, Hmida J, Goldmann G, Oldenburg J, Schildberg FA, Ossendorff R, Tomschi F, Schmidt A, Hilberg T, Strauss AC. (2025) *Physical activity and handgrip strength in patients with mild, moderate and severe haemophilia: Impacts on bone quality and lean mass*. PLOS One. doi: 10.1371/journal.pone.0319951

Preliminary Remark

Publication III was published in the journal “PLOS One” on March 26th, 2025. The impact factor of the journal “PLOS One” was 3.7 in year 2022.

Original data can be found on the enclosed CD.

Publication III evaluated the level of PA within the different haemophilia severity phenotypes and elaborated on the interplay of PA in regard to bone quality (BMD and TBS) as well as lean mass. The primary parameter was total step count taken per week. Data for this paper derived from the large prospective cohort study just as **publication I** and **II.** However, the primary parameter was total step count taken per week, so that only people who wore the electronic activity tracker were included. Accordingly, 223 people with either mild (n=45), moderate (n=46) or severe (n=132) haemophilia A or B, aged 43.6±15.6 years were analyzed within this study. Step activity was tracked electronically for 7 days consecutive after clinical examination, supported by a 7-day subjective activity diary. Along with the progress of the study, the study lead and research assistants added handgrip measurements to the clinical assessment as an overall fitness proxy. Therefore, handgrip strength was examined in n=102, of which data is displayed in the present **publication III**.

Results revealed that there was no difference between severity phenotypes neither in objective (p=0.162) nor subjective (p=0.459) PA levels. The most frequent type of PA in all severities was walking (n=72, 53.3%), followed by cycling (n=60, 44.4%).

Moreover, results showed a positive correlation between step activity and TBS (rho=0.202, p=0.005) as well as between lean mass and BMD (rho=0.309, p<0.001). Also, handgrip strength correlated with BMD (rho=0.361, p<0.001) as well as with TBS (rho=0.221, p=0.021) and lean mass (rho=0.287, p=0.003).

Concluding, in the course of **publication III** it can be seen that the majority of PwH in all severity phenotypes performed low-impact PA, such as walking or cycling. These activities most likely fail to generate sufficient strain to stimulate bone formation, explaining the lack of a relationship between PA and BMD. However, within **publication III** it could be demonstrated that handgrip strength shows significant correlations with PA, lean mass, and bone quality, serving as an indicator of PA levels and suggesting an indirect link between PA and these parameters. Additionally, there is a positive association between step activity and TBS, which is particularly relevant given that walking is a common form of PA among PwH.

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RESEARCH ARTICLE

Physical activity and handgrip strength in patients with mild, moderate and severe haemophilia: Impacts on bone quality and lean mass

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Abstract

Background

Patients with haemophilia (PwH) might be restricted in physical activity (PA) depending on the severity phenotype. It is well-known that PA affects overall health including bone quality. This study aims to evaluate the level of PA within the different haemophilia severity phenotypes and to elaborate on the interplay of PA in regard to bone quality (bone mineral density (BMD) and trabecular bone score (TBS)) as well as lean mass.

Methods

This investigation was part of a large prospective single-center cohort study examining the relation between haemophilia and osteoporosis registered at clinicaltrials.gov (ID: NCT04524481). PwH underwent a dual x-ray screening using Horizon™ to examine BMD, TBS, and lean mass. Step activity was tracked electronically for seven consecutive days after clinical examination, supported by a self-reported activity diary for seven days. Handgrip strength was examined as an overall fitness proxy.

Results

Data of 223 patients with either mild (N = 45), moderate (N = 46), or severe (N = 132) haemophilia A or B, aged 43.6 ± 15.6 years were analyzed. There was no significant difference in objective ($p = 0.162$) and subjective ($p = 0.459$) PA levels between severity phenotypes. The most frequent type of PA in all severities was walking (n = 72, 53.3%) and cycling (n = 60, 44.4%). Step activity positively correlated with TBS (rho = 0.202, $p = 0.005$) and lean mass positively correlated with BMD (rho = 0.309, $p < 0.001$). Handgrip strength correlated with BMD (rho = 0.361, $p < 0.001$) as well as TBS (rho = 0.221, $p = 0.021$) and lean mass (rho = 0.287, $p = 0.003$).

Data availability statement: All relevant data are within the manuscript and its Supporting Information files.

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Conclusion

PA does not differ significantly between the severity phenotypes. The majority of PwH in all severity phenotypes performed low-impact PA, which is most likely insufficient to positively affect BMD. However, handgrip strength correlates with BMD and TBS. Despite restrictions in movement function or reduced BMD, it is of major importance to promote PA to maintain a healthy or even increase bone quality.

Introduction

Haemophilia is a hereditary bleeding disorder, caused by a deficiency of factor VIII (haemophilia A) or IX (haemophilia B) [1,2]. Depending on the factor activity, patients with haemophilia (PwH) can suffer from either mild (5–15%), moderate (1–5%) or severe (<1%) haemophilia, which commonly determines the bleeding rates [3]. Joint bleeding is a primary consequence of haemophilia, often leading to haemophilic arthropathy, characterized by pain and restricted range of motion [4].

Nevertheless, it is well-known that physical activity (PA) represents a key factor for a healthy lifestyle with various beneficial effects on organs, muscles, and bones [5]. Though, a physically active lifestyle was not recommended for PwH until the 1970 because of a suspected increased bleeding risk at the time; PA in PwH is nowadays indispensable [6,7]. This is due to better treatment options but also to the risen awareness of general beneficial effects of exercising on the patient's health [8]. Even though PA is highly promoted in the meantime, scientific data on the extent of activity levels in haemophilic populations is controversial and data investigating the differentiation of PA levels between severity phenotypes is limited [9–12]. However, it has been described that patients affected by arthropathy show lower PA levels especially regarding higher intensities. Additionally, many PwH are affected by kinesiophobia, which is considered as the fear of pain and/or injury due to movement, especially in regard to high-impact sports because of the increased bleeding risk [13,14]. Of course, factor activity plays a key role in managing the risk profile associated with high-impact activities, though recent research suggests that PA-induced bleeding rates are relatively low [15,16].

However, in addition to favorable effects on the general individual health, PA is of major importance to maintain or gain muscle mass, also referred to as lean mass [17,18]. Lean mass is positively associated with reduced cardiometabolic risk factors, improved self-determinate aging, and healthier bone metabolism [19,20]. The overall muscle strength can be easily evaluated by assessing handgrip strength. In patient populations, such as PwH where joint pain and arthropathy can limit movement, measuring handgrip strength offers a practical way to assess overall strength without requiring more strenuous testing that might exacerbate joint issues [21,22]. Further, It has been shown that handgrip strength correlates with bone quality and it has been shown to be a valid predictor of fall risk [23]. Risk of falling is an essential concern for patients with reduced bone quality and reduced lean mass. Bone quality is represented by bone mineral density (BMD), which is positively influenced by PA [24], and trabecular bone score (TBS), an innovative measure that evaluates the trabecular microarchitecture of the bone. Good trabecular microarchitecture reduces fracture risk, despite low BMD [25]. Current knowledge on TBS in PwH is very scarce with only minimal evidence currently available [26,27]. Previous research of our group indicates that lean mass and BMD are notably reduced in patients with severe haemophilia and that TBS appears to be predominantly normal across different severity phenotypes [28]. It is known that TBS is reduced by ageing and increased weight, but further potential influencing lifestyle factors on TBS are not well-studied [29].

However, the complex interrelationships between PA and handgrip strength, TBS, lean mass, and BMD within a large haemophilic cohort of all three severity phenotypes have not been investigated yet. Based on these considerations, this study aims to fill this crucial research gap and the following three research questions were formulated:

(1) How does the severity phenotype influence the level of PA in PwH?

(2) How does PA correlate with TBS, BMD, and lean mass in PwH?

(3) How do lean mass and handgrip strength impact BMD and TBS in PwH?

Methods

Study design and participants

This investigation was part of a large prospective osteoporosis and haemophilia study. Data regarding BMD and lean mass of the entire sample (n = 255) was previously published [28,30]. In the current publication, these parameters are presented for the sample of the present study (n = 223). Adult male patients with either mild, moderate or severe haemophilia A or B were included. Patients with other bleeding disorders or younger than 18 years were excluded from this investigation. In agreement with the haemophilia joint health score (HJHS) manual, patients who experienced joint bleedings in the past two weeks were also excluded [31]. This study was conducted in accordance with the principles of good clinical and ethical practice and was acceded by the local ethic committee (339/19). Ethical approval for this study was gained at 14th of November 2019, though because of the Covid-19 pandemic, recruitment process started at 19th of August 2020 and ended at 29th of September 2022 at the University Hospital Bonn, Germany (registered at clinicaltrials.gov (ID: NCT04524481)). Along with the Declaration of Helsinki, all participants gave written informed consent after being informed about the study protocol.

Data acquisition

To evaluate a patient's daily PA level, PwH were given an electronic activity tracker (Fitbit Alta Hr, Fitbit Inc., San Francisco, USA), which was worn at the wrist for seven consecutive days after the clinical examination. The activity tracking for seven days is in line with the recommendations to collect accelerometry data over multiple days to achieve a reliable estimate of an individual's habitual PA [32–34]. The average number of steps taken within one day over the seven-day observation period (objective PA) was used for further analysis. In parallel, subjects kept an activity diary on the same seven days. Here, PwH were instructed to report performed type of PA as well as the respective duration in minutes (subjective PA) to assess daily time spend on PA. Daily activities such as housekeeping or shopping were not considered as PA and therefore excluded from further analysis. Moreover, handgrip strength, used as a correlate for the overall fitness level, was measured in n = 102 PwH using a hand dynamometer (Baseline, White Plains, NY) [35,36]. The assessment was performed bilaterally, three times in a sitting position with 90° elbow flexion. The mean of the left and right hand was used for further analysis.

The clinical examination entailed two major procedures: First, a dual x-ray (DXA) screening using Horizon™ (Hologic, USA) of the whole body, the left hip and lumbar spine was conducted. The whole-body screening revealed the subject's lean mass (g) and the left hip (neck) was used to determine the BMD (g/cm²). Additionally, the software TBS iNsight® (V. 3.1.2. Medi Maps; Switzerland) revealed the TBS based on the DXA of the lumbar spine. The TBS is measured in score points, which are classified as "normal" (TBS ≥ 1.31), "partially degraded"

(TBS between 1.30 and 1.24), and "degraded" (TBS ≤ 1.23) [37]. Second, the orthopedic joint status was clinically examined via the HJHS (Version 2.1; maximum score 124 points, 20 points × 6 joints, plus 4 points assigned to global gait; higher values indicating worse joint status), which examines the elbows, knees and ankles in regard to swelling, muscle atrophy, crepitation, stability, pain, muscle strength and range of motion [38].

Via a self-established anamnesis questionnaire, anthropometric data as well as data regarding the pharmacological treatment regime were additionally gathered.

Statistics

Descriptive statistics were calculated based on the severity of PwH and in total. The IBM© Statistical Package for the Social Sciences 29 (Armonk, NY, USA) for Mac was used for all statistical analyses. Tests for normality by Kolmogorov-Smirnov were conducted, revealing no normal distribution, which was confirmed by visual analysis of Q-Q plots. Hence, data are presented as median [1st quartile, 3rd quartile]. Thus, the Kruskal-Wallis-Test was used to examine between-group differences. In case of significant differences, Bonferroni correction was used for alpha-adjustment. To analyze potential influencing factors, Spearman's rho was calculated for correlation analyses. Here, rho = 0.10 equals a weak correlation, rho = 0.30 represents a moderate correlation and rho = 0.50 is considered as a strong correlation [39].

Regarding research questions 2 and 3, subjects were further classified into two groups based on their activity level (steps/day and PA in minutes per day (upper 50 percent, lower 50 percent)) as well as based on the amount of lean mass (upper 50 percent, lower 50 percent). A supplementary analysis was performed to evaluate group differences between patients who perform strength training compared to patients not performing strength trainings. The Mann-Whitney-U-Test was used to statistically analyze these group differences. A significance level of $p \leq 0.05$ (95% confidence interval) was established.

Results

Overall, 223 PwH were recruited and analyzed in this study. PwH showed a median age of 43 [30, 56] years. Data of patients with mild (n = 45), moderate (n = 46) and severe (n = 132) haemophilia A (n = 193) or B (n = 30) were analyzed (see Table 1). Results of the HJHS differed

Table 1. Anthropometric and descriptive data of patients with haemophilia.

Variables	Severe (n = 132)	Moderate (n = 46)	Mild (n = 45)	Total (n = 223)	p-value
Age (years) Median [Q1, Q3]	40 [29, 54]	49 [32, 59]	45 [29, 57]	43 [30, 56]	0.192
Weight (kg) Median [Q1, Q3]	82 [74, 91]	88 [80, 96]	84 [78, 95]	84 [76, 94]	0.074
BMI (kg/m²) Median [Q1, Q3]	25.2 [23.1, 27.8]	25.9 [23.6, 28.6]	26.7 [23.9, 29.1]	25.6 [23.6, 28.3]	0.149
Haemophilia form (n)	A: B:	A: B:	A: B:	A: 193 B: 30	
Viral comorbidities (n)	HIV: 29 HEP C: 23	HIV: 2 HEP C: 3	HIV: 3 HEP C: 1	HIV: 34 HEP C: 27	n/a
HJHS (score) Median [Q1, Q3]	18* [6, 34]	7* [5, 13]	7* [4, 12]	10 [5, 28]	<0.001*

Explanation: BMI = Body Mass Index; HJHS=Haemophilia Joint Health Score [2.1]; Kruskal-Wallis-tests were conducted; * indicates significant difference, Bonferroni post-hoc analysis revealed significant differences regarding the HJHS: severe-mild: $p \leq 0.001$, severe-moderate: $p \leq 0.001$.

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significantly between the severity phenotypes ($p < 0.001$). Bonferroni post-hoc testing revealed that patients with severe haemophilia had a significantly worse joint status compared to patients with moderate ($p < 0.001$) or mild ($p < 0.001$) haemophilia.

To address research question 1), objective and subjective PA data were evaluated.

A total of n = 203 patients returned the activity diary, of which n = 72 (35.4%) PwH reported to not being physically active at all. 6 (3%) PwH did not indicate the duration of performed PA in minutes. Out of the remaining 125 PwH, median PA in minutes per day was 42 [22, 81]. The activity diary was further evaluated regarding types of performed PA. In total, the most frequent type of PA in all severity phenotypes was walking (n = 72, 53.3%) followed by cycling (n = 60, 44.4%) and strength training (n = 37, 27.4%; see Table 2).

The activity tracker analysis showed a median step activity/day of 7392 [4981,10579] within the whole study cohort, which did not differ between haemophilia severity types ($p = 0.162$), analogous to the subjective PA, which neither differed between severity phenotypes ($p = 0.459$; see Fig 1). In detail, patients with mild haemophilia showed a median of 8466 [5219, 10785] steps per day and indicated to be physically active for 42 [30, 85] minutes per day. Patients with moderate haemophilia accumulated a median of 7040 [5530,11472] steps per day and

Table 2. Type of sports conducted differentiated by haemophilia severities.

PA type		N (%)	Duration (minutes/week)
Severe (n = 113)	Walking	39 (34.5)	252 [112,415]
	Cycling	32 (28.3)	135 [63,255]
	Strength Training	19 (16.8)	205 [82,242]
	Bodily exercising	10 (8.5)	60 [33,217]
	Jogging	8 (7.1)	30 [30,86]
	Physiotherapy	7 (6.2)	60 [60,240]
	Swimming	6 (5.3)	90 [32,170]
	Diverse[1]	11 (9.7)	n/a
	None	44 (38.9)	n/a
Moderate (n = 43)	Walking	20 (46.5)	210 [120,274]
	Cycling	17 (39.5)	90 [41,240]
	Strength training	7 (16.3)	120 [90,295]
	Bodily exercising	4 (9.3)	170 [130,270]
	Diverse[2]	11 (25.5)	n/a
	None	14 (32.5)	n/a
Mild (n = 41)	Walking	13 (31.7)	175 [102,594]
	Strength training	11 (26.8)	150 [69,270]
	Cycling	11 (26.8)	60 [40,180]
	Hiking	7 (17.1)	180 [120,265]
	Bodily exercising	5 (12.2)	85 [67,187]
	Diverse[3]	12 (29.3)	n/a
	None	14 (35.1)	n/a

Explanation: Data presented as absolute numbers and median [Q1, Q3]; PA=physical activity; multiple answers were possible; n/a = not applicable, bodily exercising can include rehabilitation sports as well as functional training.

[1]= Golf (n = 2), Dancing (n = 2), Hiking (n = 2), E-sports (Nintendo Switch; n = 1), Stand up Paddling (n = 1), Nordic Walking (n = 1), Tennis (n = 1), Soccer (n = 1);

[2]= Physiotherapy (n = 3), Hiking (n = 3), Jogging (n = 1), Horseback riding (n = 1), Golf (n = 1), Swimming (n = 1) Nordic Walking (n = 1);

[3]= Jogging (n = 4), Physiotherapy (n = 2), Skating (n = 1), Basketball (n = 1), Badminton (n = 1), Nordic Walking (n = 1), Pilates (n = 1), Gymnastics (n = 1).

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stated to be physically active for 42 [13, 83] minutes per day. Patients with severe haemophilia conducted 7095 [4757, 9801] steps per day with a median of 45 [22, 71] minutes per day of self-reported PA.

Concerning handgrip strength, a statistical difference between the severity phenotypes ($p = 0.030$) was observed but could not be confirmed by Bonferroni post-hoc testing. A significant correlation was further observed between handgrip strength and HJHS (rho = -.362, $p < 0.001$). The analysis of the influence of HJHS on PA revealed inverse correlation between HJHS and objective PA (rho = -0.239, $p < 0.001$), though no influence on subjective PA ($r = -0.059$, $p = 0.489$). In addition, the comparison of BMD, TBS and lean mass between the severity phenotypes, displayed in Fig 1, indicates that BMD was significantly lower in patients with severe haemophilia compared to patients with mild haemophilia ($p = 0.007$, post hoc $p = 0.006$). Also, lean mass was significantly reduced in patients with severe haemophilia compared to patients with moderate haemophilia ($p = 0.004$, post hoc $p = 0.012$). However, TBS did not differ within severity phenotypes ($p = 0.234$).

Supporting information for Fig 1 can be found as supplementary material (S1).Regarding research question 2) and 3), Spearman correlation analyses between objective and subjective PA, handgrip strength, TBS, BMD and lean mass were conducted. The results are displayed

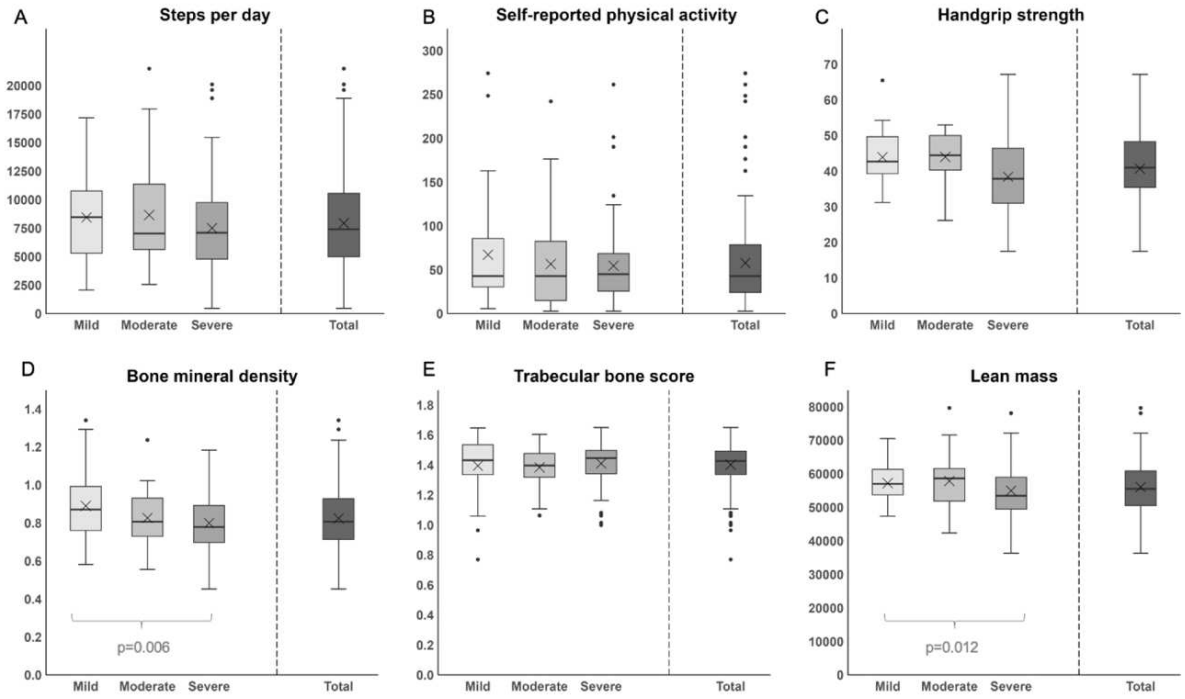

Fig 1. Boxplots showing the different severity phenotypes regarding physical activity, bone quality, lean mass and handgrip strength. Explanation: Boxplots of steps per day (in thousands; A; n = 223), self-reported physical activity per day (in minutes; B; n = 125), handgrip strength (in kilograms; C; n = 102)), Bone mineral density (in grams/cm²) of the left neck (D; n = 201)), trabecular bone score (Normal TBS ≥ 1.31, partially degraded between 1.30 and 1.24, degraded TBS ≤ 1.23; E; n = 194), and lean mass (in grams; F; n = 180). The central box signifies the interquartile range (IQR), with the mean displayed as the solid horizontal line and the median as X within the box. Whiskers display 1,5*IQR. Outliers are presented individually as dots.

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in Fig 2. Concerning research question 2), a positive correlation was found between objective PA (steps per day) and TBS (Spearman's rho = 0.202, p = 0.005). The Mann-Whitney-U-Test supported these findings, indicating a higher TBS of the patients with a higher step activity level per day (TBS median 1.442 [1.362; 1.527]) compared to the lower step activity level per day (TBS median 1.413 [1.295; 1.485]; p = 0.015). There was no significant difference regarding BMD or lean mass (p > 0.103) neither considering objective nor subjective PA. Further, a positive correlation was observed regarding handgrip strength and objective PA (rho = 0.231, p = 0.020). There was no association between hand grip strength and subjective PA (rho = 0.101, p = 0.453).

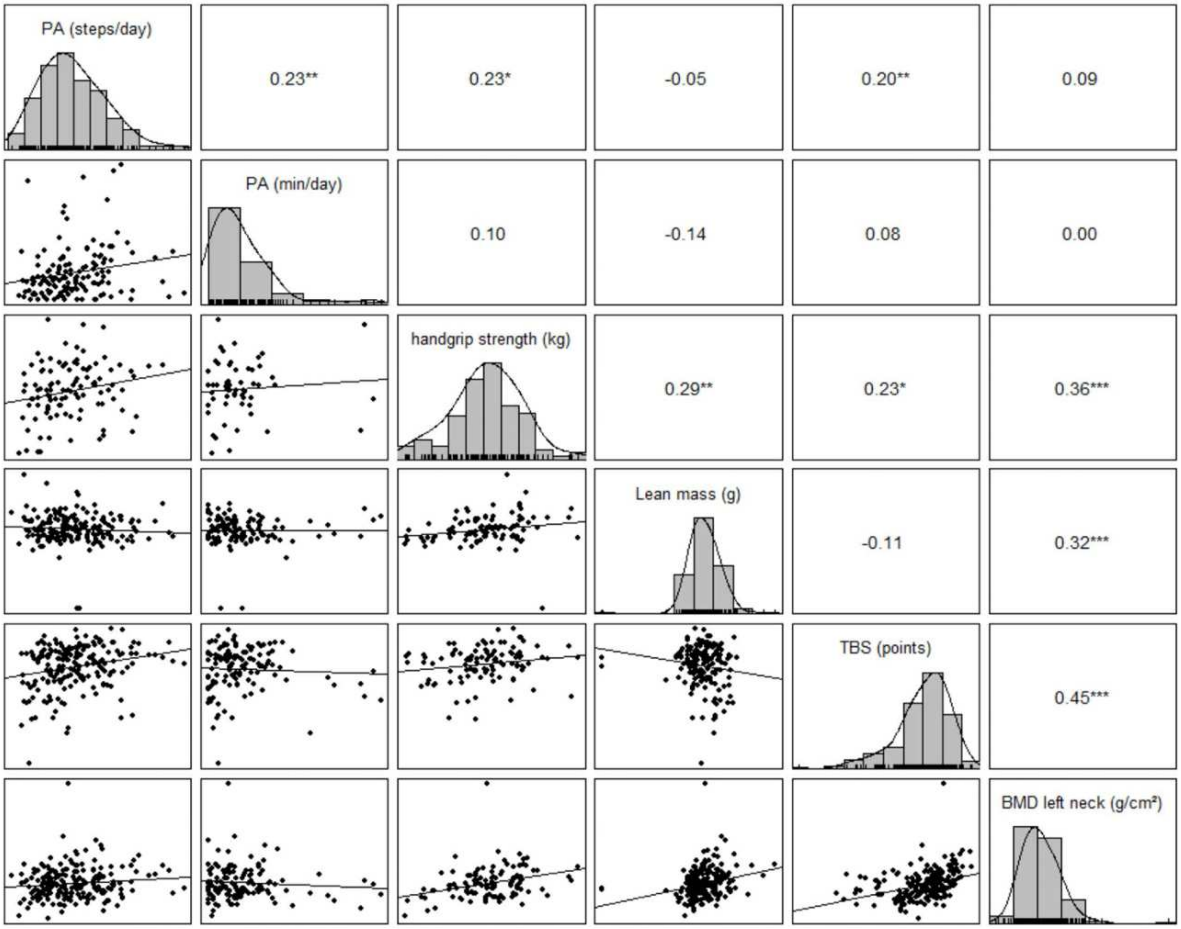

Fig 2. Spearman's Rank correlation of physical activity on bone tissue and lean mass. Explanation: * indicates significant difference (** p ≤ 0.01, *** p ≤ 0.001), PA (steps/day) = objective physical activity tracked via electronic activity tracking for the duration of 7 days (n = 223), PA (min/day) = self-reported subjective physical activity assessed via an activity diary for the duration of 7 days (n = 125), handgrip strength (mean value of left and right hand (n = 102)); lean mass (whole body (n = 180)); BMD = bone mineral density (n = 201), TBS = trabecular bone score (n = 194); Spearman's correlation coefficient was calculated and are displayed in the top right; individual data distribution of parameters is placed diagonally; scatter plots with regression line are shown in the bottom left part.

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With respect to research question 3), a significant moderate correlation was observed between lean mass and BMD (rho = 0.309, p < 0.001), though not between lean mass and TBS (rho = -0.11, p = 0.134). The Mann-Whitney-U-Test differentiating between higher amount of lean mass and lower amount of lean mass supported these findings revealing higher BMD in patients with increased lean mass (median 0.843 [0.745; 0.978]) compared to patients with less lean mass (median 0.768 [0.670; 0.869]; p < 0.001). There was no difference between the groups with regard to the TBS (p = 0.521). Handgrip strength is positively correlated with both BMD (rho = 0.361, p < 0.001) as well as TBS (rho = 0.221, p = 0.021) and lean mass (rho = 0.287, p = 0.003). The Mann-Whitney-U-Test differentiating between higher handgrip strength and lower handgrip strength confirmed that patients with increased handgrip strength show higher BMD (median 0.841 [0.755; 0.964]) compared to patients with lower handgrip strength (median 0.772 [0.684; 0.854]; p = 0.004). The same results were seen regarding the TBS scores (lower handgrip strength: 1.427 [1.333; 1.478] versus higher handgrip strength: 1.463 [1.404; 1.503]; p = 0.020) and lean mass (lower handgrip strength: 52883 [50019; 58859] versus higher handgrip strength: 56422 [51422; 62929]; p = 0.012).

A correlation analysis was conducted to evaluate the relationship between age and subjective as well as objective activity, and handgrip strength. The analysis revealed no significant associations (p > 0.05), with the exception of a weak inverse correlation observed between age and objectively measured PA (rho = -0.164, p = 0.014).

Furthermore, a Mann-Whitney-U-Test was done to evaluate differences regarding TBS, BMD and lean mass between the patients performing strength training (n = 37) and those, who did not perform strength training (n = 88). However, no statistically significant difference was found in any of these parameters (p > 0.542).

Discussion

Addressing three research questions, this investigation elaborated progressively on 1) the severity phenotype and its relationship to the level of PA, 2) the correlation of PA and handgrip strength with TBS, BMD, and lean mass, and 3) the impact of the lean mass and handgrip strength on BMD and TBS in PwH.

Regarding the first research question, the main finding of this investigation is that irrespective of the disease severity phenotypes the median step activity is 7392 [4981,10679] steps/day and 42 [22, 81] minutes/day, though both do not differ between severity phenotypes. It is assumed that the statistically significant difference is missed due to the high variability of the data in all three severity phenotypes. An inverse correlation between HJHS and objective PA was observed, which is in line with previous findings [40]. Though, the key finding is that PA levels in PwH are very heterogenic. This might be due to the fact that there are several influencing factors, as presence of pain, restriction of joint function or motivation [41]. However, there is only little research investigating daily step count in haemophilia, so that the present data of a large sample size (n = 223) can serve as orientation within the scientific community. Furthermore, handgrip strength was used as an indicator for overall fitness levels [36]. The present results of handgrip strength in PwH are comparable to previous findings [9,21,22]. The present study showed an inverse correlation between handgrip strength and the HJHS, though no significant difference between the severity phenotypes.

Considering the subjective activity diary results, most PwH in all severity phenotypes conducted low-impact activity such as walking or cycling. These findings are in line with previous research and are comparable to the European population [12,42].

Nonetheless, according to data of this study, 35.4% of the patients reported not being physically active at all, which is alarmingly high and elevated compared to the adult

non-haemophilic European (mean age 67.8 ± 8.9) population, where the prevalence of physical inactivity ranges from 4.9% in Sweden to 29.0% in Portugal [43]. The reason why PwH are physically inactive can be due to several reasons, including the presence of arthropathic related pain, fear of exercise-induced bleeding events or restricted movement function [6,9,44,45]. There might also be other non-haemophilia-specific barriers (i.e., time restrictions, low motivation) to be physically active, so that an individual approach by a comprehensive haemophilia care center is necessary to target the increase of PA.

Previous research further suggested a positive relationship between age and activity levels as well as handgrip strength [46]. To control for mediation effects, a correlation analysis was conducted within the present investigation, revealing that age only weakly correlates with objective PA, but not with subjective PA or handgrip strength.

To answer research question 2 and 3, associations between both objective and subjective activity levels and handgrip strength, TBS, BMD, as well as lean mass were analyzed. PA does have multiple short-term but also long-term effects such as increased lean mass, enhanced handgrip strength and a positive influence on bone remodeling [47,48]. Though, it needs to be emphasized that the recorded PA did not affect lean mass or BMD. With regard to the BMD, the results were expected as BMD needs high impact PA, e.g., in form of strength training, to adapt accordingly. However, the subanalysis of PwH performing strength training compared to PwH, who did not do strength training revealed no significant differences regarding BMD. This is most likely due to the fact that strength training can involve a broad variety of exercises and intensities, which has not been covered with the activity diary, meaning that there might be a measurement bias present within this analysis. However, it was observed that objective PA as well as handgrip strength are positively associated with TBS and handgrip strength also correlates with lean mass and BMD.

The present data show that lean mass does not affect the TBS, which agrees with previous literature [49]. Despite, the results of the present study confirmed that the higher the lean mass, the higher the BMD. This might be due to the fact that lean mass expresses osteogenic factors such as interleukin-6 and therefore positively influences bone remodeling [49–51]. Literature revealed that a combined training model involving resistance and weight-bearing training is recommended to effectively increase and individuals' lean mass and therefore positively impact the BMD [51]. Hereby, dynamic training with rather short stimulus duration and greater repetition frequency is highly suggested as this results in frequent loading and unloading through axial weight loading and muscle pulls [52]. This underscores the need of designing and monitoring an individualized training program for PwH by haemophilia care professionals. Such programs should prioritize bleeding prevention and address joint dysfunctions [7].

Strikingly, two different characteristics have made the TBS noticeable, which are highlighted in the following: First, TBS is positively associated with the recorded step activity while BMD is not. And second, TBS is rather normal in the haemophilic cohort though BMD is not [28]. The two components of bone quality (BMD and TBS) seem to vary in the magnitude of influencing factors. Impactful lifestyle factors on levels of BMD are well-known and studied, while only little research has been done on determinants of TBS. Though, research has shown that increased weight and low PA in childhood as well as the presence of diabetes or rheumatoid arthritis are associated with lower TBS in men [29,53]. The present investigation suggests that low impact PA is already sufficient to positively affect the TBS in PwH. Most of the PwH are able to conduct low impact PA and therefore promoting their TBS, which decreases the risk of fractures. Meaning, even though the patient is restricted in doing PA and shows reduced BMD, it is of major importance to promote step activity to decrease risk of fractures.

Strength and limitations

This investigation used valid objective activity tracking, utilizing the FitBit Alta HR and objective DXA derived data, which is considered gold standard for analyzing metrics associated with body composition, i.e., lean mass, BMD, and TBS, highlighting this study's high degree of quality [54,55]. Especially the evaluation of TBS in relation to PA is one major strength as this has not been investigated previously.

However, there are noteworthy limitations to declare. The main limitation is the recording of subjective PA as the activity diary is limited in expressiveness due to low standardization. This study lacks in investigating the nature of strength training more precisely, as strength training can encompass a broad spectrum from functional training to weight-bearing exercises either whole body or only within subregions. Furthermore, within this study a 7-day evaluation of activity was generated to get an insight in patients overall activity level, though no retrospective data on activity levels are present. As bone remodeling is a time-bound process, longitudinal studies investigating PA in PwH are necessary to check for a causal relationship of PA on BMD, but also for more information on TBS in PwH [56]. The present data of BMD are derived from the hip (neck), as these data are less prone to bias compared to BMD of the spine given a high prevalence of degenerative changes of vertebrae bodies, which can lead to false positive increased BMD scores [57]. However, this does not affect the TBS, but this needs to be noted since TBS can be determined at the spine only [57]. In addition, no data on the social or occupational status was gathered for this investigation, so that no socioeconomic facilitators or barriers for PA can be evaluated.

Conclusion

The present study showed that step activity and self-reported daily PA in minutes do not differ between the severity phenotypes. The majority of PwH in all severity phenotypes performed low-impact PA, such as walking or cycling. Most likely, these activities do not evoke sufficient strain to promote bone formation, so that no relationship between PA and BMD is observed. Handgrip strength correlates significantly with PA, lean mass and bone quality. Handgrip strength is seen as an indicator of PA level, underlining an indirect relationship between PA and the above-mentioned parameters. Moreover, there is a positive association between step activity and TBS, which is a key relevant finding as many PwH conduct walking as PA. With PwH, it is important to identify the optimal balance on PA that enhances bone quality and stimulates muscle activation, while minimizing excessive mechanical stress on the joints. Highlighting, safety measures and appropriate treatment enables PwH to conduct PA without risks of bleeding and should therefore be more promoted by respective haemophilia health care centers.

Supporting information

S1 Fig. Minimal data set for boxplots showing the different severity phenotypes regarding physical activity, bone quality, lean mass and handgrip strength.
(DOCX)

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5. Discussion

The following section will discuss the peer-reviewed **publications I-III**. First, the individual articles will be debated and viewed in the overall context and secondly, the proposed hypotheses will be tested. To get an overview on the conducted studies, a graphical abstract is displayed in figure 19.

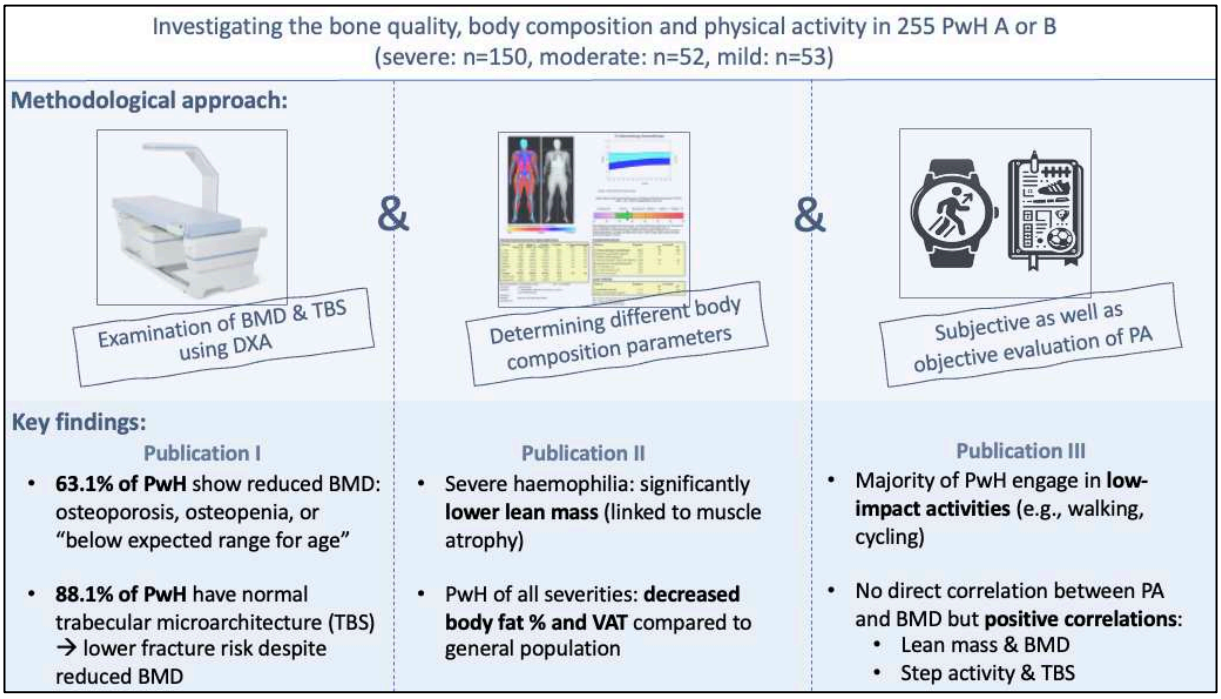

Figure 19: Graphical abstract of publications I-III

5.1 Bone quality in people with haemophilia

Publication I elaborated on the prevalence of reduced BMD in 255 PwH. In addition, the TBS was determined in a sample size of n=223 PwH. While the BMD has shown to be reduced in the total cohort of PwH, especially in people with severe haemophilia, the TBS is overall not affected. To diagnose osteopenia or osteoporosis, blood parameters have been considered as well. Results indicate a prevalence rate of 19.2% of the total cohort (n=104) affected by osteoporosis and 53.8% of osteopenic patients. Even in the younger age groups (< 50 years; n=151) 11.9% are considered "below expected range for age". It is of upmost interest to have a precise prevalence rate in PwH within a representative study cohort. 5.1% PwH of the investigated study cohort were suspected to be affected by secondary

osteoporosis (n=6, 2.3%) or secondary osteopenia (n=7, 2.7%), due to the intake of cortisone, antiepileptics or the presence of diseases such as hypogonadism. Still, after excluding these 13 PwH, the association between haemophilia and reduced boney quality is undeniable: The prevalence rate of PwH affected by reduced bone quality changes from 63.1% to 58.3%.Though, being precise, the younger aged PwH, who showed Z-scores below expected range for age must also be classified as having secondary osteoporosis or osteopenia respectively, since the primary osteoporosis per definitionem is related to age except in cases of rare genetic early-onset osteoporosis [59]. Through these epidemiological insights, the understanding of distribution of osteoporosis within PwH increases and should sensitize the health care professionals. Calling to action, an interdisciplinary team of haematologists as well as orthopedic clinicians is needed to improve haemophilia care management. In a multiple linear regression analysis, it was shown that age, followed by severity phenotype and joint status predicts the BMD best. Viral comorbidities or smoking do not seem to predict the BMD. Of course, aging is highly related to BMD in both the investigated haemophilic cohort as well as in the general population, which is well-known [59]. Though, the question arises why PwH, especially those with severe haemophilia, emphasizing the increased prevalence rate of reduced BMD also in the younger PwH, are frequently affected by osteopenia or osteoporosis.

The first person raising attention to osteoporosis in PwH was Gallacher in 1994 [47]. Ever since, researchers pointed to this association and conducted animal trials [90, 154, 155]. Within the scientific community, researchers agree on the fact that next to aging, a poor joint status in PwH is a predictor for low BMD [95, 156]. This has also been confirmed in the present findings. The mean HJHS of the investigated cohort was 17.5±16.6, which is similar to previous research investigating HJHS in different age groups [27]. The present study cohort was young (aged 43±15) in the context of osteoporosis research. But evidence states that already boys with haemophilia, whose joint status is not significantly affected show low BMD [157]. It is assumed that due to the avoidance of weight-bearing PA, peak bone mass is lower compared to non-haemophilic children [158]. If PwH do not reach the peak bone mass like non-haemophilic males, it is reasonable that BMD in both young and older PwH is lower. But neither joint status nor age hereby affect bone quality. So, to what extent does the factor itself play a role on bone quality?

It has been emphasized that different players of the hemostatic chain contribute to bone remodeling. Both factor VIII and IX deficiency cause an imbalance, which is related to reduced osteoblast activity and even increased osteoclast activity [89, 99, 154]. This imbalance is currently investigated, though the exact mechanisms are not yet defined [89]. Research of synovial tissue has shown that people with haemophilic arthropathy exhibit higher levels of RANK and RANKL as well as lower levels of osteoprotegerin compared to healthy control subjects [97]. Those molecular markers regulate bone turnover, leading to an increase of osteoclast activation [97]. Moreover, it has been shown, that (high-dose) prophylactic treatment regimen decreases the risk for low BMD, confirming the influence of the factor itself on bone quality [159]. In high-income countries most PwH have access to high-dose prophylaxis [160]. In turn, a special regard to BMD should be taken in low-income countries.

To answer the question, to what extent the factor deficiency causes disturbed bone remodeling or the consequences of the haemophilic arthropathy are the major impact for reduced BMD, future research on the microbiological level is needed.

Even though it is generally acknowledged that PwH show reduced BMD, studies investigating fracture risk and incidences in PwH are limited and inconsistent. Some investigations showed an increased risk for fractures in PwH, highlighting that the actual incidence of fractures is comparable to the general population [110]. Prospective studies investigating the incidence of fractures in PwH are scarce, though data from retrospective analyses report prevalence rates range from 4% to 37% [109]. The high prevalence rate of 37% might also include PwH experiencing non-osteoporotic-associated traumatic fractures. Still, a direct association has been observed between haemophilia severity, number of arthropathy- and haemathroses-affected joints, level of PA and the prevalence of fractures [109, 111]. Research indicates that the most common sites for fractures were femur and tibia, as well as wrist, elbow, radius and fibula. However, vertebral as well as hip and forearm fractures are considered typical osteoporotic fractures [161]. It has been stated that in most cases, fractures occurred after minor trauma, such as falling from a standing position, within PwH aged 28-30 years [109]. It can be concluded that the risk for osteoporotic fractures is increased in PwH, though overall fracture risk is

neutral compared to the general population and individual factors such as disease severity, level of PA and joint status for instance need to be considered [110, 111].

Low levels of fractures might be due to a less risk-prone lifestyle which accounts for the majority of PwH given the bleeding risk, but also because of the majorly normal status of trabecular bone [44]. **Publication I** demonstrated that TBS is decreased in 18.7% only. Meaning that even though the BMD might be reduced, the TBS is largely normal. This leads to a decrease of the overall FRAX® from a mean of 4.4% to 2.8%. Within the German non-haemophilic population, the average FRAX® score is 7.2% for males aged 50 and older [162]. What exactly happens on the microscopic level in the bone in PwH is still a matter of research. It seems that the BMD is affected by either the factor deficiency itself or by the respective consequences though the trabecular bone remains unaffected. This is a crucial finding as usually the reduction of BMD directly correlates with the TBS [163, 164]. This has been shown in non-haemophilic subjects, where the TBS decreases mostly linear to the decrease of BMD [163]. However, due to the innovative nature of TBS analysis, further research is needed in both haemophilia and non-haemophilia study cohorts to confirm the significance and generalization of TBS.

The FRAX® is a validated algorithm, which is widely used to determine risk of fractures. Nonetheless, evidence states that FRAX® is good in identifying subjects who will not experience fractures but tends to underestimate the risk in subjects who will [165]. There is recent development of a tool called *bone strain index, which* might also be helpful for the determination of first and has already been validated for predicting re-fracture risk [166, 167]. This qualitative of bone strength index calculates the stress and strain status of the bone considering the distribution of bone mineral density, geometry of the bone and the persons' body weight [166]. This way, the resistance of a bone to mechanical loads can be assessed. The bone strain index correlates with bone deformability and fatigue. In figure 20, the load on the femur is compared to using a tree and branches of different bone mineral density and geometry in standing position is displayed. In this example, the applied force at the greater trochanter is acting downwards.

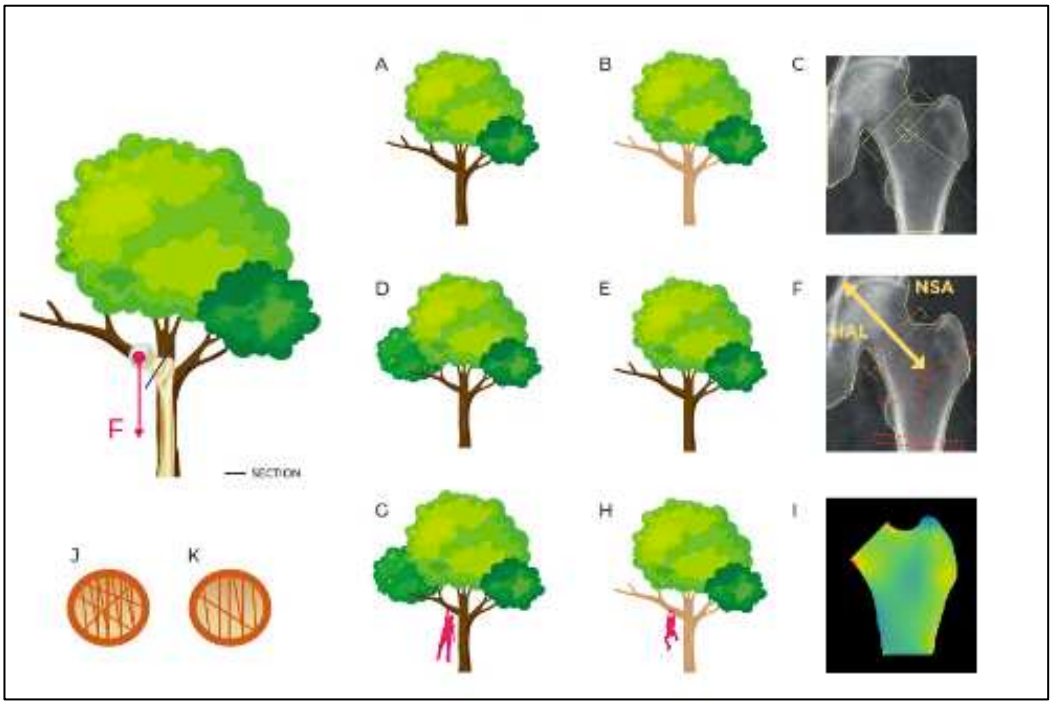

Figure 20: Simplification of the strain on the femur in a standing position comparing it with a tree with a force acting on a branch according to Ulivieri et al. (2022)

Explanation: F = Force; ***A and B*** *describe the same branch with different material density (as inferred by BMD DXA scan represented in* ***C****;* ***D and E*** *describe the branches of the same material with different shape (as described by geometry parameters (hip structural analysis and hip axis length) in* ***F****);* ***G and H*** *describe different branches in different load situations (femurs with different BMD, different geometry and different applied load as represented in femoral bone strain index analysis in* ***I****);* ***J and K*** *describe different inner structures (concept similar to TBS even though TBS does not apply to femur region)*

The bone strain index is an innovative tool derived from engineering, where this approach is commonly used to examine strain status of an object and has been adjusted on DXA-derived bone information. The index is automatically inferred from lumbar and femoral DXA-scans. It is suggested that a higher strain level indicates a higher risk for fractures. Clinical evidence on the bone strain index has been gathered since 2018 and showed positive results on the prediction of fragility fractures and refractures as well as treatment efficacy [125, 126]. Highlighting anabolic treatment showed increase of BMD going along with lower bone strain index. Of course, future research is needed to validate the bone strain index

especially across different ethnicities to provide generalization and within subjects suffering from different pathologies.

A further relevant factor for good bone quality is vitamin D. In both the haemophilic as well as the general population, vitamin D deficiency is a common phenomenon and frequently associated with low BMD [168, 169]. Within the present study cohort, 19.2% (n=49/255) had a vitamin D deficiency (<12 ng/ml). Within the European non-haemophilic population, prevalence rates of 13% are postulated [170]. Research indicates that already in children, low vitamin D levels and low BMD are observed [171].

Moreover, prior investigations showed that osteoporosis in PwH remains constant over time, meaning that the BMD does not get worse [172]. Also, PwH receiving antiosteoporotic treatment did not experience a change of the BMD [172]. It needs to be emphasized that the treatment mode of action is of particular interest. As stated within the theoretical background, osteoporosis can be treated through two different approaches: 1) decelerating bone resorption or 2) by promoting bone formation (anabolic treatment). Subsequently, BMD stays steady when treating with the first approach, though increases with anabolic treatment [173]. However, it would be of great interest to do a follow-up assessment of the study investigating osteoporosis over time in a haemophilic cohort, comparing two groups with the different treatment approaches, while also checking for incidence of fractures.

5.2 Body composition

There is only little evidence on body composition in PwH, though it is frequently suggested that many PwH are affected by obesity and reduced lean mass. **Publication II** dealt with the body composition analysis of 201 PwH of all three severity phenotypes and provides valuable reference data. One key finding is that people with severe haemophilia show significantly less lean mass compared to people with moderate or mild haemophilia. This is not surprising, as many PwH are affected by muscle atrophy because of haemophilic arthropathy. To avoid pain, PwH adopt to relieving posture, leading to atrophies as the relieved muscle does not get enough stimuli [174]. A double blind randomized controlled trial has shown that already one week of immobilization leads to a reduction of fat free mass of 3.6% in

the lower limb [175]. It is known that overall contracting muscle mass mediates myokines, such as IL-6, which positively impacts metabolic processes by increasing insulin sensitivity and promoting fat oxidation [176]. Hence, along with muscle atrophies, insulin sensitivity decreases. Though as stated in **publication II,** the extent to which local atrophies influence metabolism remains a matter of research. Nevertheless, it can be assumed that the above-mentioned metabolic alterations can occur in PwH with atrophies and should therefore be investigated in future research. Previous literature points towards the relationship of muscle strength and risk of falling due to lack of stabilization and subsequent impaired balance [177]. Recent evidence does confirm higher risk of falling in PwH compared to healthy controls [137, 178]. In regard with the findings of **publication I,** which indicated an increased prevalence of reduced BMD in PwH and the findings of **publication II**, which underlined the reduced lean mass in PwH, it needs to be emphasized that respective muscle-building measures should be promoted.

Moreover, it can be assumed that people with severe haemophilia show increased fat mass compared to people with moderate or mild haemophilia given their functional restrictions and potential less PA levels. Based on the present investigation this assumption turns out to be false, as there is no statistically significant difference between the severity phenotypes. Having a look at previous literature, it needs to be emphasized that there is no consensus. Some investigations indicated such statistical difference, showing that people with severe haemophilia have a higher BMI compared to people with non-severe haemophilia, while others did not observe this tendency [131, 179]. Hereby, muscle atrophy as well as HIV status, which both are more frequently present in people with severe haemophilia, were identified as significant predictors for higher BMI. However, it is important to note that the sample size of the study was relatively small (n=30/88) [179]. HIV has not been investigated within the present study, since the sample size of n=27/201 is likely underpowered. However, in non-haemophilic study cohorts, the relationship of increased BMI and presence of HIV has been detected and should therefore be considered in haemophilic individuals [180].

Compared to healthy controls, some investigations point to the fact that PwH show increased body fat distribution and decreased lean mass [131, 181]. The present study lacks a healthy control group, so that the present data was compared with

previous research. Concerning the overall body fat distribution, the analyzed PwH show a mean value of 29.2±6.2%, which is comparable to reference data of the European male population, showing a mean body fat percentage of 29.3±7.3% [121]. Enabling a direct comparison, table 2 shows the reference data of the non-haemophilic general European population as well as the data of the present investigation subdivided within the age groups.

For VAT, there is no consensus on reference values for the European population, but it is confirmed that VAT increases significantly with age [118, 121]. Reference data suggest a mean VAT of 424±385 g in healthy subjects aged 18-29, similar but slightly higher than the PwH data of 404±150 g. In healthy subjects aged 40-80 (65.9±9.1 years), the mean VAT is 1660±687 g, which is higher than in PwH [32, 33]. This difference might be partially explained by the older reference groups' increased BMI, leading to a systemic bias and high standard deviation [118, 121]. As already emphasized in **publication II,** the low proportion of VAT in haemophilia needs further investigation.

Despite this, the mean android/gynoid ratio of 0.6±0.2 is similar to non-haemophilic European reference data (0.7±0.2). To our knowledge, there are no previous studies on the android/gynoid ratio in PwH, though it might be comparable to waist-to-hip ratio. It has been observed that 66% (n=35/53) of PwH analyzed had an increased waist-to-hip ratio [131]. Android fat is known to indicate cardiovascular risk, while gynoid fat might be protective [121, 182].

Table 2: Reference data for body composition parameters of non-haemophilic European men based on Ofenheimer et al. (2020) compared with the present data of people with haemophilia

Variable	Total		Age 18-29		Age 30-39		Age 40-49		Age 50-59		Age 60-69	Age 70-<82	Age 60-80
	non-PwH	PwH	non-PwH	PwH	non-PwH	PwH	non-PwH	PwH	non-PwH	PwH	non-PwH		PwH
BMI (kg/m²) M±SD	26.6±4.3	26.1±4.5	24.2±3.8	24.0 ± 2.9	25.9±4.0	26.9±6.7	27.0±4.2	26.6±4.1	27.8±4.1	27.1±3.9	28.2±4.0	28.1±3.7	26.6±3.1
Fat/height² (kg/m²) M±SD	7.7±3.0	7.9±3.9	6.0±2.7	6.2±2.0	7.1±2.8	8.1±4.3	8.0±2.9	8.5±2.8	8.6±2.8	8.8±2.2	9.0±2.7	9.3±2.7	8.5±2.2
Lean/height² (kg/m²) M±SD	17.8±1.8	17.5±4.9	17.3±1.9	16.6±1.9	17.8±1.9	17.2±2.9	18.1±1.8	17.2±1.9	18.2±1.8	19.2±9.7	18.1±1.7	17.8±1.5	17.0±1.6
Body fat percentage (%) M±SD	29.3±7.3	29.2±6.2	24.8±7.4	25.9±6.0	27.7±6.9	29.5±7.1	29.8±6.5	31.2±5.3	31.3±6.2	29.5±5.1	32.7±5.7	33.6±5.9	31.9±5.0
Est. VAT mass (g) M±SD	1218±939	737±431	424±385	604±150	767±543	667±495	1243±755	808±317	1612±894	868±370	1904±914	2037±880	1092±405
Android/ Gynoid Ratio M±SD	0.7±0.2	0.6±0.2	0.4±0.1	0.4±0.1	0.5±0.2	05±0.1	0.7±0.2	0.7±0.2	0.8±02	0.7±0.2	0.8±0.2	0.9±0.2	0.8±0.2

Explanation: Age in years; non-PwH = non-haemophilic European men; PwH = People with haemophilia

As mentioned beforehand, life expectancy of the haemophilic population has fortunately increased over the past decades, so that these findings call to action for a higher awareness on age-related increased fat distribution, which should be considered at individual cardiometabolic risk assessment.

The DXA is regarded as the gold standard for body composition analysis. Consequently, this investigation (**publication II**) provides reliable results, enhancing existing literature on body composition in PwH. The study's large sample size (n=201) and wide age range (18-79 years) contribute to valuable reference data for haemophilic populations. However, this investigation does not provide reference data for the different age groups within severities due to too small sizes of the subgroups. Since body composition varies across ethnicities, it is important to note that this study's cohort is exclusively of Caucasian ethnicity, restricting the transferability to non-Caucasian populations [183]. For instance, research has shown that VAT is higher in Asians and less in Africans compared to Europeans and the prevalence of obesity in African American populations is increased compared to white Americans [184, 185]. It needs to be considered that due to rapid changes in nutrition and eating habits, cross sectional body composition analysis should be interpreted with regard to the temporal context [186, 187].

Linking together **publication I** and **publication II,** the following section will elaborate on the impact of body composition on the bone quality. Through various mechanisms, such as mechanical loading, inflammation or hormonal factors, the bone quality is influenced. Starting with mechanical loading, it is known that bones adapt in response to physical stress [139]. Physical stress can either originate from PA but also from increased weight. Surely, in the optimal case the weight should be increased due to high muscle mass rather than fat mass, though both cause mechanical stress on the bone [139]. Fat can have a protective effect for bones when falling, though excessive amounts of fat, especially VAT, can cause the expression of inflammatory cytokines [188]. These in turn can negatively affect the bone metabolism, leading to a decreased bone quality [188]. Moreover, the hormonal status is influenced by body composition parameters. The endocrine system is complex and influenced by multiple factors though some need to be highlighted as follows: A high amount of fat mass can lead to lower levels of testosterone and growth hormones, which both play a pivotal role in maintaining a

good bone quality [189]. In contrast, a higher muscle mass is positively related with hormonal balance as it can lead to higher testosterone levels as well as it might down regulate cortisol, resulting in a better bone quality [189].

5.3 Influence of physical activity on lean mass and bone quality

Publication III elaborated on PA, bone quality and lean mass. Hereby, results of **publication I** and **II** are considered together in the context of PA above all. Though first of all, the main focus was to describe the PA levels of PwH within the different severity phenotypes. Afterwards, the activity as well as handgrip strength levels were put into context with the results of **publication I** and **II.**

It is well-known that PA affects overall health including bone quality. PA in haemophilia is generally an often-debated topic. As already mentioned in the theoretical background, PA was not recommended for a long time to PwH. Luckily, today better treatment options enable PA in haemophilia. It has been observed that especially the joint status and prevalence of pain limit PA levels in haemophilia [138]. Thus, it was assumed that people with severe haemophilia are less physically active compared to people with mild or moderate haemophilia. The results of the present investigation on PA between the severity phenotypes do not show a significant difference. Though, the descriptive data suggest that the high variability of the data within all three haemophilia severities explain the missing significant difference. An inverse correlation of the joint status and step activity has also been detected in the present study cohort, confirming prior research [138]. However, this is the first study comparing the PA levels within the different severity phenotypes. Most of the investigations on PA in haemophilia either combine moderate and mild haemophilia or do not include people with mild haemophilia [101, 138]. The present results underline that PA in PwH is very heterogenic, making it impossible to generalize the results on the whole haemophilic population. Therefore, the haemophilia care takers need to target PA on an individual basis, though it needs to be considered that many PwH suffer from kinesiophobia and pain avoidance. Previous research shows that especially PwH with poor joint situations are more affected by kinesiophobia [137].

The injury risk through exercising can be categorized to cluster PA accordingly [101]. These categorizations can serve as an orientation for PwH as well as people taking oral coagulations for instance, to choose a low-risk associated type of PA. Three categories are used respectively: Category I comprises low-risk activities with minimal injury potential, characterized by low internal risk factors (e.g., joint stress, falling risk) and external factors (e.g., environmental conditions, opponent aggression). PA such as walking or swimming belong in this category. Category II encompasses activities with higher risk for injuries. But these types of PA can still be safely undertaken by PwH considering specific safety precautions (e.g., proper safety gear, professional supervision). For instance, tennis or cycling belong to category II. The third category activities pose risks for all participants, including those without bleeding disorders, featuring physical contact, hazardous equipment (e.g., hard balls), and challenging surfaces, which may result in severe traumatic injuries. Skiing or boxing belong in this category [101]. Recent studies showed that bleeding risk is not increased if the persons is well controlled with factor substitution and adheres to the treatment regimen respectively [101, 190]. The results of the present study are in line with previous research, which shows that PwH mainly do walking or cycling [100, 101, 132]. Reasons for this might vary. Either the joint function and pain are too restricting to conduct other PA or maybe just because cycling and walking are low-threshold types of PA. Meaning that almost everyone can conduct these types of PAs. In comparison, within the general non-haemophilic population, walking and cycling are the most popular types of PA as well [191].

In terms of injury or bleeding risk, walking and cycling are classified as category I or II activities. Focusing on the impact of PA on bone health, it needs to be highlighted that cycling and walking produce only light strain on the bone, which is insufficient to trigger bone remodeling responses. There is a mild to moderate positive correlation seen in step activity and TBS, which was not observed for BMD. Concerning the BMD, this outcome was anticipated since BMD requires high-impact physical activity, such as strength training, to show significant changes [192]. Nonetheless, a subanalysis comparing PwH, who engaged in strength training to those who did not, revealed no significant differences in BMD. In the present investigation, the recorded PA neither significantly impacted BMD nor lean mass, which might be due to the variability in the types and intensities of conducted

strength training exercises. The variations of the strength training were not fully captured by the activity diary, indicating a potential measurement bias. Regarding handgrip strength, which serves as a correlate for overall fitness levels, no significant differences between haemophilia severity phenotypes was detected. However, objective PA levels and handgrip strength were found to be positively associated with TBS, emphasizing that hand grip strength also correlated with lean mass and BMD.

Further, a prospective cohort study investigating the association between handgrip strength and falls showed that poor handgrip strength is associated with an approximately 3-fold increase of falls [193]. In addition, it needs to be highlighted that PwH are predisposed to fall, as they scored poorer in falls efficacy and performed worse in clinical motor performance tests compared to healthy controls, especially when advanced arthropathy is present [137, 194]. Of course, this finding is of upmost interest as risk of falling is a key relevant factor in osteoporotic subjects. This should also be taken into account when recommending PA as a haemophilia caretaker also in the context of potential kinesiophobia, which might be increased after experienced falls

The present study elaborated further on the correlation of lean mass with TBS and BMD. The gathered data show that lean mass does not affect the TBS, which agrees with previous literature [195]. However, the results of the present study confirmed that the higher the lean mass, the higher the BMD. Previous literature indicated that BMD is positively associated with BMI. It needs to be highlighted that the BMI is a biased index, which can easily deteriorate according to the individuals' body composition. Both high fat mass as well as high lean mass cause an increased BMI. Of course, an increased BMI because of a high amount of lean mass is more desirable compared to high fat mass. The beneficial effect of lean mass on BMD might be due to the fact that lean mass expresses osteogenic factors such as interleukin-6 and therefore positively influences bone remodeling [196, 197]. However, as previously described in **publication II**, people with severe haemophilia have less lean mass compared to people with moderate or mild haemophilia, which is due to the worse joint situation leading to muscle atrophy. Oftentimes, muscle atrophies occur on one side only. To oppose this, again machine training is most effective, as it allows to train an individual muscle (group) only. In contrast, most

other types of exercising involve both sides of the extremities. Furthermore, it has been highlighted in **publication I** that TBS remains relatively normal in the haemophilic cohort, despite reductions in BMD. This suggests that even low-impact PA such as walking, can positively influence TBS. Since most PwH can engage in low-impact physical activity, promoting this type of activity can enhance their TBS and reduce fracture risk. Therefore, despite restrictions on PA and reduced BMD, it is crucial to encourage step activity to lower the risk of fractures.

5.4 Hypotheses testing and answering the research questions

Publication I

1) *Research question: What is the prevalence of reduced bone quality (bone mineral density and trabecular bone score) in* people *with haemophilia?*

For research question 1, no hypothesis was postulated due to the explorative nature of the research question. However, research question 1 will be answered in the following: 19.2% of the included PwH aged > 50 years showed osteoporotic and 53.8% osteopenic values. Regarding the PwH aged < 50 years, 11.9% showed a BMD, which considered below expected range for age. However, TBS is reduced in 18.7% of PwH only.

2) *Research question:* Does the bone quality differ between mild, moderate or severe haemophilia?

   Hypothesis: People with severe haemophilia are more often affected by reduced bone quality compared to people with moderate or mild haemophilia.

This hypothesis can partially be accepted. Bone quality is represented by BMD as well as trabecular bone. Regarding the BMD, the hypothesis can be accepted though trabecular bone is equal within the different haemophilia severities. The percentage of people with severe haemophilia showing reduced BMD (either osteoporotic or osteopenic values) is 83.3 %, while in people with moderate haemophilia 66.7% are affected and only 57.7% of the people with mild haemophilia. In PwH younger than 50 years old, where the Z-score is used to check for deviations

in the same age group, the linear decrease cannot be confirmed. Here, 12.5% of people with severe haemophilia were considered as “below expected range for age”, 13.8% of people with moderate haemophilia though only 7.7% of people with mild haemophilia are affected. Concerning the trabecular bone, in people with severe haemophilia 16.7 % were (partially) degraded, in people with moderate haemophilia the percentage of (partially) degraded TBS is 23.4% and in people with mild haemophilia 20.9% are affected. There was no statistically significant difference observed.

3) *Research question:* How high is the risk of fractures in a representative haemophilic cohort based on bone quality results?

For research question 3, no hypothesis was postulated due to the explorative nature of the research question. However, research question 3 will be answered in the following: Along with BMD analysis, the FRAX® was calculated in PwH ≥ 30 years (n=186). The mean risk of suffering a fracture in the next 10 years was 4.4±.3.0%. After TBS adjustment, the FRAX® decreased to 2.8±3.7%. As FRAX® is validated in subjects aged > 40 years, a subanalysis differentiating in PwH aged between 30 and 40 years (n=69) and PwH older than 40 years (n=117), was performed. In PwH older than 40 years the FRAX® was 4.8±3.4% and decreased to 3.6±4.2% after TBS adjustment. In PwH aged between 30 and 40 years FRAX® was 3.7±2.0% and after TBS adjustment 1.5±2.1%. However, previous studies investigating risk of fractures are inconsistent and findings vary between 4 and 37% [109]. Also, compared to non-haemophilic German males ≥ 50 years, the average FRAX® score is 7.2%, which is considerably higher compared to the investigated PwH [162]. Thus, it can be concluded that the risk of getting a fracture in the upcoming 10 years within the present study cohort is rather low.

Publication II

4) *Research question:* Is the body composition in German people with haemophilia altered compared to non-haemophilic Europeans with regard to body fat percentage, estimated visceral adipose fat and lean mass?

   Hypothesis: German people with haemophilia suffer from increased body fat percentage, increased visceral adipose tissue and decreased lean mass compared to non-haemophilic Europeans.

This hypothesis can partially be accepted. The investigated study cohort of 201 PwH showed no increased body fat percentage compared to non-haemophilic European reference data (PwH: 29.2±6.2%, non-haemophilic Europeans: 29.3±7.3%). The results of the VAT vary across age groups. In the youngest age group (18-29 years), PwH showed a mean VAT of 404±150g which is comparable to non-haemophilic Europeans aged 18-29 years with a mean VAT of 424±385g. Within older age groups, European non-haemophilic males showed meaningly higher values compared to PwH. In healthy subjects aged 40-80 (65.9±9.1) years the mean VAT is 1660±687 g, while in PwH the mean VAT ranges from 808±317g (PwH aged 40-49 years) to 1092±405g (PwH aged 60-80).

Regarding the lean mass it can be stated that the overall lean mass is lower in young PwH, aged 18-29 (16.6±1.9 kg/m^2) compared to reference data of healthy subjects with 17.3±1.8 kg/m^2. However, the general European male population shows a lean/height^2 of 17.8±1.8 kg/m^2 across all age groups, which is similar to PwH (17.5±4.9 kg/m^2). Though it needs be highlighted that the standard deviation in PwH is higher compared to the reference data which limits the degree of comparability. The heterogeneity of lean mass in PwH is further underlined by the increased standard deviation in people with severe haemophilia (17.4±6.2 kg/m^2).

5) *Research question:* Does the body composition with regard to body fat percentage, estimated visceral adipose tissue and lean mass deviate within the haemophilia severity phenotypes?

   Hypothesis: People with severe haemophilia have increased levels of body fat percentage, visceral adipose tissue and show decreased lean mass compared to people with moderate or mild haemophilia.

This hypothesis can partially be accepted. Concerning the body fat percentage and VAT, there was no statistically significant difference between the severity phenotypes overserved ($p>0.474$). However, lean mass in relation to height was significantly decreased in people with severe haemophilia compared to people with mild haemophilia ($p=0.037$). Further, appendicular lean mass of the right arm was significantly less in people with severe haemophilia compared to people with moderate ($p=0.020$) or mild haemophilia ($p=0.035$). For the left arm, a statistically significant difference is missed ($p=0.058$), while the legs showed significances after calculating the Kruskal-Wallis-Test though post hoc analyses did not confirm these indications.

Publication III

6) *Research question*: Do PA levels deviate between the haemophilia severity phenotypes?

   Hypothesis: people with severe haemophilia are less physically active compared with people with moderate or mild haemophilia. There is a linearity given within the severity phenotypes.

This hypothesis can be declined. People with severe haemophilia showed equal results of step activity (median [1.IQ, 3.IQ]: 7392 [4981,10579]) as well as subjective reported active minutes per day (38 [17, 68]) compared to people with moderate (steps per day: 7050 [5530, 11472]; subjective active minutes per day: 42 [12, 82]) or mild haemophilia (steps per day: 8466 [5219, 10785]; subjective active minutes per day: 42 [25, 85]). There was no significant difference between the severity

phenotypes neither for steps per day (p=0.162) nor for subjective active minutes per day (p=0.733).

7) *Research question*: How is PA associated with bone quality (bone mineral density and trabecular bone score)?

   Hypothesis: Physical activity positively affects bone quality and lean mass. The higher the activity level, the better the bone quality and the more the lean mass.

This hypothesis can partially be accepted. There is a mild to moderate positive correlation seen in step activity and TBS (rho=0.20, p<0.005). There is no association seen in step activity or self-reported PA and lean mass or BMD (p>0.05). The Mann-Whitney-U-Test supported these findings, indicating a higher TBS of PwH with a higher step activity (TBS median 1.442 [1.362; 1.527]) compared to the lower step activity (TBS median 1.413 [1.295; 1.485]; p=0.015). There was no significant difference regarding BMD or lean mass (p>0.103) neither regarding step activity nor subjective self-reported PA

8) *Research question*: Does handgrip strength correlate with PA levels, lean mass, and bone quality (bone mineral density and trabecular bone score)?

   Hypothesis: Handgrip strength is positively associated with PA levels, lean mass, and bone quality. A high handgrip strength is seen in PwH with high PA levels, who show better the bone quality and increased lean mass.

This hypothesis can partially be accepted. A positive correlation was observed regarding handgrip strength and objective PA (rho=0.231, p=0.020). There was no association between handgrip strength and subjective PA (rho=0.101, p=0.453). Handgrip strength is positively correlated with both BMD (rho=0.361, p<0.001) as well as TBS (rho=0.221, p=0.021) and lean mass (rho=0.287, p=0.003). The Mann-Whitney-U-Test differentiating between higher handgrip strength and lower handgrip strength confirmed that PwH with increased handgrip strength show higher BMD (median 0.841 [0.755; 0.964]) compared to PwH with lower handgrip strength

(median 0.772 [0.684; 0.854]; p=0.004). The same results were seen regarding the TBS scores (lower handgrip strength: 1.427 [1.333; 1.478] versus higher handgrip strength: 1.463 [1.404; 1.503]; p=0.020) and lean mass (lower handgrip strength: 52883 [50019; 58859] versus higher handgrip strength: 56422 [51422; 62929]; p=0.012).

6. Limitations

Publication I-III derived from one large prospective monocentric cohort study with some noteworthy (methodological) limitations to declare. The idea of the study was to evaluate bone quality, body composition and the role of PA. A major approach was to hereby examine potential differences and similarities between the three severity phenotypes. Because of this study design and research focus this study did not entail a non-haemophilic cohort-group and no intervention, so that causal relationships cannot be drawn.

The study took place at the university hospital Bonn and data was gathered with the Hologic DXA system, which may limit the comparability with other DXA systems. Also, data of bone quality and body composition are only valid for the studied Caucasian ethnicity, so that this investigation lacks in transferability. Going more into detail, BMD values tend to be higher in the lumbar spine, which may be attributed to methodological measurement errors in DXA, such as artificially elevated T-values caused by degenerative sclerosis of the lumbar vertebrae.

Moreover, the study revealed that vitamin D is deficient in 19.2% of PwH and there was no significant difference of vitamin-D-levels between the severities. However, a methodological limitation of this study is the lack of information on vitamin D supplementation. Hence, the informative value of the level of vitamin D should be taken with caution as the true rate of deficiency may be underestimated due to potential supplementation.

Elaborating on the analysis of body composition a bit further, it needs to be highlighted that study includes a large sample size (n=201) covering a broad age range (18-79 years). Though, it does not provide reference data differentiated by severity and age due to the small subgroups.

Within the process of the study, the study investigation team decided to include the assessment of handgrip strength. Therefore, this relevant parameter is only measured in n=102, which limits the expressiveness of the analysis.

Another limitation concerns the subjective recording of PA through a subjective activity diary, which lacks standardization and may not accurately represent the patient’s activity levels. Furthermore, this study assessed PA over a seven-day

period to gain insight into overall activity levels in the investigated PwH. While this provides a valuable insights of daily activity habits, it still lacks retrospective data and does not account for fluctuations in activity over longer periods. Given that bone remodeling is a time-dependent process, short-term assessments are limited in the expressiveness of the long-term effects of PA on bone quality.

7. Implications and prospects

The **publications I-III** provided multiple new insights regarding the bone quality, body composition and the PA levels in PwH. Emphasizing the large sample size of n=201-255 PwH, which enabled to depict representative prevalence rates for reduced BMD, reference values for body composition parameters as well as PA level within the different type of haemophilia severity. Especially data of people with mild haemophilia is scarce, emphasizing the benefit of this investigation for the scientific community. Thus, this explorative cohort study closes a major research gap regarding the respective interplay of bone quality and body composition considering effects of PA in people with mild, moderate and severe haemophilia.

The main question remaining is if the disease itself is the cause for developing osteoporosis or if the association between haemophilia and reduced bone quality is based on the consequences of the disease. The question arises whether haemophilia itself can or should be recognized as a cause for secondary osteoporosis. It is generally differentiated between causes for secondary osteoporosis and risk factors for osteoporosis. This differentiation is essential for osteoporotic treatment, as for secondary osteoporosis, commonly there is an underlying disease, which might be treated first or in combination with the osteoporosis. It gets even more complex when looking at the risk factors, of which some can be influenced, like PA levels, while some are a matter of fact, such as age [59]. Being affected by haemophilia is an underlying disease, which increases the risk to suffer from osteoporosis. Hence, haemophilia should be recognized as a risk factor for osteoporosis at least. Taking a glance on the future, it is assumed that better treatment options enable higher factor levels also during childhood and less physical restrictions during adulthood. Thus, it is highly suggested that the high prevalence of reduced BMD in haemophilia will decline.

For now, to ensure optimal clinical care and protect PwH from the consequences of undiagnosed osteoporosis, such as fracture risk, reduced mobility and spine misalignments, the present findings should be integrated into clinical practice. Particularly for the aging PwH it is crucial to incorporate osteoporosis screenings into clinical routine starting at age 30. Additionally, vitamin D supplementation should be provided as needed. As orientation for clinicians, a decision tree is

attached in figure 21. The term “DXA” might be specified to highlight the inclusion of TBS measurement and fracture risk assessment respectively. Further, the anamnesis should include the level of PA now as well as within the past years and during childhood to get an insight into possible peak bone mass alterations.

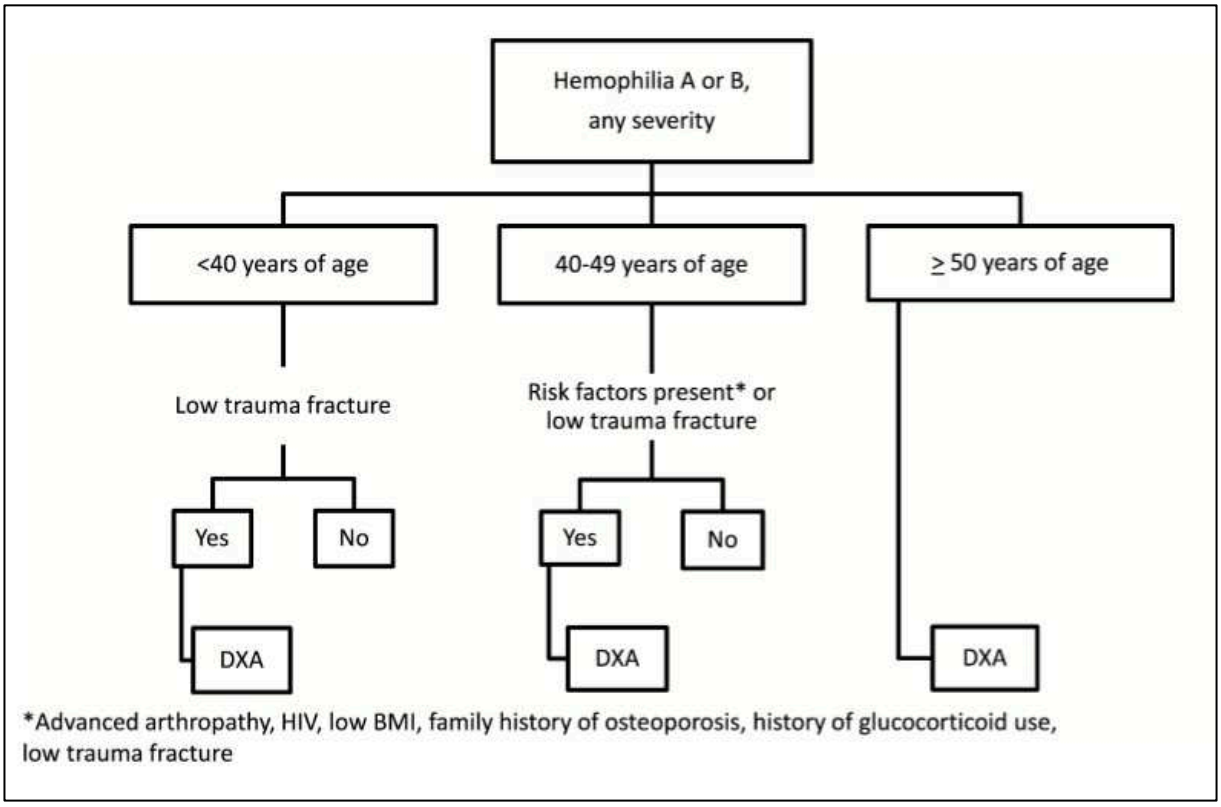

Figure 21: Decision tree for screening bone mineral density in people with haemophilia according to Kempton, et al. (2015)

Explanation: BMI = Body mass index, DXA = dual x-ray absorptiometry, HIV = human immunodeficiency virus

The present investigation is a cross-sectional method. This way, only the current status can be depicted though no causal relationships can be detected. So far, only little is known about the drug antiosteoporotic therapy in haemophilia. One relevant study dealing with this issue has recently been published though includes a small sample size of PwH affected with osteoporosis only (n=7) [172]. Future longitudinal studies are needed to investigate the outcome of comprehensive antiosteoporotic treatment and/or vitamin D supplementation in PwH. Along with reduced BMD, a prior study showed worse immune status in PwH (unpublished data). Clinicians

should be aware of this fact in order to control for the individual's immune status next to osteoporosis screening. Of course, PA has a major impact on the immune system, underlining the importance of engaging in regular PA again.

According to the German Guidelines of Osteoporosis the indication of drug therapy in osteoporosis is based on a specific risk calculator [64]. Hereby, different parameters such as experienced previous fracture, risk of falling based on an underlying neurological or geriatric disease (e.g. morbus Parkinson, multiple sclerosis) and presence of another disease (e.g. hypogonadism, rheumatoid arthritis) are considered. As a further implication for the future, it should be discussed whether hemophilia should be integrated into the risk calculator for the indication of drug therapy.

Moreover, with recent developments highlighting the importance of females with haemophilia, it is reasonable to also investigate their heightened risk of osteoporosis. Given the potential impact of chronic bleeding, factor deficiencies, and hormonal influences on bone health, studies should investigate the role of estrogen levels, vitamin D metabolism, and inflammatory markers in osteoporosis risk. Especially inflammatory markers should be of major interest in future studies with male PwH too. Whether haemophilic arthropathy, including the presence of (chronic) synovitis leads to local or systemic reduced bone quality is of major interest. But also, the role of atrophies and potentially increased VAT on bone quality in affected PwH could be investigated further to better understand these relationships. Both, the relationship between VAT and bone as well as the rather low VAT values, which were found in the present study cohort should be investigated further.

Additionally, research lacks in prospectively investigating bone quality and lean mass following a PA intervention in PwH [198]. It would be necessary to conduct a long-term randomized controlled trial in order to depict the most effective types and intensity of exercising for PwH. Osteology research has confirmed that combined training focusing on resistance training should be conducted to increase bone quality. Hereby, intensity plays a higher role than the duration of exercising and further, explosive strength should be trained through dynamic exercises. Figure 22 shows a forest plot evaluating the effects of different training characteristics on the

BMD as well as muscle strength, underlining that a combined training of resistance training and weight-bearing exercises has the strongest effect on BMD [196]. Regarding the weight-bearing exercises, it can be seen that repetitions do not matter as much as the load to affect BMD. Future investigations like the presented meta-analysis, examining the effect of the different training characteristics on TBS are needed. So, an optimized training schedule can be planned respectively.

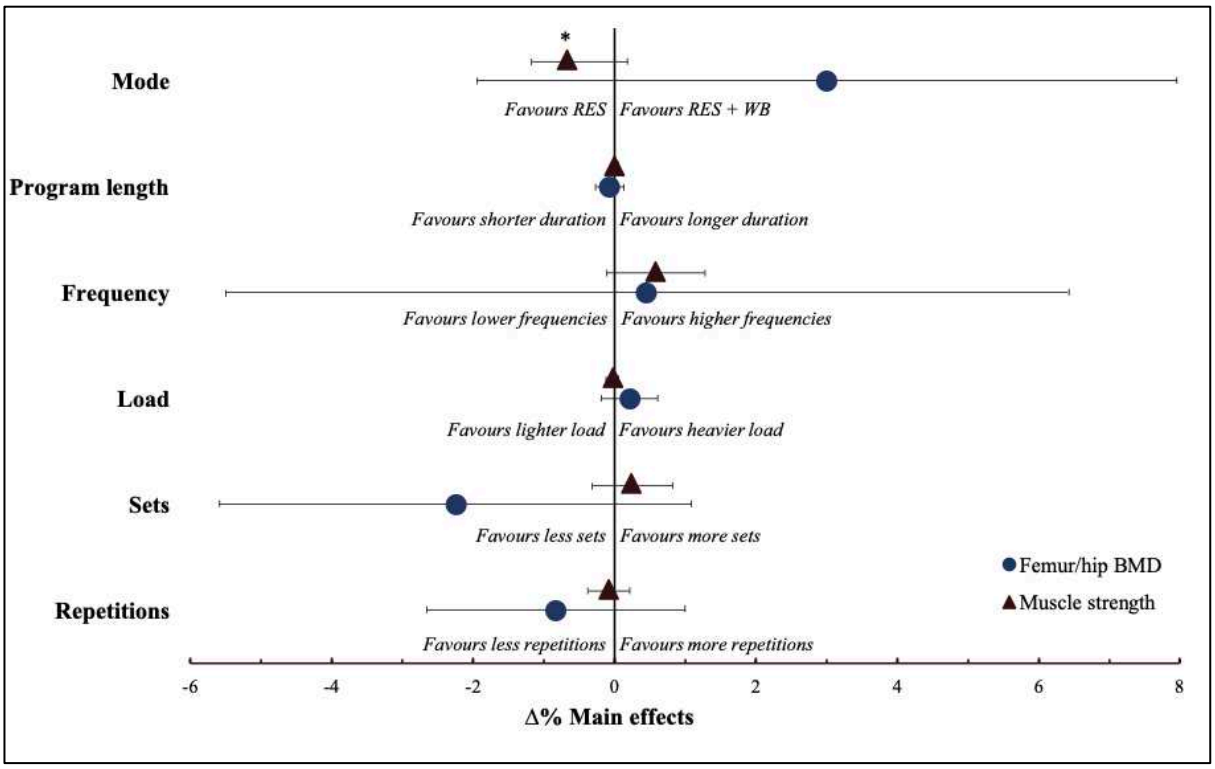

Figure 22: Effect of different training characteristics on the combined changes in muscle strength and bone mineral density according to O'Bryan et al. (2022)

Explanation: RES = resistance training only, RES + WB = combined resistance training plus weight-bearing/impact loading exercises

In 2014, a study investigating the effects of short-term resistance training on bone metabolism in people with severe haemophilia A was published [199]. It has been stated that within six weeks resistance training, including chest and shoulder press, hip flexion, extension and abduction as well as knee extension, squats and back extension using free weights and dumbbells leads to an improvement of bone

formation as the bone-specific alkaline phosphatase was increased in the intervention group [199]. Bearing in mind that based on the restrictions of many PwH, training options are limited. Though, previous research investigating six months of PA in PwH has shown that they are able to improve their physical performance regardless their joint status [200]. This training program included mobility, coordination, strength and endurance, which was carried out in a gym for 90 minutes. Highlighting, positive effects were especially seen in the patient's strength [200]. Machine training is seen as the most effective and most feasible way for people with individual limitations as many adaptions are possible [136, 200]. Leg press or cable pull exercises should be in focus to trigger bone remodeling based on machine training [201]. Moreover, exercising with additional electromyostimulation is used in people with motion restrictions to simplify muscle gain [202]. Research lacks in investigating electromyostimulation training in PwH focusing on the bone formation, though there is evidence that a whole-body electromyostimulation training has positive effects on the bone in osteopenic patients [203, 204]. In haemophilic populations, there is not much evidence on electromyostimulation, as haemophilia is considered an absolute contraindication to whole-body electromyostimulation [205]. Though a study published in 2006 showed that a six-weeks of local surface electromyostimulation training increases diameter of the rectus femoris by 24.3%, with no adverse events, indicating a new treatment approach [206]. These findings are in line with another study investigating local electromyostimulation for eight weeks in 15 patients with haemophilic arthropathy [207]. Of course, future studies are needed to first validate (local) electromyostimulation training in PwH and second to elaborate on the needed intensities to approach bone formation.

Another key finding regarding activity in PwH is that PA levels are not generalizable. The range within all three severity types respectively is large. Merely the variance of the subjectively recorded PA level of the people with severe haemophilia is not as large compared to people with moderate or mild haemophilia. Still, there is no significant difference between the severity phenotypes regarding their subjectively recorded PA, emphasizing too heterogenous data. Thus, it is highly suggested that haemophilia care takers need to evaluate the level of PA on an individual basis.

Moreover, it was shown that handgrip strength correlated significantly with BMD, TBS and lean mass. These findings are in line with previous research in both the haemophilic as well as non-haemophilic population, also showing a correlation of reduced handgrip strength to increased fall risk [193, 208-210]. Therefore, it is concluded that handgrip strength is an appropriate surrogate for body composition and bone quality as well as for risk of falls as mentioned before. Proposing, it could be an easy and feasible way to include the assessment of handgrip strength in clinical settings, to get an insight into the overall fitness of PwH.

The present analysis does not entail subjective data on physical performance, psychological effects of the presence of comorbidities or falls efficacy, for instance. Generally, the presence of orthopedic comorbidities in PwH has already been investigated by several researchers [36, 87, 168]. Though, with regard to the future, a further aspect to be investigated could be the subjective perception of body composition in relation to quality of life, for instance. This allows to evaluate the psychosocial domain of reduced BMD, lean mass, handgrip strength or PA with regard to quality of life.

Concluding, haemophilia can be seen as a risk factor for reduced BMD of which both, clinicians as well as PwH should be aware of. Haemophilia and its consequences are complex and have multiple facets, emphasizing the diverging joint situations, which need to be considered on an individual basis. Especially haemophilic arthropathy leads to altered body composition (i.e. increased prevalence of muscle atrophy). Though, according to the slogan “sport is medicine”, PA should be integrated into the people’s daily life to improve bone quality and multiple body composition parameters, which results in the increased overall quality of life. Future longitudinal studies are needed to elaborate on the effect of specific PA interventions on bone quality and the relation to body composition to be able to give precise recommendations for training programs.

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List of abbreviations

a.o.	among others
BMD	Bone Mineral Density
BMI	Body Mass Index
COX-2	Cyclooxygenase-2
DXA	Dual X-ray absorptiometry
e.g.	Example given
FRAX®	Fracture risk assessment tool
HIV	Human immunodeficiency virus
HJHS	Haemophilia joint health score
i.e.	id est
IL-6	Interleukin 6
L1-4	Lumbar vertebral bodies 1 through 4
OPG	Osteoprotegerin
PA	Physical activity
PwH	People with haemophilia
RANK	Receptor activator of nuclear factor Kappa-B
RANK/L	Receptor activator of nuclear factor Kappa-B ligand
ROM	Range of motion
TBS	Trabecular bone score
VAT	Visceral adipose tissue

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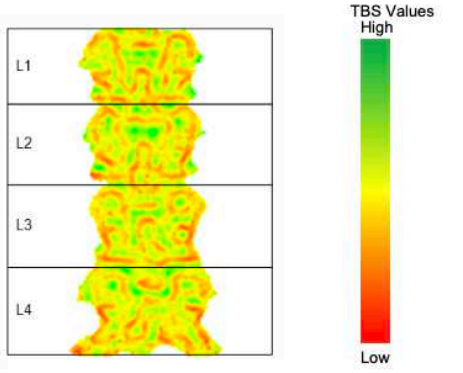
Appendix 1: Example of the Evaluation of the Trabecular Bone Score

BONE HEALTH REPORT

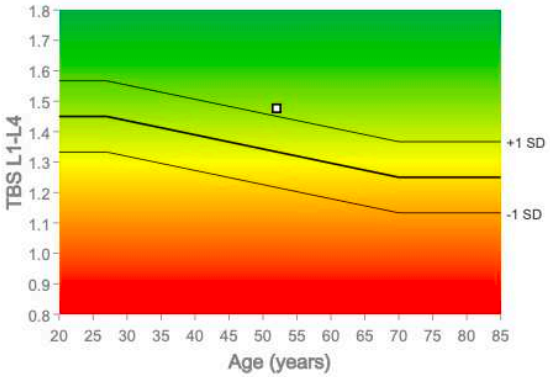
1 TBS Mapping



Non-diagnostic image

2 TBS Spine Results

TBS L1-L4 = 1.477 - Normal microarchitecture



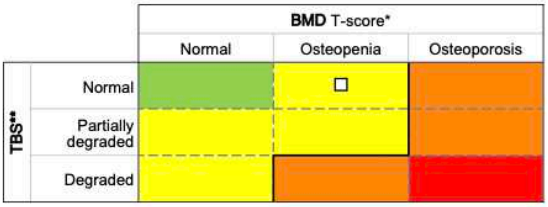
Reference population: USA (NHANES / Medimaps) - White

3 Skeletal Status Assessment

Osteoporosis is 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]

The TBS is derived from the texture of the DXA image and has been shown to be related to bone microarchitecture and fracture risk. It provides information independent of BMD.

For purpose of clarity, "Bone Resilience Index" is defined as the combination of BMD T-score and TBS categories. The Bone Resilience Index zones are established based upon level of fracture risk.[2]



* BMD T-score is the min value of spine, total hip and femoral neck

** Spine TBS L1-L4 Normal microarchitecture > 1.31; Degraded ≤ 1.23

Color coded Bone Resilience Index zones based on Fracture Risk[2]

4 Therapeutic Decision Tools

The FRAX® 10-year probability of fracture:

Type of Fracture	Risk	Risk adjusted for TBS*
Major Osteoporotic	5.6 %	1.3 %
Hip	0.3 %	0.1 %

* Validated only for Caucasian and Asian women and men [3]. Refer to local guidelines before using these values.

Reported Risk factors beside BMD: parent fractured hip

The BMD T-score:

Bone Site	BMD T-score	BMD T-score adjusted for TBS*
Spine	-1.3	-
Femoral Neck <•	-1.2	-
Total Hip <•	-0.7	-

* Validated for Caucasian women only [4]. The greyed cell is the minimum value. The arrow displayed near the hip bone sites represents the hip side of the exam : <• for left hip, •> for right hip.

TBS osteo

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BONE HEALTH REPORT

5 Detailed Spine Results

Region	TBS	TBS Z-score	BMD (g/cm²)	BMD T-score
L1	1.460	-	0.977	-0.9
L2	1.509	-	0.978	-1.1
L3	1.467	-	0.958	-1.3
L4	1.471	-	0.911	-1.6
L1-L4	1.477	1.2	0.953	-1.3
L1-L3	1.479	1.2	0.971	-0.9
L1-L4(L3)	1.480	1.3	0.951	-1.2
L1-L4(L2)	1.466	1.3	0.945	-1.3
L2-L4	1.482	1.1	0.946	-1.5
L1-L2	1.484	1.3	0.978	-0.7
L1-L3(L2)	1.463	1.3	0.967	-0.8
L1-L4(L2L3)	1.466	1.4	0.940	-1.3
L2-L3	1.488	1.0	0.968	-1.2
L2-L4(L3)	1.490	1.2	0.941	-1.6
L3-L4	1.469	1.2	0.932	-1.7

6 Conclusion

The Lumbar spine TBS is 1.477 which suggests a normal microarchitecture compared to reference population.

The patient's associated BMD and TBS values suggest a Moderate resilience to fracture.

Furthermore, the minimum BMD T-score (either adjusted or not for TBS), positions the patient in the Osteopenia category equivalent.

The patient's FRAX results should be interpreted in regard to the intervention thresholds provided by national medical guidelines.

Final decision regarding diagnostic or therapeutic recommendations should include BMD, TBS, additional clinical risk factors as well as the clinical context of the patient.

7 Notes & References

Date of report generation: 11/30/2022 5:50:25 PM
Date of analysis: 5/2/2022 – TBS iNsight version 3.1.2
DXA: Horizon W #303754M – File: PA22502A.p05

1. Consensus Development Conference, Am J Med 94, 646-650 (1994)
2. Adapted from J. Bone Miner. Res. 26, 2762–2769 (2011)
3. Calcif Tissue Int. 96, 500-509 (2015)
4. Adapted from Osteoporos Int. 29, 751-758 (2018)

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