Diploma Thesis

Trabecular Bone Score in non-diabetic, prediabetic and type II diabetic subjects

Multiparametric results from a population-based large-scale cohort, the "BioPersMed" cohort

Submitted by

Alexander Alois Karl Wagner

For the academical degree

Doctor of medicine (MD)

at the

Medical University of Graz

Division of Endocrinology & Diabetology

Dept. Internal Medicine

First and Second Supervisors:

Univ.-Prof.in Dr.in med.univ. Barbara Obermayer-Pietsch

Dr.in med.univ. Verena Schwetz, PhD

Graz, 03.11.2017

Declaration

I hereby declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 03.11.2017 *Alexander Wagner eh*

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Acknowledgement

First and foremost, I would like to thank my supervisor and mentor Prof. Dr. Barbara Obermayer-Pietsch for always having an ear and eye open, for all the times she provided the help I needed in an instant, even right after stepping out of an airplane, and for simply being a great person to work with. Not once she failed to fascinate me with her special field and her tremendous knowledge. Never have I seen a doctor or researcher with a similar enthusiasm, fascination and power, while yet being incredibly friendly and helpful. Thank you very much!

In addition, I am very grateful to all the people from my times of study and for all the help from everyone involved in this endeavour, with special regards to Christoph Haudum and Bernd Tschapeller for editing the data and helping me whenever requested.

Furthermore, I want to thank my parents, Maria and Alois, for the support you've granted me in my lifetime, my aunts for all the times you bore witness to my problems, hopes and dreams, and my grandmother for lightening up my mood by simply being herself. Specific thanks to my girlfriend's parents, Ruperta and Norbert, for granting me more support than I could ever dearly hope for. Also, I would like to thank my brother-in-law Dieter for all the help he provided over these past years.

A big "Thanks" to my friends for all the great memories and times you were at my side.

Special thanks to my siblings, Jasmin and Stefan, for encouraging me to pursue my goals, for being honest and keeping me in line whenever needed. You are simply great!

The greatest thanks are due to my partner and love, Nora, without whom this would not have been possible. I love you.

To everyone – Thank you so very much!

Abstract

Introduction: Bone mineral density (BMD) in type II diabetes mellitus (T2DM) patients tends to be normal or even elevated, while the fracture risk is severely increased. The cause for this increase is assumed to be an enhanced risk of falls due to vascular complications but also a substantial change in bone texture and composition. Therefore, BMD as measured by dual energy X-ray absorptiometry (DXA), the current gold standard for diagnosing osteoporosis (OPO), should be supported by obtaining information on bone quality in terms of imaging and fracture risk assessment, whereas DXA-derived planar BMD only displays the quantity of bone mineralisation. The trabecular bone score (TBS) is a grey-level texture analysis derived from DXA scans of the lumbar spine and enables a more detailed picture of the biomechanical composition of bone. Thus, the predictive value of TBS for T2DM patients at risk for bone fractures requires a closer evaluation, including the utilization of parameters involved in bone and glucose metabolism.

Materials & Methods: The data of 232 volunteers (86 male, 148 female) from the "BioPersMed cohort", a longitudinal study cohort at the Division of Endocrinology and Diabetology and Cardiology at Medical University of Graz between 2010 and 2015, are used to calculate and assess potential associations between clinical (BMD, TBS, BMI, age), functional (oGTT) and biochemical parameters (hormones, bone markers, cholecalciferol). The cohort has been stratified for non-diabetic, pre-diabetic and diabetic participants according to their oGTT's to evaluate glucose metabolism and its effects on bone from healthy individuals via intermediate to overt T2DM patients.

Results: Prediabetes was characterized by a mild but significant reduction of TBS compared to non-diabetic patients, whereas the diabetic subgroup showed a more pronounced decrease. Furthermore, bone metabolism was found to be negatively associated to increased 2h-oGTT levels. Hormonal parameters such as FSH and LH were negatively associated with increased blood glucose levels. TSH, PTH and 25(OH) vitamin D did not show significant effects on glucose metabolism and imaging parameters.

In addition, negative associations between impaired glucose control and bone markers (CTX, P1NP, OC, cholecalciferol), as well as postmenopausal elevated hormones (LH, FSH) have been found. PTH, TSH and testosterone did not show significant relations to TBS values.

Discussion: Our findings suggest that TBS is a valid parameter for bone quality assessment in patients with T2DM, showing that both prediabetes and T2DM are negatively correlated to TBS in a progressive manner. The development pattern of TBS in prediabetes towards T2DM showed that even at an early stage bone structure is impaired, hence preventive efforts need to be in place as soon as possible. Other parameters, such as bone biomarkers or vitamin D might not have sufficient predictive value, probably due to common vitamin D supplementation. Further studies and investigations, especially including age-adjusted TBS measurements, will clarify open questions in this complex, hence clinically important interaction.

Summary (German)

Einleitung: Die Knochendichte (BMD) bei PatientInnen mit Typ II Diabetes mellitus (T2DM) ist normal oder erhöht, wobei das Frakturrisiko insgesamt allerdings stark erhöht ist. Es wird angenommen, dass diese Erhöhung durch ein gesteigertes Sturzrisiko aufgrund von vaskulären Veränderungen aber auch durch deutliche Änderungen der Textur und der Zusammensetzung des Knochens bedingt wird. Die Messung der Knochendichte mittels Dual-Röntgen-Absorptiometrie (DXA), welche den derzeitigen Goldstandard in der Diagnostik von Osteoporose (OPO) darstellt, sollte daher durch zusätzliche Informationen über die Knochenqualität unterstützt werden. Dadurch kann eine präzisere Beurteilung des Frakturrisikos gewährleistet werden, da die DXA-Knochendichte lediglich die planare Quantität der Knochenmineralisierung darstellt. Der Trabecular Bone Score (TBS) wird über eine Graustufen-Texturbeurteilung von DXA-Messungen der Lendenwirbelsäule ermittelt und ermöglicht eine genauere Analyse der Biotextur des Knochens. Folglich bedarf es einer genaueren Evaluierung des prädiktiven Wertes des Trabecular Bone Score (TBS) bei PatientInnen mit T2DM, aber auch den vorausgehenden Stadien, wobei Parameter aus dem Knochen- und Glukosestoffwechsel miteinbezogen werden sollten.

Materialen & Methoden: Die Daten von 232 ProbandInnen (86 männlich, 148 weiblich) aus der „BioPersMed-Kohorte“, die von den Klinischen Abteilungen für Endokrinologie & Diabetologie und Kardiologie an der Medizinischen Universität Graz für eine longitudinale Studienkohorte gesammelt worden waren, wurden verwendet, um potentielle Assoziationen zwischen klinischen (Knochendichte, TBS, BMI, Alter), funktionellen (oGTT) und laborchemischen Parametern (Hormonspiegel, Knochen Biomarker, Vitamin D) zu berechnen und zu bewerten. Die Kohorte wurde in Nicht-DiabetikerInnen, PrädiabetikerInnen und T2DM-PatientInnen stratifiziert um den Glukosestoffwechsel und dessen Effekte auf den Knochen in Bezug auf die mutmaßlich kontinuierlichen Veränderungen bis hin zum manifesten T2DM zu evaluieren.

Ergebnisse: Prädiabetes zeigte, im Vergleich zu Gesunden, bereits eine beginnende Reduktion des TBS, wobei die T2DM-Subgruppe den stärksten TBS-Abfall im Vergleich zur DXA-Messung zeigte. Die Serumbiomarker des Knochenstoffwechsels

wiesen eine negative Korrelation zu erhöhten 2h-oGTT auf. Ebenso zeigten FSH und LH negative Assoziationen zu erhöhten Blutglukosespiegeln. TSH, PTH und Vitamin D waren nicht signifikant mit den Parametern assoziiert.

Diskussion: Wir haben beschrieben, dass TBS als Parameter für die Beurteilung von Knochenqualität bei T2DM verwendet werden kann, wobei bereits bei Prädiabetes eine deutliche Verminderung des TBS gefunden wurde und daher eine osteoprotektive Prävention und Therapie der Grunderkrankung sobald wie möglich einsetzten sollte. Andere Parameter, wie Biomarker des Knochenstoffwechsels oder Vitamin D-Spiegel haben möglicherweise einen zu geringen prädiktiven Wert, zumal Vitamin D häufig bereits supplementiert wird. Weitere Studien und Untersuchungen, z.B. mit altersadjustiertem TBS, können weitere Einsichten in dieses auch klinisch wichtige Thema bringen.

Abbreviations

OPO - Osteoporosis
BMD - Bone Mineral Density
OS - Oxidative Stress
RANK - Receptor Activator of NF-κB
RANKL - RANK-Ligand
OPG - Osteoprotegerin
PBM - Peak Bone Mass
WHO - World Health Organization
LRP5/6 - Low-density Lipoprotein Receptor-Related Protein 5 / 6
ER-α - Estrogen Receptor-α
TGF-β - Transforming Growth Factor-β
HAC - Hydroxyapatite Crystals
PTH - Parathyroid Hormone
DXA - Dual X-Ray Absorptiometry
mSv - Milli-Sievert
OP - Osteopenia
FRAX - Fracture Risk Assessment Tool
CT - Computed Tomography
HR-pQCT - High Resolution-Peripheral Quantitative Computer Tomography
pQCT - Peripheral Quantitative Computer Tomography
vBMD - Volumetric Bone Mineral Density
QUS - Quantitative Ultrasound
SOS - Speed of Sound
BUA - Broadband Ultrasonic Attenuation
MRI - Magnetic Resonance Imaging
H-MRS - Proton-Magnetic Resonance Spectroscopy
CTX - C-terminal Type I Collagen
NTX - N-terminal Type I Collagen
OC - Osteocalcin
ucOC - Undercarboxylated Osteocalcin
PICP - C-terminal Pro-Peptide of Type I Collagen
PINP - N-terminal Pro-Peptide of Type I Collagen

bALP - Bone-Specific Alkaline Phosphatase

TRAP - Tartrate-Resistant Acid Phosphatase

SERM - Selective estrogen receptor modulator

T2DM - Type 2 Diabetes Mellitus

DM - Diabetes Mellitus

HbA1c - Haemoglobin A1c

oGTT - Oral Glucose Tolerance Test

OEDG - Austrian Diabetes Society

ADA - American Diabetes Association

EASD - European Association for the Study of Diabetes

GLP-1 - Glucagon-like Peptide-1

DPP-4 - Dipeptidyl Peptidase-4

SGLT-2 - Selective Sodium-Glucose Transporter-2

Figures & Tables

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1. INTRODUCTION

1.1. Osteoporosis

1.1.1. Definition

Osteoporosis (OPO) is a progressive metabolic bone disease, characterized by decreased bone mass, impaired microarchitecture and degraded bone tissue, resulting in a consequent increase in fragility and fracture risk. Regarding the various causes, it can be categorized into primary and secondary OPO. (Sözen, et al., 2017)

1.1.1.1. Primary Osteoporosis

In general, there are two main types of primary OPO. Considering the pathways affecting bone metabolism, we differentiate into postmenopausal / postandropausal and age-related genesis for the loss of bone mass and bone mineral density (BMD). In addition, there is a special form called idiopathic juvenile OPO, which occur in children and young adults only. Despite the primary form being the most common type of OPO, secondary causes need to be ruled out before making the diagnosis to engage in subsequent actions. Responsible factors can be revealed by further measures, such as laboratory evaluations, precise physical examinations and a detailed patient history. (Sözen, et al., 2017)

As the most frequent form, **postmenopausal OPO** is mainly caused by estrogen deficiency, resulting in an imbalance of bone formation and resorption. This leads to thinner cortical bone with increased porosity and a microarchitectural impaired trabecular bone structure. (Eastell, et al., 2016) The responsible mechanisms are not fully explored yet, nevertheless, due to ongoing research, certain hypothesis, explaining possible pathogenetic factors, have been revealed. (Weitzmann & Pacifici, 2006) (Qiaozhen, et al., 2016) One of these describes a potential key role for sexual hormones as antioxidants and an according protection against oxidative stress (OS) and its involvement in the development of OPO. (Manolagas, 2010) (Qiaozhen, et al., 2016) Another theory exhibits the increased osteoclastic maturation caused by estrogen deficiency, leading to an imbalance in bone resorption and bone formation.

It involves an estrogen-regulated RANK ligand (RANKL) and the RANKL/RANK/Osteoprotegerin (OPG) pathway, responsible for the regulation of bone resorption. (Kaunitz, et al., 2009) (Boyce & Xing, 2007)

Senile OPO is characterized by a continuous age-related loss of bone mass. In the first years, human bone undergoes a modelling process until reaching its peak bone mass (PBM) in puberty. At this point, the loss of bone mass begins and bone resorption is starting to prevail. Despite of this, bone resorption is a physiological process and is a counterpart of bone formation, a rebuilding process. In older age however, these developments result in lower cortical and trabecular bone mass and impaired, more fragile, bone structure. While other risk factors of increased age are not included yet, a considerable number of fractures already occur due to these circumstances. (Sözen, et al., 2017)

As a rare form of primary OPO, the **idiopathic juvenile Osteoporosis** (IJO) is characterized by vertebral fractures and sub-metaphyseal fractures of the long bones. It presents with chronic pain of the back, the hip and lower limbs. Affected children may also have problems with walking caused by proximal muscle weakness. The clinical appearance needs to be differentiated from other primary bone diseases, such as osteogenesis imperfecta, presenting blue sclerae, hypermobility, hearing impairment and dentinogenesis imperfecta. The reasons causing those fractures include generalised osteopenia, thin cortical bone and areas of sclerosis at the metaphyses of long bones. The exact aetiology is still unclear and precise information about the genetical involvement has not been identified yet. (Franceschi, et al., 2015)

1.1.1.2. Secondary Osteoporosis

Secondary OPO is less common and has its underlying cause in other diseases, resulting in an indirect decrease in bone quantity and quality. The pathogenesis is often multifactorial. An insufficient explanation by traditional risk factors may also suggest considering a secondary cause. The clinical appearance can be very misguiding, therefore signs like atypical fractures or specific clinical features, such as anaemia, amenorrhoea or signs of specific endocrinopathies, suggest further investigation.

Based on proven drug-induced osteoporotic-like conditions, a careful drug history is recommended as well. The following table gives an overview of drugs with a proven osteoporotic effect. (Sheu & Diamond , 2016)

Drugs that induce an increase in fracture risk
Corticosteroids (≥5 mg prednisolone daily or equivalent for ≥3 months)
Antiepileptics (Carbamazepine, Phenytoin, Phenobarbitone)
Diabetes medication (Thiazolidinediones, Empagliflozin)
Selective Serotonin Reuptake Inhibitors
Excess Thyroxine
Aromatase Inhibitors
Tamoxifen (when used in pre-menopausal women)
Gonadotropin-releasing hormone
Chemotherapy
Immunosuppressants (Cyclosporine, Tacrolismus, Methotrexate)
Lithium
Heparin
Proton Pump Inhibitors
Aluminium-containing Antacids
Depot Medroxyprogesterone acetate
Antipsychotics

Tab.1: Drugs that induce an increase in fracture risk (Sheu & Diamond , 2016)

1.1.2. Epidemiology

Based on several estimations, more than 200 million people suffer from OPO, currently the most common bone disease in humans and on the increase. The World Health Organization (WHO) announced that more than 8.9 million fractures occur annually on account of OPO worldwide. (Cabral, et al., 2016) Whereas all groups of ages and ethnicities can be affected by OPO, it is more common in Caucasians, women and older people. Due to an increasing in life span and a consequently aging population, current statistics from the International Osteoporosis Foundation reveal that 1 of 3

women and 1 of 5 men are facing an osteoporotic fracture in their lifetime. (Sözen, et al., 2017)

In addition to the significance in morbidity and mortality, the rising economic costs, reaching 37 billion Euros annually in Europe in 2010, should be considered as well. (Vandenbroucke, et al., 2017) (Eastell, et al., 2016) Even though prevalence and incidence for fragility fractures are increasing, the detection rate of OPO is still too low, resulting in many patients being undiagnosed. Public Health Authorities utilize BMD and age as the most relevant determinants in case of managing OPO. (Boschitsch, et al., 2017)

1.1.3. Etiology

OPO is a multifactorial disease, with men and women being affected similarly in terms of causes. The most common causes are hypogonadism, unhealthy diet, vitamin D deficiency, treatment with glucocorticoids or anticonvulsants, abuse of alcohol and nicotine and specific diseases of the gastrointestinal tract, the endocrine system, kidneys or liver. (Misiorowski, 2017)

Hormonal deficiencies in women is one of the most common causes for secondary OPO. The menopause results in a rapid decrease of estradiol concentration levels and their subsequent functions in the bone metabolism. The effects of hypogonadism in men, however, are not of less importance, with androgen-deficiency being the equivalent to the menopausal effect. (Misiorowski, 2017)

Further causes for OPO are genetic variations, affecting the quantity and quality of bone mass with a resulting impairment of bone strength. Studies of twins and family heritability demonstrated that 50 to 70% of an individual’s bone mass is inherited. In addition, genome-wide association studies, revealed that BMD is associated to 56 loci, whereas 14 of them also show relations to fracture risk. According to other studies from the year 2015, the loss of bone mass and the increase in fracture risk are influenced by more than 500 genetic variations. So far, associated regulating factors only explain a small amount of causes in comparison to the wide field, yet to be revealed and tested.

These factors include low-density lipoprotein receptor-related protein 5 and 6 (LRP5 / LRP6), vitamin D receptor, estrogen receptor-α (ER-α), transforming growth factor-β (TGF-β) and components of the bone matrix, such as collagen type I α I. At this point, the individual fracture risk assessment cannot incorporate these variations due to lack of a proven functional effect leading to impaired bone mass or increased fragility of bone. (Eastell, et al., 2016)

1.1.4. Pathogenesis

Most fractures in the elderly population are not solely caused by osteoporotic fragility, but the processes are at least partly involved. The most important predilection sites for osteoporotic fractures are the proximal femur, the distal radius and the vertebrae. (Eastell, et al., 2016) To avoid fractures, however, a hierarchical structure is needed to make bone stiff and flexible at the same time. In general, fractures occur once the applied force surpasses bone strength. (Boyce & Xing, 2007) (Curtis, et al., 2015) Once the bone is weakened by increased bone loss, further factors can lower or enhance the fracture risk: (Sözen, et al., 2017)

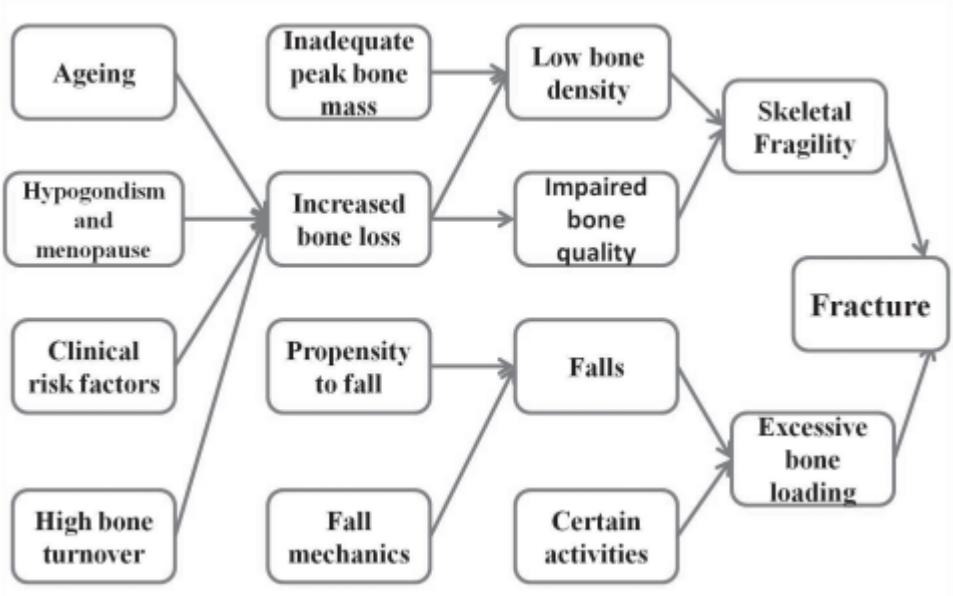


Fig.1: Pathogenesis of osteoporotic fractures (Sözen, et al., 2017)

Based on the linking of non-collagenous proteins with collagen type I fibrils, wound in a triple helical structure, shearing can be prevented. To enhance bone strength,

hydroxyapatite crystals (HAC) get deposited within the collagen structure. In addition, enhanced bone strength can be achieved by an increased number of cross-links between non-collagenous proteins and collagen fibrils. In OPO, cross-links are reduced in number and the HAC tend to be larger, making the bone porous and prone to fracture. (Curtis, et al., 2015) (Boyce & Xing, 2007)

Bone cells can be subgrouped in 3 main types: osteocytes, osteoblasts and osteoclasts. Osteocytes are former osteoblasts, embedded in the bone mineral structure, and represent 90-95% of the bone cells. Osteoblasts are responsible for bone formation and can either mature into osteocytes or line up on the bone surface. Osteoclasts, on the other hand, are in charge of bone resorption, embodying the counterpart of osteoblasts. Despite their counterpart function, osteoblasts and osteoclasts work together by modelling and remodelling bone structure, regulated by osteocytes around the Haversian canal. Those cells function as a mechanosensory system and allow communication with adjacent osteocytes and other bone cells, enabled by endocrine, paracrine and autocrine processes. RANK-RANKL (RANK-Ligand) signalling is involved in the regulation of osteoclastic activation and survival during bone remodelling. Excessive bone resorption can be inhibited by OPG binding to RANKL, which cannot bind on its receptor as a consequence. (Curtis, et al., 2015) (Boyce & Xing, 2007)

Estrogen plays an important role in bone metabolism, both in men and women. Studies show that women tend to lose 3 to 5% of their, mainly trabecular, bone mass annually due to diminished ovarian estrogen production during menopause. In comparison to female patients with normal BMI, obese age-matched women, defined by higher BMI values, tend to have higher estrogen levels in the postmenopausal phase and therefor are protected against the development of OPO. This circumstance is based on the fact that the increased amount of white fat tissues enables the augmented conversion of adrenal androgens into estrogen by producing the responsible enzyme aromatase, also called CYP19A1. Transient estrogen deficiency, on the other hand, can be rather common during lactation or post-partum in general. The calcium levels needed for the infant stems from the amount obtained by bone resorption and thereby can prove the protective value of estrogen in terms of bone metabolism. (Eastell, et al., 2016)

Estrogen receptors (ER) appear in all types of bone cells. ERα and ERβ are two isoforms and can be differed in location, effects of mediation and distribution. ERα are mainly produced by cells of the cortical bone and are responsible for mediating ligand effects of natural estrogen. On the other hand, phytoestrogens, found in soy products, needs ERβ, which is expressed by cell of trabecular bone primarily, to mediate their impact. Estrogen deficiency leads to an imbalance between bone formation and resorption in favour of resorption by regulating cellular differentiation, their activity and the induction of apoptosis. Additionally, the secretion of interleukin-1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor (TNF) and the activation of immune cells enhance the imbalance because of estrogen deficiency, therefore also leading to an inflammatory process. (Eastell, et al., 2016)

According to the specific bone cells, estrogens affect each type of bone cell in a different way. Estrogens regulate differentiation of osteoblasts and augmentation of lifespan in osteoblasts and osteocytes, stimulate the synthesis of collagens and act as antiresorptive hormones in order to maintain a dynamic metabolism. In addition, they induce the expression of RANKL and OPG, serving as an antagonist to RANKL. Furthermore, oestrogens also affect osteoclasts by interfering in their signalling pathways, inducing apoptosis and preventing them of differentiating. Concerning osteocytes, estrogen deficiency leads to structural variations due to impaired mechanosensing and lack of connectivity between adjacent cells. (Eastell, et al., 2016)

Serum oestradiol levels are strongly associated with bone health in men, even more important that testosterone levels seem to be. (Eastell, et al., 2016) Men with a proven deletion in estrogen receptor-related genes also show signs of OPO, implying the need of estrogen. Studies suggest that the correlation between low concentrations of oestradiol and fractur risk become effective once a certain threshold value (below 16-20 pg/ml) is undercut. (Misiorowski, 2017)

Androgens, on the other hand, induce proliferation and differentiation of osteoblasts, while inhibiting functions of osteoclasts and serve as regulators in terms of interactions between these. In addition, their role as stimulators include the increase of sensitivity to IGF-1, the secretion of growth hormone and the production of bone itself. The levels of androgens seem to be of special importance for the manifestation of OPO. High-risk

individuals with very low testosterone levels during adolescence never reach a normal peak bone mass, needed to avoid an accurate fracture risk. These main reasons for such low concentrations are castration or hyperprolactinaemia, especially caused by prostate cancer treatment. OPO in men seems to manifest a few years later compared to women. The rapid decrease of estrogen in the course of menopause is not equivalent to the decrease of androgens in men, which happens over time and in a more linear fashion. The decrease, however, points out the anabolic effects of androgens in bone and leads to older men having a higher risk for biomechanical fractures. In addition, androgens also have an important effect on muscle mass and therefore increase an individual's bone and muscle strength. (Misiorowski, 2017)

Insufficient calcium supply leads to secondary hyperparathyroidism with a subsequent increase of bone resorption, lowering the bone mass, whereas supplementation is associated with an increase of BMD. Reduction of menopausal bone loss has not been proven by calcium supply only but bone loss during the ageing process has been by calcium supplements. Vitamin D deficiency results again in secondary hyperparathyroidism with typically increased secretion of parathyroid hormone (PTH), hence increasing bone turnover, especially in the elderly population. A serum concentration of 25(-OH)- vitamin D below 50 nmol per litre, however, does not induce the evolution of OPO but osteomalacia, hence is a potential secondary gain for other causal factors. (Eastell, et al., 2016)

1.1.5. Diagnosis

For many years, the main approach in diagnosing OPO consisted in the quantitative measurement of the BMD by dual-energy X-ray absorptiometry (DXA) and obtaining information on previous fragility fractures of the hip or vertebra or fractures without a major trauma as its cause. So far, no information about bone quality could be included into the process, mostly caused by the lack of accurate measurement options. Further imaging technologies and measurements were revealed and used to assess fracture risk and result in a more detailed diagnostic process. (Sözen, et al., 2017)

There are several indicators to start a measurement of BMD in adults, especially depending on the patient's age. Men and women between the age of 50 to 64 are recommended to be tested, if women are menopausal or certain risk factors apply on adults of both sexes. These risk factors include fragility fractures in adults above the age of 40, a prolonged therapy with glucocorticoids or other medications with a proven harmful effect on bone, smoking, an excessive alcohol intake, a pre-existing weight below 60 kilograms or a major loss of weight (>10% at an age of 25), osteopenia or conditions strongly related to OPO or the loss of bone mass. The medical history of the patients and their family should be inquired in addition because parental fractures of the hip are strong indicators in this age group. Adults with an age below 50 years mostly have the same indicators, but with a focus on specific disorders, such as primary hyperparathyroidism, malabsorption syndromes or hypogonadism, including premature menopause. In case of a BMD measurement being not available, a vertebral X-ray imaging can be performed. Further indications are shown in the following table: (Sözen, et al., 2017)

Criteria for vertebral imaging
Age (women ≥70 years, men ≥80 years)
Loss of height (deviations between current height and peak height (at age 20) ≥4cm
Prospective height loss (deviations between current height and prior measurements)
Prior vertebral fractures
Glucocorticoid treatment (recent or prolonged)

Tab.2: Vertebral Imaging (Sözen, et al., 2017)

1.1.5.1. Dual X-Ray Absorptiometry

DXA is a X-ray-based imaging method to determine the BMD using ionised radiation. Due to its importance in daily routine investigation, the radiation exposure must be kept as low as possible, in terms of reducing the risk of consequential damages. Despite of the exposure dose and effective dose being very low compared to other techniques, the effective doses, stated in millisievert (mSv), can vary significantly by age, whereas the exposure dose of a 5-year-old child can be 2-3 times higher than an adult's. In addition, radiation exposure also depends on other aspects, such as patient size,

number of images, imaging parameters (length, width, speed), tube-related variations and other differences within models of various manufacturers. An exemplary effective dose from a peripheral DXA scan is <0.01 mSv, whereas the worldwide average dose caused by nature is 2.4 mSv per year. (Damilakis, et al., 2010)

The clinical measurement of BMD by DXA at the femoral neck and lumbar spine is currently the most widespread method to asses fractur risk and to diagnose OPO. (Oei, et al., 2016) It is used as an investigation method to determine osteopenia (OP) or OPO, as well as to monitor the response to a certain therapy or the evolution of OP/OPO itself. The exact constitution of a tissue will not be determined by DXA due to not considering shape, size and distribution within the bone. It will merely give information about the quantity of electrons, serving as a barrier for X-rays. Consequently, a denser tissue with more electrons result in fewer X-rays reaching the detector, i.e. a higher BMD. This circumstance affects the accuracy of the interpretation value, so new methods of obtaining information on bone quality are highly sought after. (Schacter GI, 2017)

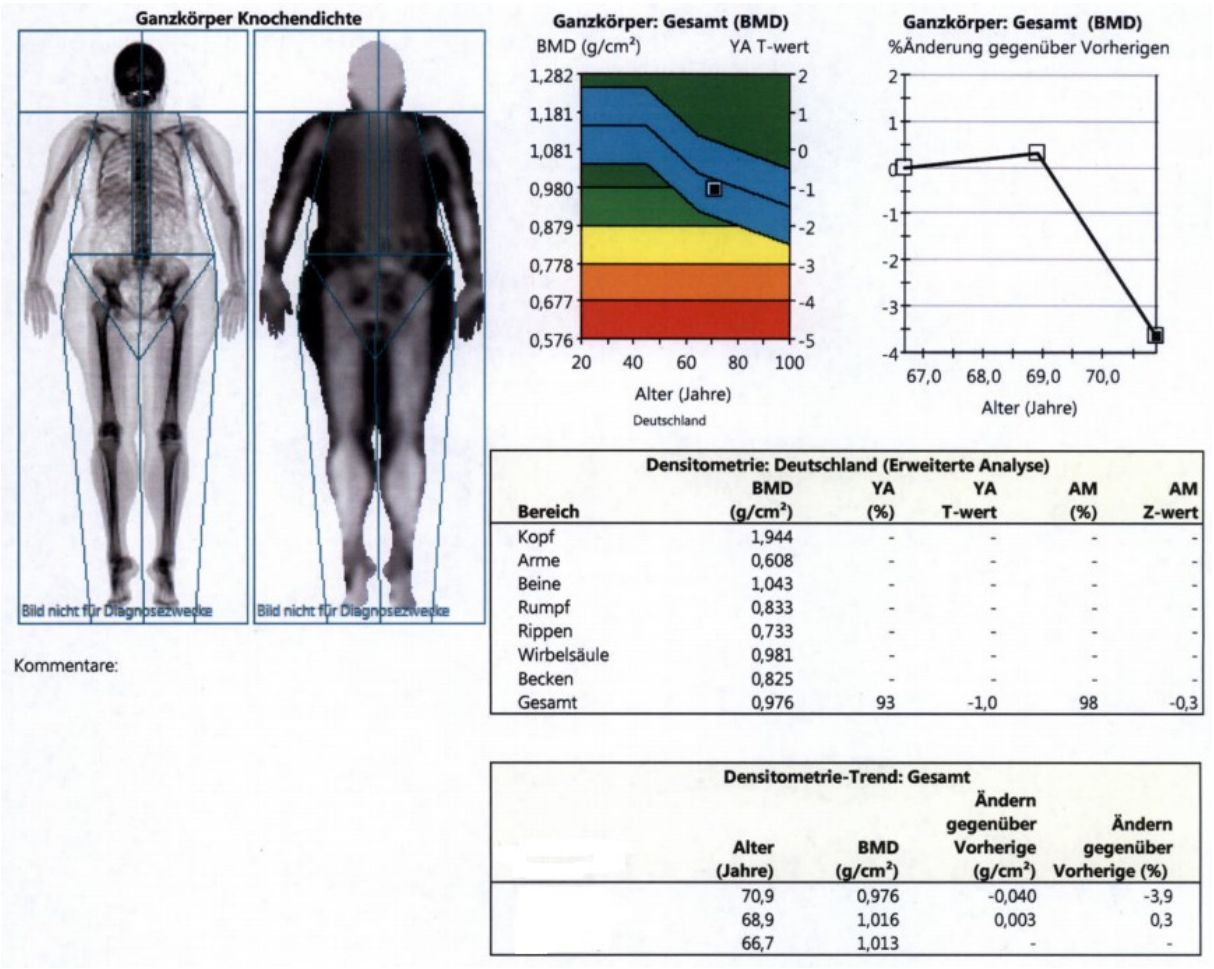
While providing information about bone quantity only, the utilization of other radiographic methods and techniques, mostly of quantitative nature too, declined as being replaced by DXA. There are additional modes of measurement using other skeletal sites, such as skull, distal radius or full body measurement, but these are conducted for research mainly. (Oei, et al., 2016)

The areal BMD is measured in g/cm² but more commonly presented by the T-Score, expressing the number of standard deviations (SD) above or below a certain standard, referring to a healthy 30-year-old adult of same sex and ethnicity. (Oei, et al., 2016) Once the DXA scan shows a decrease in BMD of 2.5 or more SD from the young adult mean population, defined as a T-Score below -2.5, or a fragility fracture of the hip or vertebra or fractures without a major trauma as its cause already occurred, the diagnosis of OPO can be made. (Sözen, et al., 2017) Due to an unproportional loss of bone mass in age, represented by a lowered T-Score, many people would be diagnosed with OPO because of reaching a certain age. Subsequently, the Z-Score has been developed to compare the subject’s BMD to controls of same sex and age.

(Schacter GI, 2017) A lowering of the T-Score between -1.0 and -2.5 at any skeletal site is described as OP, a potential precursor to OPO. (Oei, et al., 2016)

As an example of BMD enabling a rough estimation for the fracture risk, a study could show that a decrease of one SD caused an increase in fracture risk by 1.4 – 2.6-fold. DXA scans of the femoral neck and a unified database for white women, age 20 to 29, provided by the NHANES, short for National Health and Nutrition Examination Survey, served as a reference standard. (Schacter GI, 2017)

The following example displays a DXA-derived full body total BMD measurement, including the DXA scan, a progression curve and the score table:



Densitometrie: Deutschland (Erweiterte Analyse)

Bereich	BMD (g/cm²)	YA (%)	YA T-wert	AM (%)	AM Z-wert
Kopf	1,944	-	-	-	-
Arme	0,608	-	-	-	-
Beine	1,043	-	-	-	-
Rumpf	0,833	-	-	-	-
Rippen	0,733	-	-	-	-
Wirbelsäule	0,981	-	-	-	-
Becken	0,825	-	-	-	-
Gesamt	0,976	93	-1,0	98	-0,3

Densitometrie-Trend: Gesamt

Alter (Jahre)	BMD (g/cm²)	Ändern gegenüber Vorherige (g/cm²)	Ändern gegenüber Vorherige (%)
70,9	0,976	-0,040	-3,9
68,9	1,016	0,003	0,3
66,7	1,013	-	-

Fig.2: Full body total BMD - anonymized patient data provided by the Medical University of Graz (left to right: DXA full body scan, BMD reference curve, BMD progression curve)

1.1.5.2. Trabecular Bone Score

Trabecular Bone Score (TBS) is a parameter of a grey-level texture measurement derived from DXA scans of the lumbar spine. It measures local grey variations within two-dimensional (2D) projection images to approximate the three-dimensional (3D) microarchitecture of the scanned vertebrae. Thereby, it can provide more detailed information on the biomechanical state of bone, subsequently contributing to the fracture assessment. The hypothesis states that if DXA scans result in the same BMD, microarchitectural variations, potentially determined by deviated TBS, may decrease bone stability and make it prone to fracture. (Oei, et al., 2016) Bone structures with higher homogeneity tend to have lower fluctuations in photon absorption, resulting in higher TBS values. Higher fluctuations, however, are demonstrated by lower TBS values and more likely to represent poor microarchitecture in comparison. (Martineau & Lesie, 2017)

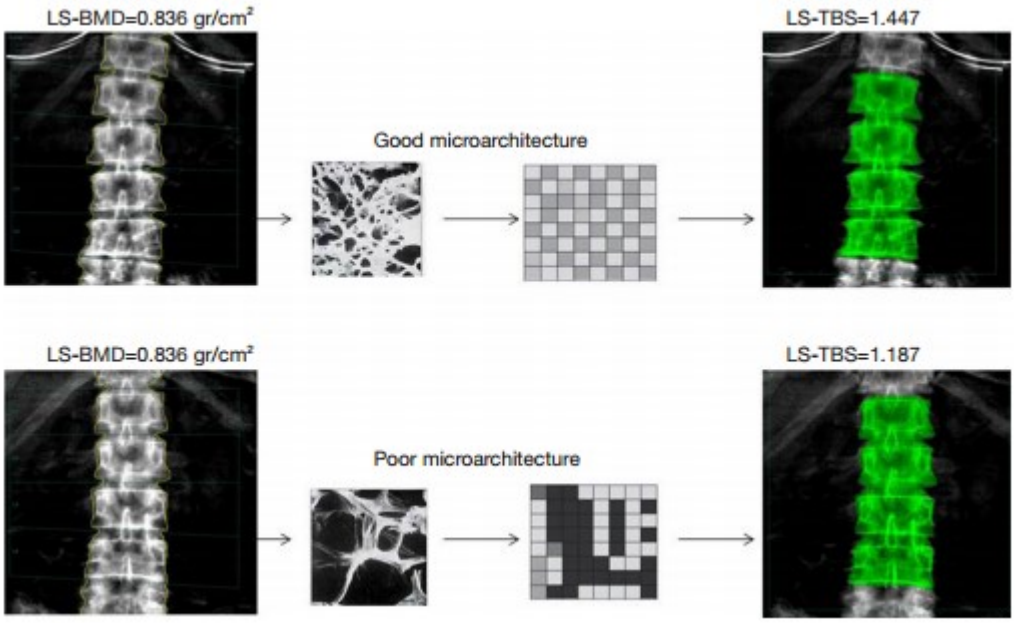


Fig.3: TBS scan (L1-L4) provides information on microarchitecture (Oei, et al., 2016)

TBS values are unitless and can be calculated retrospectively from standard DXA scans by specific commercially available software. There are some factors to interfere in an accurate determination. Earlier TBS software versions generated lower TBS values for men compared to women due to lack of adjustment in terms of soft tissue thickness. While having the same body mass index (BMI), men tend to have a higher

soft tissue thickness leading to the assumption of a higher fracture risk. Consequently, the software had been adjusted by using the BMI as an index for the abdominal soft tissue, enabling these gender variations to be insignificant as a result. However, recommendations of the manufacturers imply to only use on patients with a BMI between 15 and 37 kg/m². Additionally, degenerative processes cause deviations in measurement and evaluation of these parameters. Sclerotic alterations, especially increasing with age, can lead to higher BMD, whereas studies show the TBS values to be unaffected in comparison. In case of artefacts, the affected vertebral levels need to be excluded to maintain an accurate calculation. (Martineau & Lesie, 2017)

Multiple studies imply TBS to have a remarkable predictive value in addition to the FRAX model and to be of importance in terms of guiding clinical decisions in the future. As an example, a meta-analysis stated that a TBS decrease of one SD increases the major osteoporotic fractures up to 1.44 times. Apart from OPO, TBS may also be used in the monitoring of treatments or to be of predictive value concerning other diseases. (Oei, et al., 2016)

1.1.5.3. Other Imaging Technologies

The **computed tomography** (CT) is a promising tool to analyse both cortical and trabecular bone in a more separate way due to the belief of specific characteristics, depending on the skeletal site, in both structures with the potential of enhancing or reducing the fracture risk. Disadvantages include the CT equipment being way more expensive than other imaging procedures and a higher extent of radiation exposure. Additionally, limitations in access must be considered as well. The data is also very complex and requires specific software in analysis. (Oei, et al., 2016)

The individual analysis of the cortical and trabecular bone structure can be accomplished by performing a **high resolution-peripheral quantitative CT** (HR-pQCT), which is also used for muscle measurements. (Oei, et al., 2016) (Stagi, et al., 2016) Furthermore, HR-pQCT enables a more detailed evaluation of morphological changes within the trabecular bone, which can decrease bone strength tremendously.

Peripheral quantitative CT (pQCT), on the other hand, uses low radiation doses and calculates a volumetric BMD (vBMD), whereas DXA uses the areal projection. (Stagi, et al., 2016) The following figure shows an example of the HR-pQCT-imaging used to assess cortical and trabecular structures changings:

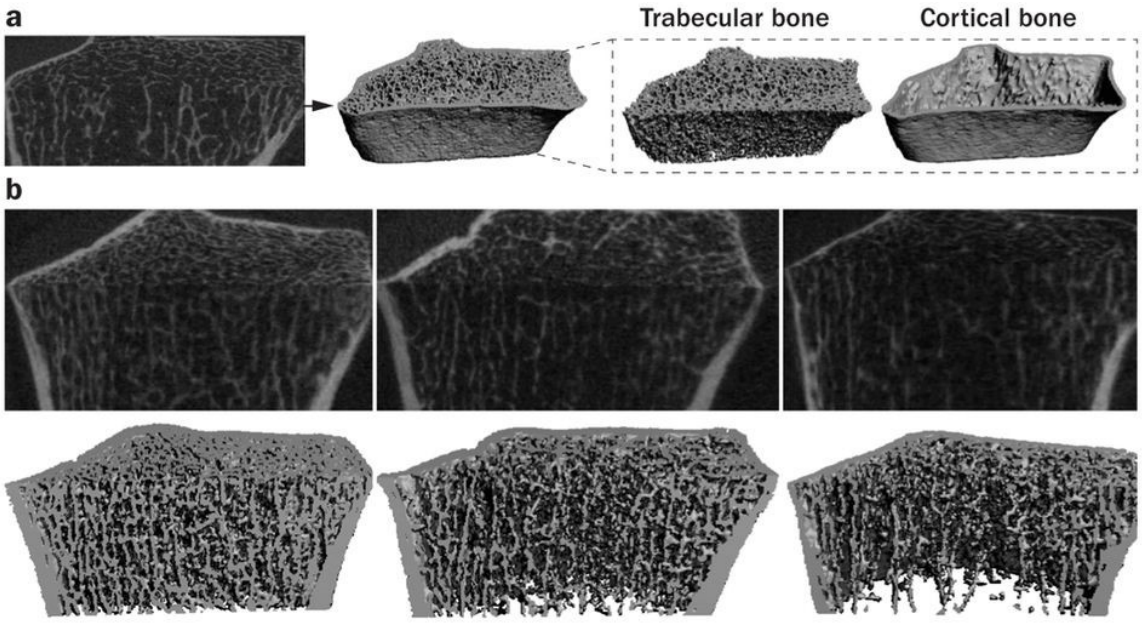


Fig.4: HR-pQCT-Imaging of cortical and trabecular structures (Geusens, et al., 2014)
a Segmentation of a forearm **b** Deterioration in the ultra-distal radius due to ageing

Quantitative Ultrasound (QUS) is a radiation-free, easily accessible and inexpensive method for in vivo assessment of the bone structure to estimate the associated fragility. (Oei, et al., 2016) Besides detecting bone mass, QUS also provides information on properties of mechanics and structure, wherefore DXA and CT are not capable of. (Gong, et al., 2014) On the other hand, the parameters on various devices were not clearly defined with accurate reference ranges, resulting in variations between devices of different manufacturers, even when applied to the same bone structure. Therefor it was used mainly as a supplement to the current gold standard DXA and is usually applied only to peripheral skeletal sites, such as calcaneus, radius, tibia and the phalanges. (Otani, et al., 2017) (Oei, et al., 2016)

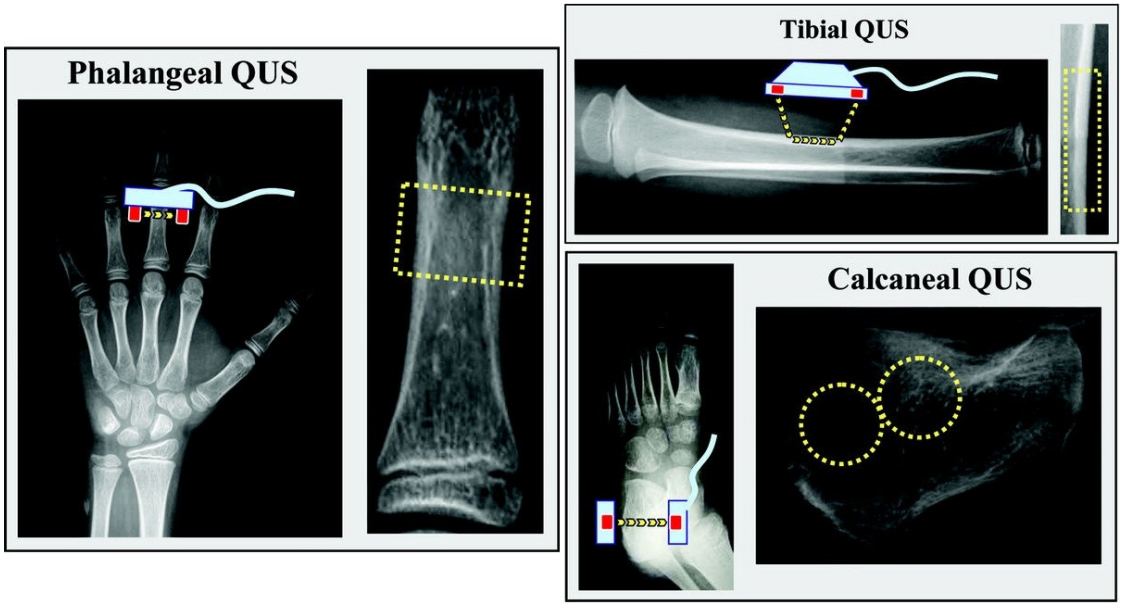


Fig.5: QUS devices used to assess bone mineral state in children (Baroncelli, 2008)

A standardization process has been established by the Japanese Osteoporosis Society in 2007, enabling the conversion of parameters from various devices into standardized values, increasing the predictive significance of QUS worldwide. (Otani, et al., 2017) Nevertheless, DXA maintained to be the gold standard due to giving more detailed and reliable information. (Malavolta, et al., 2004)

The frequency of the ultrasonic waves range between 0.5 and 1.25 MHz. (Gong, et al., 2014) The two main parameters, speed of sound (SOS) and broadband ultrasonic attenuation (BUA), are quantitative parameters and used to determine the density of a bone, not equal to BMD but highly correlated. SOS is measured in m/s and describes the time, in which the waves passes through the bone. The attenuation in the bone, mostly caused by absorption by the cortical bone and scattering in the trabecular bone, is represented by BUA, expressed in dB/MHz. (Malavolta, et al., 2004) Patients affected by OPO tend to have a decreased SOS and DUA, correlating with other statistic parameters, such as vertebral bone density. (Funke, et al., 1995) Due to QUS using elastic waves, these parameters are utilized to evaluate the elastic features of the bone and subsequently to give information on the bone strength. (Otani, et al., 2017)

According to studies, **magnetic resonance imaging** (MRI) is another promising investigative process for reproducing trabecular structures in high resolution. Despite being quite expensive and time-consuming, the utilization is free of ionized radiation, therefore making it an attractive tool to obtain further information on bone properties. The spatial resolution of a HR-pQCT is higher by comparison. The MRI spectroscopy serves as an indirect MRI application and is used for the assessment of osseous structures without applicating contrast agents. The golden standard for quantitative measurements of the bone marrow fat is the proton-magnetic resonance spectroscopy (H-MRS). Ongoing developments in software and hardware, e.g. using 3-Tesla devises, enable a more precise look on the microarchitecture of the trabecular bone compartment, as well as displaying bone at a molecular level. (Oei, et al., 2016) (Link, 2012) The trabecular structure derived from MRI measurements correlate with CT-scans, histology and the biomechanical strength. As a result, it is possible to differentiate patients with same BMD and variations in terms of previously occurred fragility fractures. Unfortunately, accurate imaging is limited to peripheral sites. Distal radius, tibia and calcaneus are the locations MRI is usually applied to. (Link, 2012)

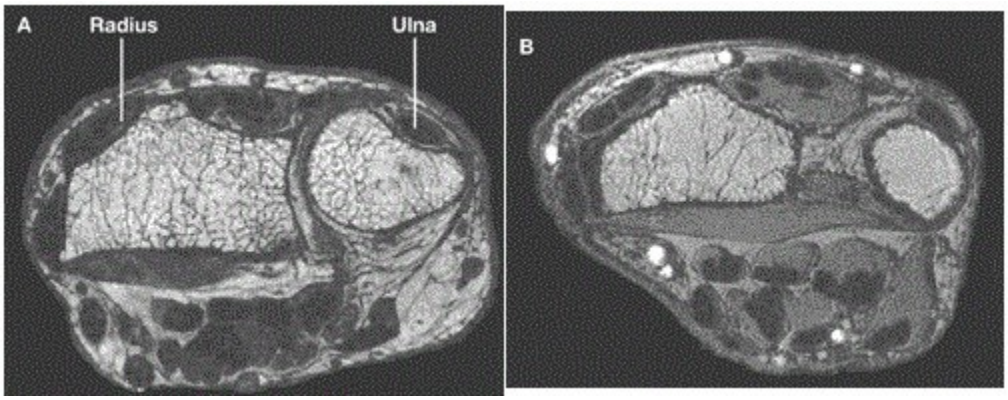


Fig.6: Radius and ulna displayed by MR-Imaging (Rosen & Bouxsein, 2006)
A 34a, female, healthy condition **B** 64a, female, osteoporosis

A direct analysis of bone cells and their behaviour on various conditions can only be obtained by bone biopsies with subsequent histomorphometry. Due to invasiveness and potential infection risk, this does only serve as a special option to diagnose and monitor OPO and other bone-related conditions in a clinical setting. (Gong, et al., 2014)

1.1.5.4. Biochemical Measurements

In general, biochemical markers can be grouped in biomarkers of bone resorption and bone formation. These markers are mostly products derived from metabolic processes involving type I collagen or non-collagenous proteins with an association to bone cells. (Naylor & Eastell, 2012)

C-terminal type I collagen (CTX) and **N-terminal type I collagen** (NTX) are collagen degradation products and are used as stable indicators for bone metabolism. NTX has been measured from urine samples with monoclonal antibodies, whereas CTX is one of the most common serum markers. CTX concentrations mirror the amount of peptide fragments released into circulation and therefore is used as the reference marker for bone resorption by the International Osteoporosis Foundation. Both products are stable at room temperature but due to circadian rhythms and variations caused by food intake, standardized conditions need to be maintained. (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012)

25(OH)-Vitamin D, also named **cholecalciferol**, is a secosteroid with a structural similarity to cholesterol and cortisol and currently the most important parameter in routine diagnostics of vitamin D. Systemic concentrations of 25(OH)-vitamin D below 20ng/ml are defined as vitamin D deficiency, a very common condition mostly caused by lack of sunshine exposure. The synthesis of vitamin D can only be conducted by UV radiation reaching the skin, thereby raising a potential problem in our societies. Systemic variations in cholecalciferol levels have been with autoimmune diseases, cancer, diabetes and other diseases, whereby most of these associations aren't fully explored yet. The highly active form of vitamin D is named calcitriol (1,25(OH)2D3. Its effects include the increase of intestinal calcium uptake, the decrease of renal calcium elimination and the increase in bone turnover to augment serum calcium levels. The measurement of calcitriol as an active form used to be very difficult but new automatized assays and mass spectrometric measurement improved the analyzation intensively. Higher levels could be determined in conditions like idiopathic hypercalciuria, sarcoidosis or paraneoplastic hypercalcaemia. (Obermayer-Pietsch & Schwetz, 2016)

PTH is produced in the cells of the parathyroid gland and accumulates in their secretory granules, serving as a depot. Once the serum calcium levels drop, PTH is released into circulation to raise the calcium concentration by bone degradation. (Obermayer-Pietsch & Schwetz, 2016)

Osteocalcin (OC) is the most common non-collagenous bone-specific protein and was the first bone metabolism marker. Even though it is bound to hydroxyapatite of the mineralized bone matrix, 20-30% are released into the circulation and therefore act as a biomarker for bone formation during the osteoblastic differentiation. The synthesis is dependent on vitamin K and vitamin D and requires these vitamins for the carboxylation to enhance the binding ability. Undercarboxylated OC (ucOC) is one option of measuring vitamin K insufficiency. According to studies, increased level of undercarboxylated OC are associated with hip fracture risk in elderly women, while other studies propose the enhanced secretion of insulin via undercarboxylated OC in mice and men. OC in general is said to have additional value in terms of steroid and glucose metabolism (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012)

Pro-peptides of type I pro-collagen, with a c-terminal (PICP) or a n-terminal (PINP), are stable serum markers in terms of bone formation. PINP serves as an internationally standardised routine parameter and is relatively easy to measure. During degradation of collagen type I, these markers are cumulating in liver cells and therefore an adequate liver function is needed for accurate concentration measurements. Additionally, the renal function is important as well due to renal elimination of collagen products. (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012)

Bone specific alkaline phosphatase (bALP) is part of the membrane-bound ALPs, which are content of every tissue in the human body. It has several isoforms, which differ by glycosylation, and can be found in the liver, the bile system, the kidneys or in the placenta. bALP is expressed by osteoblasts during bone formation and thereby serves as a marker for the assessment of metabolic bone diseases, such as Paget disease of bone. (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012)

Tartrate-resistant acid phosphatase (TRAP) is a monomeric metalloenzyme and can be divided into 5a and 5b, depending on the source. TRAP Type 5a is expressed

by osteoclastic-activated macrophages and dendritic cells, whereas type 5b is produced by osteoclasts and measured by immunoassays. In addition, TRAP 5b is activated by RANKL and shows an activity ten times as high as TRAP 5a. (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012)

Pyridinoline and **deoxypyridinoline** are markers of bone resorption and components of crosslinks between fibrils formed by amino acids to enhance strength. They can be found in all collagen-depended fibrils (I, II, III) and get released into circulation by matured collagens as a metabolic product. The concentration of these crosslinks can be measured in urine by high-performance liquid chromatography, free deoxypyridinoline on the other hand can be determined by Enzyme-linked Immunosorbent Assay, known as ELISA, too. (Naylor & Eastell, 2012)

Hydroxyproline is also a marker of bone resorption. It is a collagenous amino acid found in bone, skin and other tissues that contain various types of collagens. The big disadvantage is that values tend to be elevated once gelatine or food that is rich on collagens is consumed, therefore the clinical use of this markers has been reduced during the past decades. (Naylor & Eastell, 2012)

1.1.5.5. Fracture Risk Assessment

Fractures are the most severe OPO consequence in terms of health, having an enormous impact in a patient's daily life and outcome. (Sözen, et al., 2017) For many years, DXA-based measurement of BMD has been the main investigative process to assess fracture risk in patients with certain conditions like OPO due to having a high specificity. The sensitivity, on the other hand, is quite low and therefore additional assessment tools were needed. Furthermore, most of the fractures in postmenopausal women occur in patients with a T-score above -2,5, i.e. women not categorized as OPO patients due to not undercutting the threshold value set by the WHO. To include other clinical risk factors that are involved in mechanisms resulting in enhanced fracture risk, FRAX, short for fracture risk assessment tool, has been created. It uses an algorithm to estimate the 10-year fracture risk for hip fractures or major OPO fractures of hip, spine, wrist or humerus, based on various risk factors that haven't

been included yet and can be used in addition to BMD. (Compston, 2016) (Sözen, et al., 2017)

First, the algorithm contains basic information like age, gender and the body mass index, especially if BMI <21kg/m2. Secondly, the patient history of prior fractures and parental history concerning hip fractures are included as well. Furthermore, the BMD T-Score of the femoral neck, the presence of conditions like rheumatoid arthritis or causes to secondary OPO, the early onset of menopause, any prior or current treatment with glucocorticoid with an equivalent to ≥5mg prednisolone for at least 3 months, previous or current smoking and the individual alcohol intake (≥3 alcoholic beverages daily) are part of the FRAX algorithm as well. (Schwartz, et al., 2011) (Sözen, et al., 2017)

For example, for a 70-year old woman (170cm, 90kg = ~ BMI 31) with a T-Score of -2,5 in femoral neck and spine, no osteoporotic treatment but affected by secondary osteoporosis, previous fragility fracture after the age of 45 and previous parental hip fracture, current smoking and more than 3 alcoholic beverages daily, the 10-year risk assessment is demonstrated in the following figure (Figure 6).

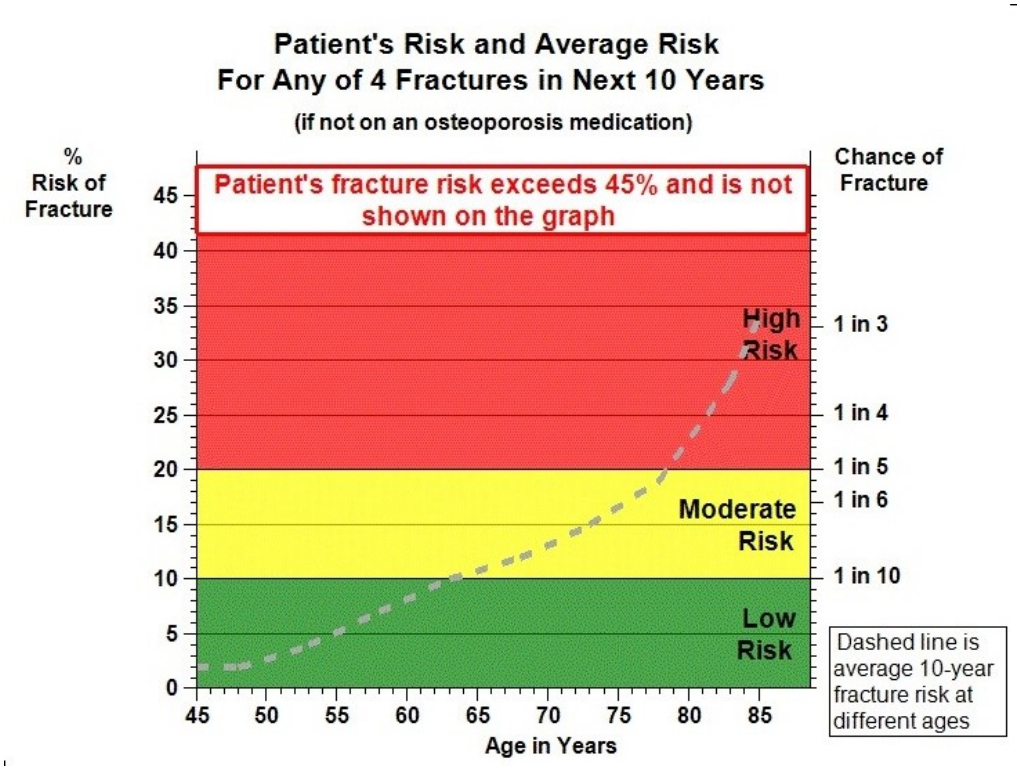


Fig.7: FRAX 10-year risk assessment (FORE , 2014)

Despite of FRAX being a major advance in clinical fracture risk assessment, not all risk factors are included yet and therefore, clinical users and practitioners must be aware of the state as an additional helping tool. (Oei, et al., 2016)

1.1.6. Treatment

If one of the following terms is met in patients of both genders, a specific treatment is in order. Guidelines, created by DVO in 2014 and revised in 2017, suggest that a specific treatment is recommended, once one (2nd or 3rd grade of Genant) or multiple (1st to 3rd grade of Genant) low-traumatic vertebral body fracture (2nd or 3rd grade of Genant) occurred and the T-Score of specific sites (lumbar spine, femoral neck or proximal femur in total) is below -2,0, while other causes are unlikely. Low-traumatic fractures are fractures, which causes include the fall from a standing position, the fall from low heights or no real force impacts took place. In addition, low-traumatic fractures of the proximal femur, with a T-Score below -2,0 in the sites mentioned above, suggest treatment measures as well. Furthermore, guidelines also recommend drug treatment if high-dose oral glucocorticoids, equivalent to ≥7,5mg of prednisolone, were prescribed for at least 3 months or if the treatment is estimated to take at least 3 months, with less time passed, and a T-score ≤-1,5 on of the mentioned sites or one/multiple low-traumatic vertebral body fractures, with same criteria described above, or more than 3 peripheral fractures occurred. In these cases, the glucocorticoid therapy should be re-evaluated after 3 months to adjust medication or stop treatment at all. (DVO, 2017)

1.1.6.1. Antiresorptive Medication

Antiresorptive drugs are used as first-line treatment in OPO and their effects basically consist of inhibiting the function and development of osteoclasts, primarily responsible for bone resorption. However, uncertainties remain about how long the optimal duration of treatment is, how the post-discontinuation phase may be handled and if the risks and benefits of antiresorptive treatment are somehow balanced in the end. (Meier, et al., 2017)

Bisphosphonates are the most common antiresorptive drugs and have their effect in inhibiting osteoclasts from development and subsequently reducing their lifespan. Alendronate, Risedronate and Zoledronate are used to prevent and treat postmenopausal and male OPO, as well as glucocorticoid induced OPO. Ibandronate, on the other hand, has its main purpose in the treatment of postmenopausal OPO. (Sözen, et al., 2017) The DVO guidelines recommend Ibandronate to be used on women with a T-score below -3,0 on the femoral neck. (DVO, 2017) Apart from irritation of the upper gastrointestinal tract, bisphosphonates show high effectivity, with reducing fractures rates up to 50%, and are well tolerated as well. (Gallagher & Tella, 2014)

Denosumab contains monoclonal antibodies against RANKL and is part of the postmenopausal OPO treatment, especially used in patients with high fracture risks, previous occurred fractures and insensitivity regarding other therapy options. (Sözen, et al., 2017) Furthermore, it is recommended in male patients with intensive bone loss caused by hormonal ablation in terms of treating prostate cancer. However, there is a considerably increased risk for vertebral fractures after discontinuation, so in practical use, this should be included in the therapy decisions. (DVO, 2017)

Selective estrogen receptor modulators (SERMs) are used to prevent OPO development in postmenopausal women. (DVO, 2017) Until reaching the age of 60 or 10 years past menopause, it is recommended for women to be treated with drugs like Raloxifene or Bazedoxifene. (Meier, et al., 2017)

1.1.6.2. Osteoanabolic Treatment

Vitamin D and **calcium** supply are important factors in terms of prevention of OPO development. The combination proved to have a positive effect on the fracture risk of the femoral neck and fractures of other sites. For patients without a specific drug treatment, 1g calcium should be granted by food intake. In case of not reaching this amount, however, supplements are in order. Concerning vitamin D3, 800 to 1000 IE should be supplemented on a daily basis as well. If treated with antiresorptive drugs, hypocalcaemia is a side effect that needs to be eliminated beforehand. Patients that

receive treatment, the same amount of calcium should be gained to avoid hypocalcaemia, while also maintaining a solid vitamin D3 intake. (DVO, 2017)

Teriparatide is a synthetic form of the human PTH and is mainly used as differential therapy. The most promising response monitoring is achieved by measuring PINP because of its dynamic variations. (Naylor & Eastell, 2012) In glucocorticoid-induced OPO, it showed a more effective fracture reduction than oral bisphosphonates in terms of vertebral body fractures. However, the maximum of treatment duration is limited to 24 months due to costs and the fear of bone tumors due to some previous animal experiments, which were never proven in human patients. (DVO, 2017)

Strontium ranelate is an optional drug for severe OPO in men with high fracture risk and postmenopausal women in general, in case other drugs are not applicable due to incompatibilities or contraindications. (DVO, 2014)

1.1.6.3. Other Treatments

In general, the main approach in secondary OPO is to treat the causal condition and to avoid further development of osteoporotic fragility, for example by changing or adjusting the current medication. In case of a glucocorticoid therapy, the aim is to adjust the medication to the lowest possible drug dose for further prevention, while still treating the primary disease accurately. Additionally, anxiety from previous falls arises in some individuals and results in additional falls, a condition in which psychosocial treatment may break this vicious circulus. (DVO, 2017)

1.2. Type II Diabetes Mellitus

Type 2 Diabetes mellitus (T2DM) is a chronic metabolic syndrome of heterogeneous nature and affects the carbohydrate and fat metabolism. (Scheen, 2003) Internationally, millions of people suffer from T2DM and the number of affected is increasing at a shocking rate. Currently, T2DM is one of the leading causes of mortality worldwide and is highly associated with lifestyle, eating habits and living conditions. (Messina, et al., 2017) The complications resulting from T2DM are quite severe and despite having multiple disciplines concerned with treatment and management of this disease, it persists in the role as main cause of blindness, cardiovascular conditions, end-stage renal diseases and non-traumatic amputations of the lower limb. (Veves, et al., 2013) (Temneanu, et al., 2016)

A special form of diabetes mellitus (DM) is gestational diabetes, characterized by occurring during pregnancy. Compared to type 1 diabetes mellitus, T2DM is more common and represents approximately 90% of diabetes cases. It occurs mainly in adults above the age of 40 years and in obese people. The symptomatic in both conditions may appear highly variable, but in general, T2DM tends to show fewer symptoms with less characteristics. Additionally, the risk of diabetic ketoacidosis is much higher in type 1 diabetes mellitus. Concerning treatment, insulin is used in T2DM as a late option for glucose control due to the phenomena of insulin resistance and hyperinsulinaemia, whereas patients with type 1 diabetes need to be treated instantly with insulin due to their lack of own insulin production. In general, the functional deficiencies are present far sooner than the clinical manifestation occurs, implying that prediabetic patients, with an elevated blood glucose level not yet reaching the threshold value of 200 mg/dl, need to be detected and managed as soon as possible for optimal effect. ((OEDG), 2016) (Mutie, et al., 2017)

1.2.1. Epidemiology

Currently, DM is the 5th leading cause of mortality worldwide, with most reported cases with T2DM, with an important tendency to increase. (Messina, et al., 2017) Calculations from 2011 suggest that there are nearly 366 million people with DM, with approximately

50% of them being unaware of their condition. By 2030, the number of patients affected by DM is estimated to be doubled. (Veves, et al., 2013)

Due to the increase in life expectancy, the earlier onset of T2DM and the population getting older on average, prevalence and incidence are reaching proportions of epidemic nature, with estimation suggesting the incidence to increase about 4.5-fold between 2005 and 2050. (Yakaryilmaz & Öztürk, 2017) (Scheen, 2003) An additional problem is the health care issue of prediabetes with currently more than million Europeans suffering from this condition. Subsequently prediabetes is highly associated with the progressing towards T2DM, whereas 5 to 10% of these patients develop T2DM on an annual basis. (Mutie, et al., 2017)

Apart from T2DM being diagnosed in adults predominantly, the frequency in children and adolescents are increasing significantly as well. The prevalence in terms of gender reflects the situation in the elder population, with a prevalence being higher in girls than boys. (Temneanu, et al., 2016)

1.2.2. Etiology

T2DM is a metabolic syndrome with multiple causes linked to environmental conditions and genetic predispositions resulting in reduced production and secretion of insulin, as well as impaired insulin sensitivity with additional attention to peripheral tissues. (Scheen, 2003) The genetic influence in the development of T2DM is supported by increased prevalence in first-degree relatives and in certain ethnic group. (Temneanu, et al., 2016) Apart from genetical variations with heterogeneous impacts on an individual's metabolism and cumulative effects shown in family histories and ethnics, lifestyle-based risk factors for T2DM have been studied for their believed significance and proven by showing strong associations with poor nutrition, including lack in diversity, and a sedentary lifestyle, often resulting in obesity with especially high intraabdominal fat content. (Messina, et al., 2017) (Scheen, 2003) The influence of media, marketing strategies and technical evolution is of additional importance due to being omnipresent and the rising effect on people. Other conditions, like metabolic syndrome, obstructive sleep apnoea, osteoarthritis, non-alcoholic fatty liver disease

and some types of cancer, that are associated to obesity or based on poor lifestyle in some extend are closely correlated with T2DM as well. (Temneanu, et al., 2016)

1.2.3. Pathogenesis

T2DM is characterized by decreased production and sensitivity of insulin with a subsequent disruption of communication between the responsible tissue types, such as pancreas, liver, adipose tissue, skeletal muscle, intestine and the central nervous system. As a result, the altered homeostasis of glucose embodies the development of T2DM. (Scheen, 2003)

Beside liver, muscle and adipose tissue, pancreatic beta-cells of the islets of Langerhans are probably the most afflicted tissue in terms of T2DM and are no longer able to produce the required amount of insulin needed to balance glucose levels after dietary intake. After glucose has been ingested by the intestine or produced in the liver by gluconeogenesis, the carbohydrate enters the circulation and induces further actions. To balance glucose levels, insulin is produced to lower and therefore balance the amount of glucose in the bloodstream by forcing glucose uptake in cells. The amount of insulin, on the other hand, mainly depends on the rate, in which glucose is metabolized in various tissues, and the glucose levels entering circulation. (Mutie, et al., 2017)

Obesity, especially of central visceral nature, has an important role in the development of T2DM and appears in most patients. Numerous mechanisms, including elevated non-esterified fatty acid levels, toxic effect of ectopic fat storage in liver, pancreas and muscle tissue and the effect of various adipocytokines, are involved in the disruption of insulin activity with interference in both secretion and sensitivity of action. These abnormalities in fat metabolism are additionally associated with other conditions, like the metabolic syndrome. (Scheen, 2003)

The issue of increased glucose tolerance is enhanced by the decreased beta-cell capacity, found in elderly patients. Therefore, the aging process is an additional factor in the evolution of T2DM. (Yakaryilmaz & Öztürk, 2017)

1.2.4. Diagnosis

The main approach in detecting patients with T2DM, and DM in general, is the measurement of glucose, either random or fasting blood glucose, or haemoglobin A1c (HbA1c), a glucose-related parameter that provides information on long-term glucose metabolism. To obtain further information, the oral glucose tolerance test (oGTT) is a routine test to unmask impaired glucose tolerance by testing the metabolic response after glucose intake. Subsequently, the blood glucose level will be detected after certain time intervals, most commonly after 30, 60 and 120 minutes, to show the individual's metabolic response. Concerning a healthy metabolism, the initial elevation of blood glucose is followed by an exponential decline. A metabolism affected by DM, however, will not be able to reduce the blood levels in time due to reduced insulin sensitivity and lack of insulin secretion. By means of these parameters, the diagnosis can be made once certain terms of the following guidelines, provided by the Austrian Diabetes Society (OEDG), are met. ((OEDG), 2016)

	Manifested DM	High risk for DM
Random blood glucose (non-fasting)	≥200 mg/dl (11,2mmol/L) + classic symptoms OR ≥200 mg/dl	-
Fasting blood glucose (venous plasma)	≥126 mg/dl on 2 different days	Impaired fasting glucose ≥100 mg/dl (5,6 mmol/L) + ≤125 mg/dl (6,9 mmol/L)
OGTT glucose after 2h (venous plasma)	≥200 mg/dl on 2 different days	Impaired glucose tolerance ≥140 mg/dl (7,8 mmol/L) + ≤125 mg/dl (11,0 mmol/L)
HbA_{1c}	≥6,5 % (48 mmol/mol) on 2 different days	≥5,7 % (39 mmol/mol) + ≤6,4 % (46 mmol/mol)

Tab.3: Diagnosis Guideline by the Austrian Diabetes Society ((OEDG), 2016)

According to the guidelines, the screening of specific groups and patients with certain risk factors should be enabled and carried out to maximize the preventive effect. High risk patients, as mentioned in the table above, should be considered as prediabetic and therefore, screened to determine the state of prediabetes or to monitor progression

towards DM. In asymptomatic patients above the age of 45, measurements of fasting blood glucose are indicated every 3 years, whereas HbA1c and oGTT serve as an option. Patients with a lowering in HDL-cholesterol (<35 mg/dl) and/ or elevated triglycerides (>250 mg/dl) should be considered as well. Individuals affected by arterial hypertonia, with a blood pressure surpassing 140/90 mmHg, should be tested due to the association with vascular conditions. Furthermore, women should be considered once the diagnosis of polycystic ovarian syndrome is made or a child is born to them and its weight exceeds 4,5kg. Apart from other conditions, associated to DM, that indicate further investigation, certain ethnicities of asian, african or latin-american descent are part of high-risk populations and require additional awareness due to genetic background. ((OEDG), 2016)

1.2.5. Treatment

The basic principle of treating T2DM is the lifelong modification of the patient's lifestyle. ((OEDG), 2016) Studies suggest that 80% of T2DM cases can be prevented entirely only by weight management, exercise and adjust diet. (Messina, et al., 2017) In addition, certain drugs are available to manage blood sugar level, whereas fasting glucose levels should be kept below 130 mg/dl, ideally <110 mg/dl, and postprandial levels, measured 2 hours after intake, should not exceed 180 mg/dl. The aim to control the glucose metabolism is to keep HbA1c below 6,5% to ensure higher life expectancy, a low risk for hypoglycaemia and to avoid additional cardiovascular comorbidities. If it is not possible to reach this state without complications and enhanced risk for hypoglycaemia, a target value of 7% is sufficient. In case of severe hypoglycaemia in the past, limited life expectancy and other late complications, 8% should not be surpassed. ((OEDG), 2016)

Patients diagnosed with T2DM should receive drug treatment as soon as possible to avoid further progress with subsequent early and late consequences. Guideless recommend making the decision based on HbA1c value. In case of undercutting the 6.5 %, no drug treatment is needed but metformin could be used in addition to lifestyle modification. In the range between 6.5% to 9%, metformin serves as first line drug but is combined with an additional antidiabetic drug, called dual therapy. Once HbA1c

values above 9% are measured, a triple therapy with metformin should be executed. Symptomatic hyperglycaemia and decompensation are indications for swift transfer to a hospital. ((OEDG), 2016)

Metformin is recommended as first line drug therapy of T2DM by many guidelines, including guidelines by the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD) and OEDG, and considered to be an optimal initial drug to treat chronic elevations of blood sugar levels. The exact mechanism of effect is unclear to this day but is thought to involve the lowering of hepatic glucose production. The perks of metformin involve a high efficiency, a good safety profile, weight neutrality, reduction of cardiovascular complications, improvements in lipid levels and inflammatory markers, as well as being a low-cost drug. (Sanchez-Rangel & Inzucchi, 2017) ((OEDG), 2016)

In terms of dual therapy, metformin is combined with glucagon-like peptide-1 (GLP-1) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, selective sodium-glucose transporter-2 (SGLT-2) inhibitors, α-glucosidase inhibitor, sulfonylurea, glinides, pioglitazone or insulin. Triple therapy includes one further drug, whereas GLP-1 agonists and SGLT-2 inhibitors are not yet recommended as combination. ((OEDG), 2016) (Veves, et al., 2013)

The indication for insulin treatment is given once dietary measures and oral antidiabetic drugs do not the achieve the required effect or contraindications for specific drugs exist. Further reason suggesting insulin therapy are severe comorbidities or in perioperative settings. The blood sugar target values are not changing during insulin treatment. The required dose depends on the type of treatment, whether other antidiabetic drugs are in use or special conditions prefer higher or lower doses. There are several forms of insulin, vary in start, duration and maximum of effect. ((OEDG), 2016)

Additionally, comorbidities and potential complications should be treated as well. Lipid levels and blood pressure should be controlled to prevent the onset or progression of further complications. (Veves, et al., 2013) Lower blood pressure (<120/80 mmHg) are recommended in patients with high risks for strokes or diabetic nephropathy but may enhance the risk for cardiovascular events. Most diabetic patients do not reach the

aimed pressure, whereas complications because of low blood pressures tend to happen on lower rates as expected. The target pressure currently is 130-140 / 80-90 mmHg. Concerning fat metabolism, the surpass of threshold values of LDL-cholesterol (>70 mg/dl), triglycerides (>200 mg/dl) and non-HDL-cholesterol (>100 mg/dl) indicate drug treatment to reduce further complications. Patients with T2DM with additional risk factors, however, can be treated with statins without regarding LDL-cholesterol values. ((OEDG), 2016)

1.3. Osteoporosis and Type II Diabetes Mellitus

1.3.1. Context and relations

Despite of higher BMD, T2DM patients display an enhanced fracture risk, whereas the increased fall risk, especially caused by vascular complications, is thought to be the primary cause. In order to prevent further fractures, additional assessments and strategies for keeping balance, enhancing muscle strength and maintaining a specific visual acuity have been established. Nonetheless, the underlying cause why T2DM is making bone to seem weaker and prone to fracture despite of accurate BMD values needs to be examined more closely and therefore the relation between bone and glucose metabolism embodies the key role of many studies nowadays. (Hofbauer, et al., 2007) (Hamann, et al., 2012)

Abnormalities in the bones of T2DM patients are due to direct effects of insulin-related deficiencies, increased glucose level and enhanced production of adipokines, with leptin being the best-characterized adipokine, and cytokines with subsequent effects. However, the displayed BMD in patients with T2DM is paradox, which may be explained by the positive effect of obesity and the close correlation between these two conditions, therefore masking the actual impairment of the bone structure. (Hofbauer, et al., 2007)

One of the most significant parameters in this relation is osteocalcin, a protein derived from osteoblasts and odontoblasts and mainly bound to hydroxyapatite, while the rest is released into circulation and measured to gain information on bone formation. Posttranslational carboxylation, which depends on vitamin K, enables OC to bind free calcium. Therefore, ucOC is additionally providing information on the vitamin K supplies, whereas supplementation results in a subsequent decrease of ucOC levels. Furthermore, decarboxylation of OC diminishes the affinity to bind to hydroxyapatite. (Obermayer-Pietsch & Schwetz, 2016) (Naylor & Eastell, 2012) (Schwetz, et al., 2012)

Besides the utilization as a biomarker, OC is involved in glucose metabolism as well. Studies determined positive correlations between serum OC concentrations and insulin secretion, as well as proliferation of pancreatic beta-cells. Furthermore, OC improves insulin sensitivity by enhancing the expression of adipokine in adipocytes,

resulting in an insulin-sensitizing effect. In addition, OC is positively associated to serum adiponectin levels and lowered insulin resistance. On the other hand, inverse associations to plasma glucose levels, body fat mass and, in general, the development of T2DM have been established. (Hwang, et al., 2012) (Schwetz, et al., 2012)

Leptin is the most-recognized adipokine in terms of skeletal effects and is produced in adipocytes, while functioning in the brain by inhibiting appetite in order to favour functions of reproductive nature. Concerning bone metabolism, however, its effects on osteoblasts are accomplished via 2 pathways. Firstly, the reduction of serotonin further leads to decreased osteoblastic proliferation and subsequently increased bone resorption, while limiting the bioactivity of OC. Secondly, leptin reduces the insulin secretion by direct influence on pancreatic beta-cells. Besides leptin, adiponectin and resistin are additional adipokines and mediators known to be partly involved in the positive effects of obesity on bone. Resistin-receptors are expressed on osteoblasts and osteoclasts and the effects include the stimulation of osteoclastogenesis and the increased pre-osteoblastic proliferation, whereas the differentiation of osteoblasts is not influenced. Adiponectin has its receptors on both cell types as well and functions by supressing osteogenesis, whenever insulin is not present. In general, however, studies showed that adipokines show adverse effects and cannot be solely used to explain BMD values in T2DM. (Hofbauer, et al., 2007)

Markers of bone resorption, like NTX, CTX and deoxypyridinoline vary quite strongly, either being elevated, lowered or not altered at all, depending on the study and DM conditions. (Hamann, et al., 2012)

In addition, hormonal factors are involved in the development of OPO and contribute to the effects of T2DM on bone metabolism. Primary hyperparathyroidism and chronic PTH excess result in bone loss, especially affecting cortical bone and skeletal sites consisting higher amounts of cortical bone. The diagnosis of OPO and osteoporotic fractures are indicating parathyroid surgery in patients without any other symptoms. (Hofbauer, et al., 2010) (Bilezikian, et al., 2009) Hyperthyroidism is additionally involved in the loss of bone mass by activation of thyroid hormone receptor α, leading to increased resorption of bone. (Hofbauer, et al., 2010)

Furthermore, hypogonadism is a central part of the development of OPO in both men and women. Androgens, for example, are needed to gain a certain peak bone mass and upkeep bone strength afterwards. Androgen-deprivation is a common option for treating prostate cancer but shows rapid loss of bone mass as a side effect. Besides postmenopausal OPO, pregnancy-associated OPO is affecting women as well, whereas the causal factors are not yet fully understood. A combination of vitamin D and calcium deficiencies, low bone mass and high bone turnover due to increased PTH-like proteins. (Hofbauer, et al., 2010)

1.3.2. Case report

The following illustrations (Tab. 3, Fig. 8–10) show the case of a 71-year-old Caucasian woman, who suffers from T2DM and obesity, with a height of 155,6 cm, a weight of 79,4kg and a BMI of 32,8 kg/m2. The graphics display the paradox BMD-TBS-relation in which patients with T2DM have accurate BMD but a decreased TBS-Score. Therefore, the explanation of increased falling risk as the main causal factor of subsequent increased fracture risk in patients with T2DM does not fit entirely.

Region	TBS	TBS T-Score	TBS Z-Score	BMD	BMD T-Score
L1	1,117	---	---	1,024	-1,0
L2	1,109	---	---	1,044	-1,4
L3	1,200	---	---	1,195	-0,2
L4	1,234	---	---	1,211	-0,1
L1-L4	1,165	-3,4	-0,9	1,130	-0,5
L1-L3	1,142	-3,9	-0,8	1,094	-0,7
L1-L2	1,113	-4,1	-0,7	1,034	-1,2
L2-L3	1,154	-4,0	-1,2	1,125	-0,7
L2-L4	1,181	-3,3	-1,0	1,158	-0,5
L3-L4	1,217	-2,6	-0,8	1,204	-0,1

Tab.4: 71-year-old female T2DM patient with normal BMD but decreased TBS

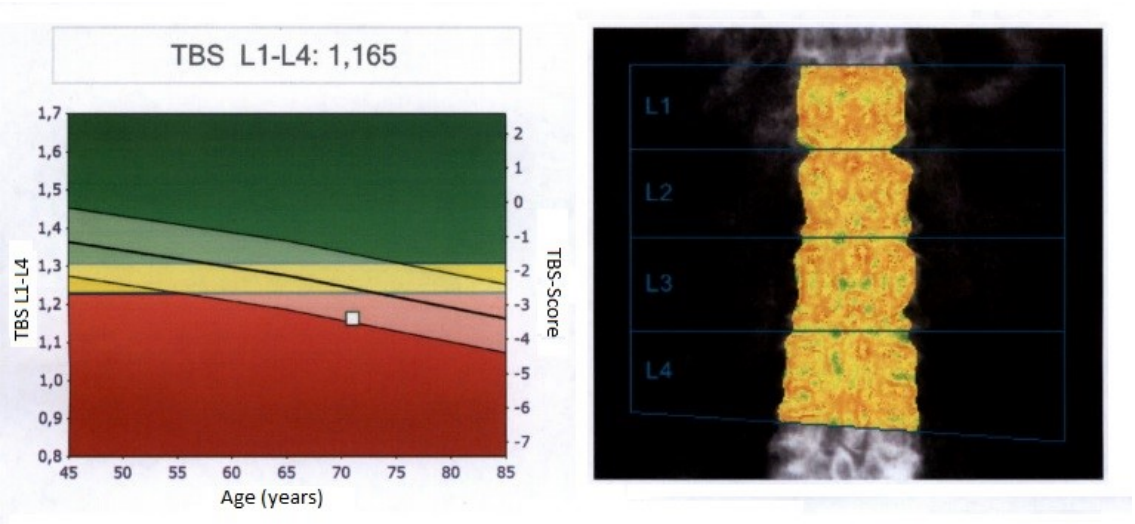
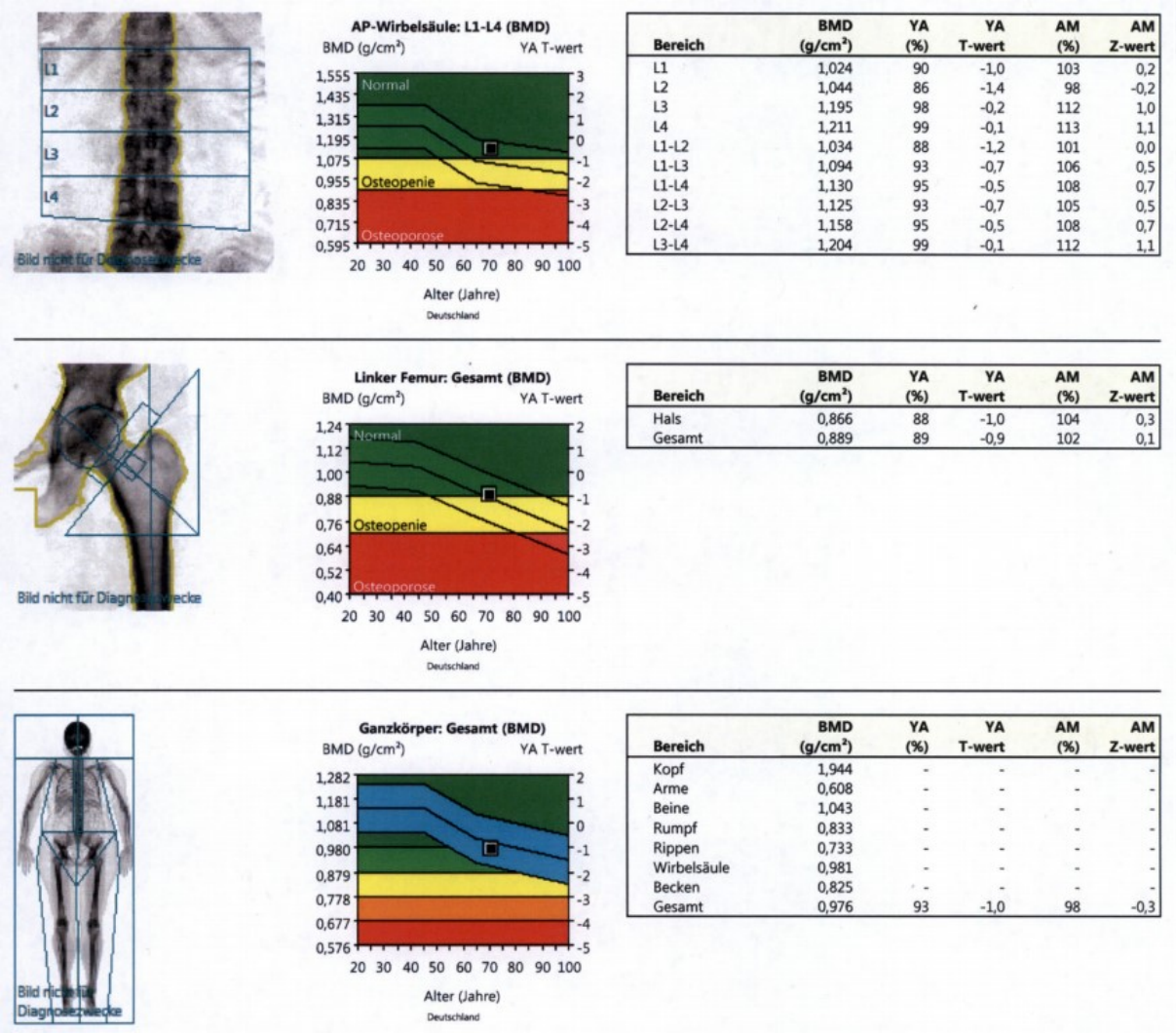


Fig.8: TBS measurement and reference curve



Bereich	BMD (g/cm²)	YA (%)	YA T-wert	AM (%)	AM Z-wert
L1	1,024	90	-1,0	103	0,2
L2	1,044	86	-1,4	98	-0,2
L3	1,195	98	-0,2	112	1,0
L4	1,211	99	-0,1	113	1,1
L1-L2	1,034	88	-1,2	101	0,0
L1-L3	1,094	93	-0,7	106	0,5
L1-L4	1,130	95	-0,5	108	0,7
L2-L3	1,125	93	-0,7	105	0,5
L2-L4	1,158	95	-0,5	108	0,7
L3-L4	1,204	99	-0,1	112	1,1

Bereich	BMD (g/cm²)	YA (%)	YA T-wert	AM (%)	AM Z-wert
Hals	0,866	88	-1,0	104	0,3
Gesamt	0,889	89	-0,9	102	0,1

Bereich	BMD (g/cm²)	YA (%)	YA T-wert	AM (%)	AM Z-wert
Kopf	1,944	-	-	-	-
Arme	0,608	-	-	-	-
Beine	1,043	-	-	-	-
Rumpf	0,833	-	-	-	-
Rippen	0,733	-	-	-	-
Wirbelsäule	0,981	-	-	-	-
Becken	0,825	-	-	-	-
Gesamt	0,976	93	-1,0	98	-0,3

Fig.9: Case example - BMD reference curves of spine, left femur and full body

Despite having normal BMD at the spine, with the increasing degenerative disease potentially masking the “real” bone density measurement, the curves of the BMD of total left femur and total full body (Fig. 10) point out the development of OPO or in this case the progression of OPO nonetheless. The mineralisation of bone is still enhanced in the DXA scans of the spine, whereas the bone quality is very likely to be impaired, suggested by a TBS of 1,165 displayed in Fig. 8.

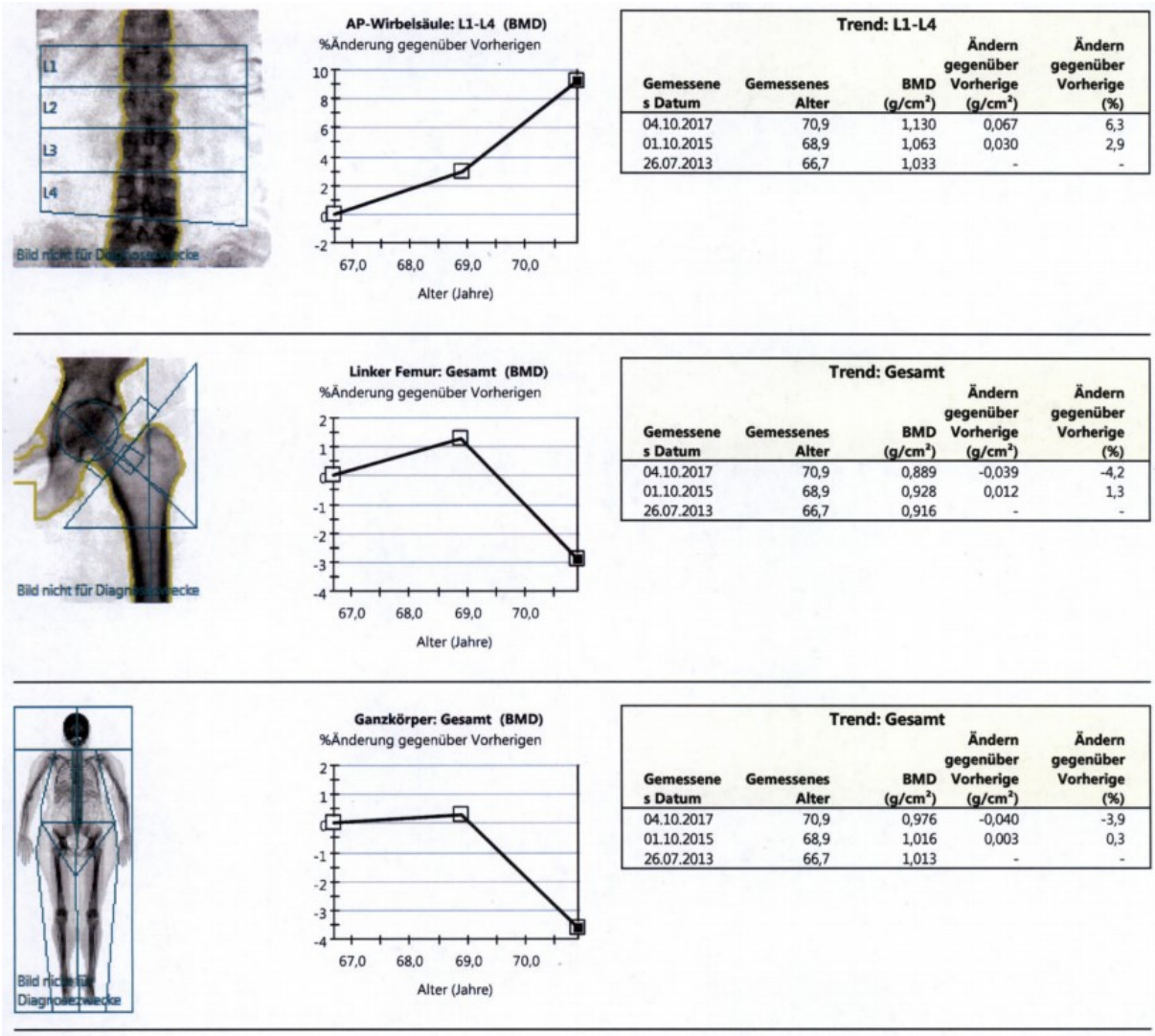


Fig.10: Case example - BMD progression curves

2. AIM OF DIPLOMA THESIS

The aim of this diploma thesis is to show the development of impaired bone structure in prediabetic patients in comparison to other participants with T2DM or healthy individuals. Therefore, TBS should provide additional information on the biomechanical microstructure and texture of bone to unmask the actual state of bone. Furthermore, we use additional parameters, with an alleged or proven involvement in the process of glucose metabolism and bone, to gain more information and potential clarification to describe our findings.

3. MATERIALS & METHODS

The data used in this diploma thesis are based on a longitudinal study cohort of a COMET K-project, the “BioPersMed” (Biomarkers for Personalized Medicine) cohort, started by the Divisions of Endocrinology and Diabetology and Cardiology of the Medical University of Graz in 2010, whereas planned and submitted in 2009. At first, various parameters and assessments of cardiological interest were included only but after a few months the Department of Endocrinology became a part in this endeavour as well. From this point, DXA scans, TBS calculations, biochemical measurements of hormones, electrolytes and additional markers were determined and assessed additionally. In 2012, the study location needed to be changed and Prof. Obermayer-Pietsch took over the study management. The assessment of data was continued and the team started to edit them in 2014, including myself. The cohort contains more than 450 clinical, functional and biochemical parameters of 966 volunteers from originally 1025 persons (531 female, 435 male) with a mean age of 58.

In general, the plan consisted of biannual visits with all participants going through screening, follow-up 1 and 2, with telephone visits in the year between. In the first visit (“baseline visit”), most volunteers underwent a cardiological screening only, therefore, the endocrinological part of the screening process was initially done in the upcoming visit. Over time and based on organisational changes, study analyses were planned and thus the data needed to be made accessible. As during several years, some participants unfortunately passed away and some left the cohort due to personal reasons, in 2015, the data of 966 people could be converted for upcoming editing. Due to problems during the data collection and logistic complexity, the amount of data was limited to a certain degree, resulting in data shortfall. To resolve this issue, the data will need to be converted anew and the gaps could be filled in by manual search.

The current available cohort for this diploma thesis consists of the data of 232 participants (n=232), including 148 women and 86 men. Besides TBS-Score and BMD-related parameters (T- and Z-Score, BMD of lumbar spinal segments) and body anthropometric parameters (height, weight, BMI), blood levels of hormones, biomarkers, vitamin D and lactose intolerance tests are part of the data as well.

4. RESULTS

Out of the “BioPersMed” cohort, 232 volunteers (n=232) were available to be stratified into non-diabetic (>140 mmol/L), prediabetic (140-200 mmol/L) and diabetic (>200 mmol/L) patients using 2-hour oGTT glucose levels.

BMD T-Score (based on the WHO classification of OPO) and TBS were included into the calculations, whereas BMD Z-Score data had to be excluded unfortunately due to lack of age-adjusted TBS values. Anthropometry data is represented by height, weight, and BMI. Additionally, bone biomarker (CTX, P1NP, OC, 25(OH) vitamin D) and hormonal blood levels (parathyroid hormone, thyroid stimulating hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone) were included to explore changes of bone metabolism more closely. FSH and LH are parameters used to include menopausal effects, whereas testosterone is used here equally for men. Due to high age being highly associated with bone status, the significance of age needs further clarification as well.

First and foremost, the data was used to calculate descriptive values (Tab.4) for each of the three subgroups to display the effects of T2DM and prediabetes on the respective parameters. The normal-distributed parameters are represented by the number of patients (n), the mean value and standard deviation, whereas the others are presented by the median, as well as the 25 and 75 percentiles.

Secondly, correlations between the parameters were calculated to describe an overview of the data set and to examine significant relations in further detail. (Tab.5)

		non-diabetic	prediabetic	diabetic	Total
Age	n	188	22	22	232
	Mean	57,89	57,18	61,89	58,20
	Std. Deviation	8,68	11,18	8,75	8,98
Weight	n	188	22	22	232
	Median	72,00	81,00	86,50	79,83
	25 Percentile	62,00	69,75	79,50	70,42
	75 Percentile	83,00	95,25	106,00	94,75
Height	n	188	22	22	232
	Mean	169,38	169,05	173,23	169,71
	Std. Deviation	8,50	10,70	9,40	8,85
BMI	n	188	22	22	232
	Median	24,97	27,90	29,57	27,48
	25 Percentile	22,44	26,29	26,26	25,00
	75 Percentile	27,77	32,56	33,34	31,22
TBS	n	188	22	22	232
	Mean	1,34	1,33	1,24	1,30
	Std. Deviation	0,107	0,109	0,087	0,101
BMD T-Score	n	188	22	22	231
	Mean	0,47	1,18	0,77	0,56
	Std. Deviation	1,052	0,858	1143,000	1063,000
oGTT 2h	n	187	22	22	231
	Mean	89,81	162,73	297,50	116,54
	Std. Deviation	21,33	17,39	57,36	67,92
Osteocalcin	n	187	22	22	231
	Median	22,30	18,20	13,25	17,92
	25 Percentile	18,55	14,80	10,70	14,68
	75 Percentile	28,25	20,80	17,00	22,02
P1NP	n	187	22	22	231
	Median	49,40	37,45	30,55	39,13
	25 Percentile	38,50	31,40	21,70	30,53
	75 Percentile	62,90	48,50	35,60	49,00
CTX	n	183	22	22	227
	Median	0,37	0,27	0,20	0,28
	25 Percentile	0,27	0,18	0,13	0,19
	75 Percentile	0,75	0,48	0,32	0,52
Cholecalciferol	n	187	22	22	231
	Median	29,80	26,95	24,90	27,22
	25 Percentile	24,40	22,10	20,20	22,23
	75 Percentile	36,85	33,70	36,00	35,52
PTH	n	185	22	22	229
	Median	46,20	41,80	37,50	41,83
	25 Percentile	36,80	32,30	30,10	33,07
	75 Percentile	55,70	62,00	41,50	53,07
TSH	n	185	22	22	229
	Median	1,73	1,36	1,85	1,64
	25 Percentile	1,14	0,88	0,85	0,96
	75 Percentile	2,51	1,65	2,52	2,23
Testosteron	n	185	22	22	229
	Median	0,33	0,30	2,16	0,93
	25 Percentile	0,14	0,15	1,37	0,55
	75 Percentile	2,57	2,01	3,17	2,58
FSH	n	155	16	14	185
	Median	61,60	16,20	8,92	28,91
	25 Percentile	8,18	6,40	7,06	7,21
	75 Percentile	94,90	66,50	11,25	57,55
LH	n	155	16	14	185
	Median	25,30	13,31	5,90	14,83
	25 Percentile	6,24	5,31	4,51	5,35
	75 Percentile	36,20	35,35	7,39	26,31

Tab.5: Descriptive Table including subgroups and total values

Correlations

	oGTT 2h	TBS	BMI	Height	Weight	BMD T-Score	OC	P1NP
oGTT 2h		-0,288	0,413	0,127	0,358	-0,047	-0,251	-0,301
TBS	-0,288		-0,121	0,078	-0,051	0,453	0,111	0,190
BMI	0,413	-0,121		0,248	0,872	0,174	-0,231	-0,181
Height	0,127	0,078	0,248		0,666	0,120	-0,249	-0,284
Weight	0,358	-0,051	0,872	0,666		0,198	-0,309	-0,274
BMD T-Score	-0,047	0,453	0,174	0,120	0,198		-0,167	-0,120
Osteocalcin	-0,251	0,111	-0,231	-0,249	-0,309	-0,167		-0,258
P1NP	-0,301	0,190	-0,181	-0,284	-0,274	-0,120	0,707	
CTX	-0,175	-0,031	-0,280	-0,217	-0,316	-0,088	0,594	0,472
Cholecalciferol	-0,196	0,079	-0,124	-0,181	-0,167	0,004	0,120	0,123
PTH	-0,115	-0,009	0,049	-0,005	0,038	-0,091	0,153	0,051
TSH	-0,024	0,131	-0,067	0,113	0,024	0,153	0,001	-0,080
Testosteron	0,133	-0,024	0,294	0,518	0,499	0,016	-0,232	-0,210
FSH	-0,187	-0,130	-0,317	-0,515	-0,506	-0,228	0,365	0,373
LH	-0,188	-0,073	-0,228	-0,546	-0,438	-0,112	0,362	0,345
Age		-0,446				-0,303		

	CTX	Chole-calciferol	PTH	TSH	Testo-steron	FSH	LH	Age
oGTT 2h	-0,175	-0,196	-0,115	-0,024	0,133	-0,187	-0,188	
TBS	-0,031	0,079	-0,009	0,131	-0,024	-0,130	-0,073	-0,446
BMI	-0,280	-0,124	0,049	-0,067	0,294	-0,317	-0,228	
Height	-0,217	-0,181	-0,005	0,113	0,518	-0,515	-0,546	
Weight	-0,316	-0,167	0,038	0,024	0,499	-0,506	-0,438	
BMD T-Score	-0,088	0,004	-0,091	0,153	0,016	-0,228	-0,112	-0,303
Osteocalcin	0,594	0,120	0,153	0,001	-0,232	0,365	0,362	
P1NP	0,472	0,123	0,051	-0,080	-0,210	0,373	0,345	
CTX		0,023	0,064	0,096	-0,085	0,248	0,139	
Cholecalciferol	0,023		-0,138	0,069	-0,123	0,047	0,102	
PTH	0,064	-0,138		-0,018	0,076	-0,022	0,005	
TSH	0,096	0,069	-0,018		0,077	-0,066	-0,004	
Testosteron	-0,085	-0,123	0,076	0,077		-0,596	-0,581	
FSH	0,248	0,047	-0,022	-0,066	-0,596		0,848	
LH	0,139	0,102	0,005	-0,004	-0,581	0,848		
Age								

significant Correlation
insignificant Correlation

Tab.6: Parameter correlations

The correlations (Tab.6) display that bone markers share negative associations to increased 2h-oGTT levels, whereas OC and P1NP show stronger negative correlations. Vitamin D and CTX, however, show significantly negative correlations of a lower extent. FSH and LH are negatively associated with increased blood glucose levels as well. Testosterone, on the other hand, does not share this relation. TSH and PTH appear not to correlate with blood glucose levels and T2DM parameters. TBS correlates negatively with age and increased oGTT levels and positively with P1NP and, of course, BMD T-score. BMI, as an anthropometric parameter, showed correlations to P1NP, OC, CTX, FSH, LH and BMD T-Score, whereas a significant relation to TBS has not have been found.

The following figure (Fig.11) includes the histograms and distribution patterns of our subgroups. Non-diabetic patient data show an approximation to a normal-distribution. The other two subgroups, however, were distributed differently, potentially caused by a lower number of participants.

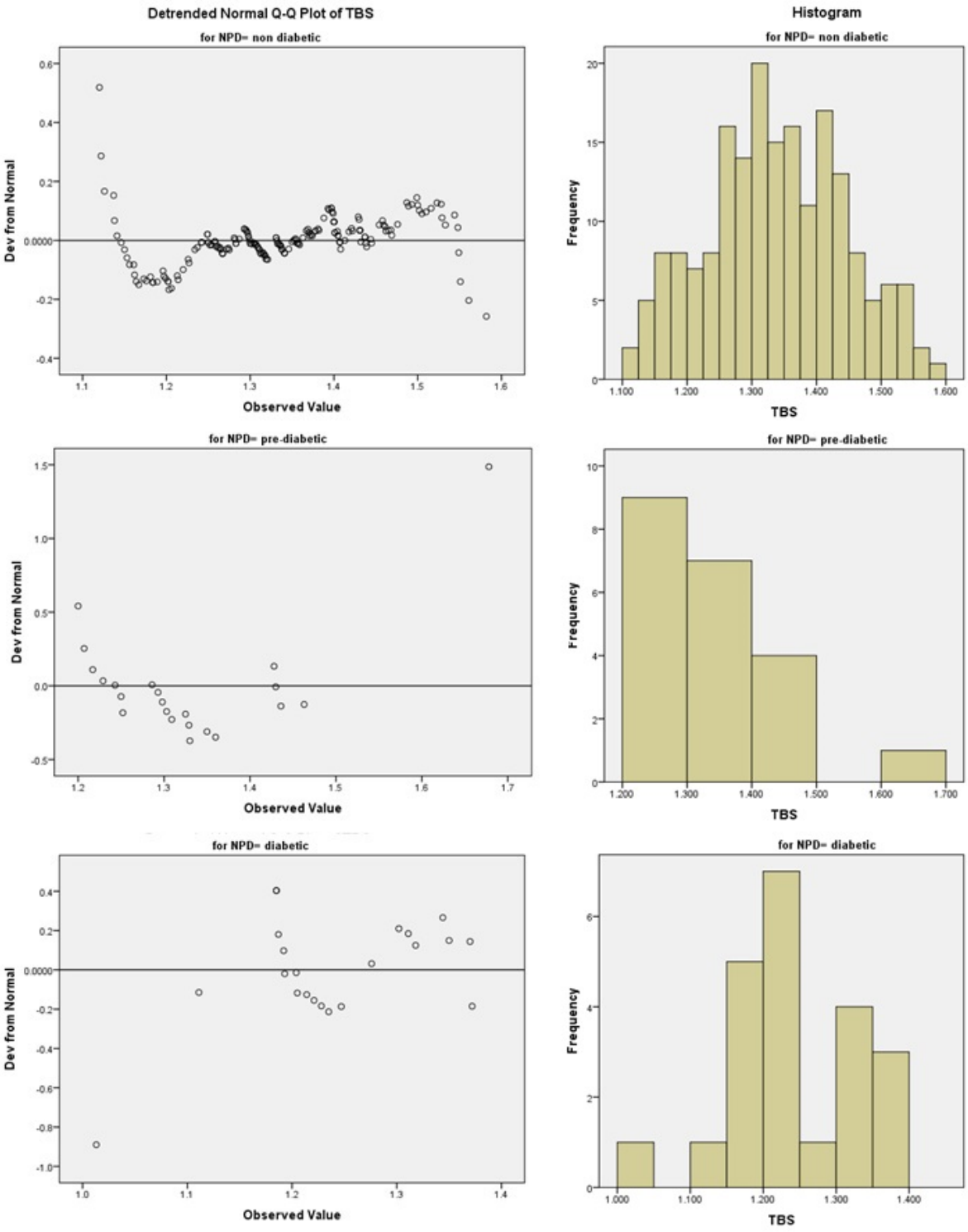


Fig.11: Detrended Normal Q-Q Plot and histogram of TBS in subgroups

TBS group comparison (Fig.12) shows lower values in prediabetes displaying a progression towards T2DM, represented by a mild reduction of TBS and a p-value lower than 0.001. In addition, two data outliers could be determined in the prediabetic and diabetic subgroup, with no background of the underlying cause to date.

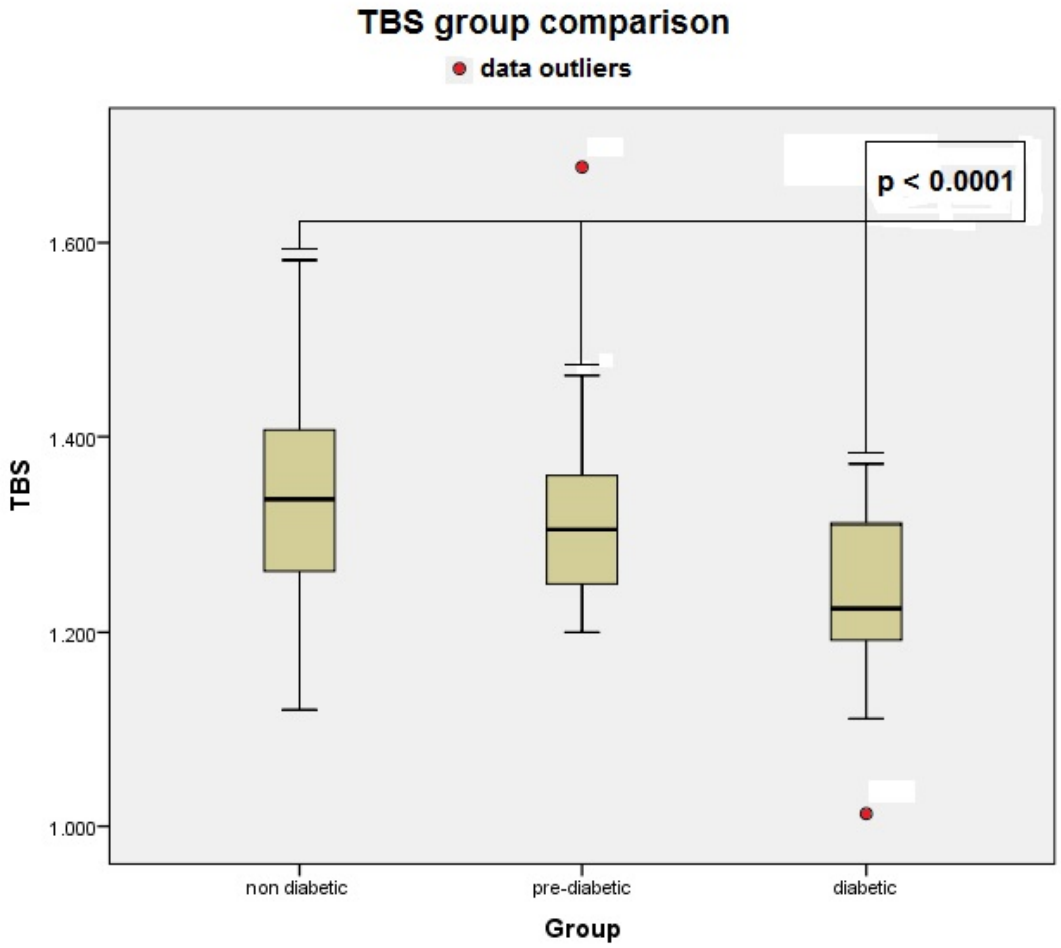
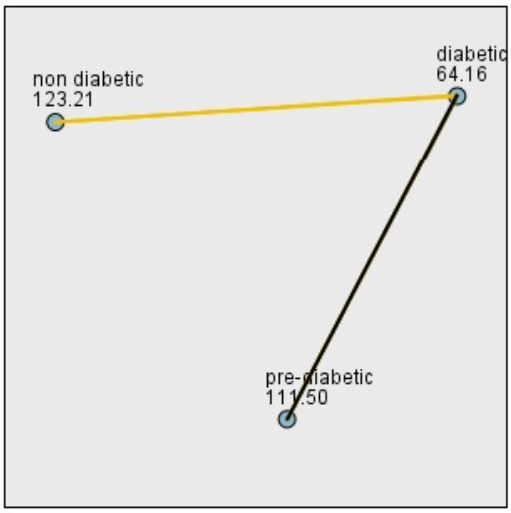


Fig.12: Group comparison of TBS

Pairwise comparison calculation (Fig.13), on the other hand, determined a significant comparison between non-diabetic and diabetic patients, while the comparison to pre-diabetic patients showed a clear trend.



Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
diabetic-pre-diabetic	47.341	20.236	2.339	.019	.058
diabetic-non diabetic	59.051	15.123	3.905	.000	.000
pre-diabetic-non diabetic	11.710	15.123	.774	.439	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Fig.13: TBS pairwise group comparison

5. DISCUSSION

Both OPO and T2DM are progressive chronic metabolic diseases with increasing prevalence, especially affecting the elderly population with severe consequences. (Scheen, 2003) (Sözen, et al., 2017) Due to their multifactorial conditions and the number of patients suffering from diabetic conditions, the risk factors and etiological processes have been studied to optimate diagnosis, medication, monitoring and other medical care-related aspects in order to improve health care in general. So far, the management of T2DM is well-established, still with some potential for improvement, whereas prediabetes, the rather unknown precursor of T2DM, has not been studied accordingly due to identification and access problems. The manifestation of T2DM can be quite asymptomatic and, therefore, many patients are diagnosed in rather far-progressed state with irreparable damage and many complications based on the underlying conditions. (Hofbauer, et al., 2007) The focus of managing T2DM should be set on early detection of glucose intolerance and insulin resistance, with more preventive potential as a benefit, subsequently having impact on health care, medical outcomes and health economy as well.

DXA-derived BMD and the patient's fracture history are the basics in diagnosing OPO, characterized by the loss of bone mass and impairment in bone structure leading to bone fractures with the well-known multiple consequences. The lack of involvement of bone quality assessment has shown a paradox state in patients with T2DM, who appear with normal or enhanced BMD in DXA measurements but increased fracture risk as well. (Sözen, et al., 2017) (Oei, et al., 2016) According to previous studies, the primary cause for the increased occurrence of fractures is alleged to the elevated fall risk due to micro- and macrovascular complications, derived from T2DM but also due to texture and material changes. Diabetic bone is, nonetheless, impaired in structure and prone to fracture, while BMD measurements being by trend elevated e.g. due to the frequently occurring obesity in T2DM patients. Studies have shown that obesity has a protective effect on bone density and, therefore, quality assessment needs to be involved to provide more information on the actual bone strength. (Hofbauer, et al., 2007) (Hamann, et al., 2012)

Our analyses suggest that TBS is a valid parameter for quality assessment in patients with T2DM, but also in pre-diabetes, showing that prediabetes and T2DM are negatively correlated to TBS in a progressive manner. Due to a lack of age-adjusted TBS values, the correlation to BMD Z-Score and the group comparisons adjusted for age could not be calculated, but will follow at a later stage of data acquisition. The progression of prediabetes towards T2DM via group comparison showed that prediabetes is already characterized by impaired bone structure and shows a strong trend, hence preventive efforts need to be established and carried out as soon as possible. In general, early screening and diagnosis with subsequent measures will enable to counter these lifestyle and age-related diseases at an early stage for optimal benefit.

Vitamin D enables enhanced intestinal calcium uptake and decreases renal calcium elimination with the effect of more calcium being available in the circulation. (Obermayer-Pietsch & Schwetz, 2016) Our results did not show strong associations to TBS, which is potentially based on the fact that most of the patients were supplemented with vitamin D. So, the cohort vitamin D levels tended to be quite accurate to recommended reference ranges due to treatment with vitamin D. Consequently, the contributing effect of vitamin D deficiency to OPO could not be determined. However, a number of studies suggest a central role of vitamin D in bone health. (Obermayer-Pietsch & Schwetz, 2016) (DVO, 2017)

FSH and LH showed negative correlations to TBS, implying that postmenopausal conditions are affecting both bone structure and BMD in women. Testosterone, on the other hand, did not show significant relations in men. Further investigation of BMD and TBS in meno- and andropause are on the way. (Eastell, et al., 2016)

Two data outliers appeared in the TBS group comparison, one prediabetic patient with elevated TBS and one diabetic patient with severe reduction of TBS. There are several potential explanations, e.g. a potential error due to data conversion, a mistake along the editing process or any currently unknown biological condition contributing to the extensive reduction or increase of the patients' TBS. The actual cause for the two data outliers could not be determined, but will be followed in future.

The major problem observed in this study has been the complexity of the cohort, disabling some parameters to be included at all. Consequently, body composition measurements with regard to fat and lean mass - while present - were hardly were hardly included, calcium blood levels were not evaluable after conversion due to technical reasons and other tests, e.g. handgrip values, need to be included and converted anew with careful editing to be analysed in a larger group of probands out of the BioPersMed cohort in the future.

In conclusion, our findings suggest that TBS is a valid parameter for bone quality assessment in patients with T2DM, showing that both prediabetes and T2DM are negatively correlated to TBS in a progressive manner. The development pattern of TBS in prediabetes towards T2DM showed that even at an early stage, bone structure is impaired, hence preventive efforts need to be in place as soon as possible. Other parameters, such as bone biomarkers of vitamin D might not have sufficient predictive value, probably to common vitamin D supplementation. Further studies and investigations, especially including age-adjusted TBS measurements and several additional conditions and parameters, will clarify open questions in these complex, hence clinically important findings relating bone health to glucose metabolism.

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