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A literature review of articles concerning fracture risk in Diabetes, and a population based follow up study on $HbA1_c$ values and fracture risk in "The Tromsø 4 health survey".

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Abstract

In this study the first aim was to do a literary review on the relation between diabetes and fracture risk. Second aim is to study the association between $Hba1_c$ and fracture risk in the dataset from Tromsø 4.
For the literature study PubMed was used, and search words were “fracture, risk and diabetes”. The search gave 370 articles, approximately 59 of these have been used. Data on fracture risk in diabetes and pathophysiological models are presented.
The follow up study was based on the Tromsø 4 health survey, in which 7127 subjects participated. The fracture rates were investigated during a mean follow up time of 517 weeks. A COX-regression model, which was stratified for gender and adjusted for age, was used to study fracture risk in relation to $HbA1_c$ values. The COX- regression study also gave survival plots for both genders.
A graphical presentation of fracture risk in the $HbA1_c$-quartiles across all age groups, in both genders, was made to study the possible interaction of $HbA1_c$*age and fracture risk.

The text study showed consistency with regards to increased fracture risk in type 1 diabetes, in type 2 diabetes the numbers were not so conclusive, some studies even found a decreased fracture risk in t2DM. In diabetics with diabetic complications, fracture risk was increased in both diseases. Pathophysiological mechanisms in diabetic bone disease has been explored.

In the follow up study there was a total of 799 fractures. The cumulative fracture incidence was 11% in the study population, 15 % females and 6 % in men during follow up.
Fracture risk decreased with increasing $HbA1_c$ among women: by each unit increase in $HbA1_c$ fracture hazard ratio (HR) decrease by 14%, CI (-26-0%), (p=0,048). In men HR was decreased by 6%, CI (-25,5-18%), but the relation was insignificant at (p=0,611).

The interaction of age*$HbA1_c$ and fracture risk was very weak in both genders and showed no significance at p=0,735 in females and 0,436 in males.

Introduction:

The study is divided in two parts, the first part is a literature based text rewiew that looks at recent studies regarding the topic of fracture and diabetes. Epidemiological studies that show fracture risk in diabetes are includeed, there are also articles that look into the possible pathophysiological mechanisms that govern bone health in diabetes. The aim is that the articles included will shed light on what has been found so far concerning fracture and diabetes, but also inspire the reader to think of the underlying causes of this topic, in terms of pathophysiological mechanisms in diabetic bone disease.

The next part of the study is a statistical analysis of a dataset from the "Tromsø 4 Health survey". The study had 7127 participants. Fracture rates were studied in this population. And COX regression was used to look at the relationship between fracture rates and the $HbA1_c$ (long time blood glucose value). A hazard ratio was calculated. The fracture risk were stratified for gender and adjusted for age. Survival plots were made. A clustered bar chart of fracture risk was made to look for a interaction between age, and the $HbA1_c$-fracture risk relation.

Some facts about diabetes

Diabetes mellitus is a condition in which a person is unable to lower his or her blood glucose level, hence the name mellitus (sweat).

Diabetes is diagnosed either by a glucose loading test in which the subjects drinks a solution containing 75 grams of glucose and a blood sample measures the blood glucose level, which is supposed to be below 11,1 mmol/L glucose. A fasting blood glucose level of more than 7,0 is also indicative of diabetes.

Glucose levels between 7,0 and 11,1 mmol/L is a sign of insulin resistance, typical of diabetes type 2, more on that to come.

$HbA1_c$ is often called the long term blood sugar value. It is the percentage of the red blood cells that are glycocylated at the haemoglobin site. A normal non-diabetic HbA1C is 3.5-5.5%. In diabetes about 6.5% is a good value. Red cells live for 8 -12 weeks before they are replaced. By measuring the HbA1C it can tell you how high your blood glucose has been on average over the last 8-12 weeks[1].

Type 1 diabetes (t1DM) presents itself in young people, from early childhood and into the early twenties. In Norway there are approximately 25000 type 1 diabetes patients, and 600 new cases are registered each year. During the last 30 years, newly recorded cases of diabetes have doubled. Norway is one of the countries with highest t1DM rates in the world[2]. The disease mechanism is known, an inflammatory condition seems to underlie the destruction of the insulin secreting cells of the pancreas. The lack of insulin obviously leads to hyperglycemia. Treatment in t1DM is lifelong subcutaneous insulin administration.
To what extent the inflammatory condition affects other tissue e.g bone is not well known, but of obvious interest for this paper. The underlying cause of the disease is not known.

Type 2 (t2DM) diabetes mainly affects the older generation. The elevated blood glucose levels seen in t2dm patients are due to an insensitivity to insulin at a cellular level. In most cases insulin resistance presents itself as a part of the metabolic syndrome which is a cluster of the following disorders: hypertension, obesity, elevated blood lipids and an increased insulin tolerance. In response to the ever increasing blood glucose the pancreas is stimulated to produce even more insulin, due to this, the t2dm patients are often hyper insulinemic. The pancreas cannot withstand this production rates, and over years it wears out, insulin production deteriorates in advanced states of the disease and the endocrine pancreas is left in a hyposecretory state. In the early stages of t2dm treatment is directed at the metabolic syndrome, reducing weight and exercise has a positive effect on the persons blood glucose levels, in more advanced states of the disease the treatment is directed at two targets, one the pancreas, trying to boost whatever is left of the persons insulin secretion, and the muscle and fat cells, trying to make them more sensitive to insulin and thereby reducing blood glucose. In the most advanced state, insulin support by injection is needed to control the blood sugar. The development of t2dm is well studied, and the current understanding points to genetic factors and a unhealthy lifestyle as risk factors.

As mentioned, high blood glucose levels are a common trait for these diseases, (when not treated, or poorly controlled). Microvascular complications, in the form of rethinopathy and glomerullopathy, and macrovascular disease in the form of heart and vascular disease are the long term effects. The direct and indirect effect of blood glucose on bone metabolism and fractures is not well understood, but is of special interest when studying fractures in diabetes.

Long term blood glucose is monitored with Hba1c measurements in both types of diabetes.

Some facts about osteoporosis

The National Institute of Health (nih) defines osteoporosis in this manner; “A skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture.

Dual energy X-ray absorptiometry (DXA, formerly DEXA) is considered the gold standard for diagnosis of osteoporosis. Diagnosis is made when the bone mineral density (BMD) is less than or equal to 2.5 standard deviations below that of a young adult reference population. This is translated as a T-score. The World Health Organization has established diagnostic guidelines as T-score -1.0 or greater is "normal", T-score between -1.0 and -2.5 is "low bone mass" (or "osteopenia") and -2.5 or below as osteoporosis[3].

Bone quality is defined by the National Instute of Healt as: “The total sum of characteristics of the bone that influence the bone’s resistance to fracture”. In clinical trials with antiresorptive biphosphonate therapy (aledronat and risedronat) and selective estrogen reseptor modulator therapy (raloxifene), which work on the microarcitesture of the bone, the reduction in fracture rates could not fully be explained by the relative small changes in BMD. BMD measures many aspects of the bone (thickness of the cortical bone, trabecular bone and mineralization), but denotes nothing specifically. This means that BMD and most bone quality characteristics are intertwined and largely inseperable.
Changes in the bones microarchitecture, is not measured by BMD. It can be measured by markers of bone resorption in blood and urine. This shows that BMD did not fully explain bone quality, and it has later been shown that the inclusion of bone resorbtion markers have improved the ability to predict fractures in osteoporotic patients.

BMD has been shown to correlate strongly to whole bone strength in laboratory settings, with r up to 0,9. In a meta analysis with 39000 subjects, Johnell, o., J. A. Kanis, et al 2005[4] showed that the risk for any osteoporotic fracture increased by 1,41 per SD decrease in BMD and by 1,38 in women, (CI 1,33-1,51 and 1,28-1,48, respectively)[4]. But in studies of fragility fractures overall, the proportion of patients with WHO classified osteoporosis remain modest (0-44%). Estimates have shown that BMD is only a modest contribution to fracture risk in general, with as much as 85 % of the fracture risk being unrelated to BMD.[5]

During the last 50 years the incidence of osteoporosis associated fractures in the elderly has

increased. In Tromsø 3 out of 10 women above 50 years, and 6 out of 10 above 70 years were osteoporotic. This increase in fracture rates has attracted a lot of attention in the medical society, and society as a whole, for several reasons.

It is a disease that mainly affects women, a fact that is reflected in the incidence of the three most common osteoporotic fractures; hip fractures, vertebral compressions and wrist fractures[2]. The latest numbers from the Norwegian patient register report approximately 9000 hip fractures yearly in Norway. 15000 Norwegians break their wrist(s) every year. Recent numbers from the Norwegian patient register show that eight out of ten hip fractures occur in women[6].

Fractures in the elderly, especially hip fractures have poor outcomes. A study in Orkdal in Norway showed a 9% mortality rate after one month of hospitalization, 24% after a year and 42% after three years[7].

Bones are dynamic structures, constantly being broken down and built up again, existing in equilibrium between these two forces. Changes in either of these two forces upset the architecture of the bone. During life bone architecture and density follows a slow route of change, building up during childhood and peaking in size and density during late adolescence and adult life. From the age of 35-40 and after, BMD suffer a slow and steady decline in both sexes, but in female the decline is greatly exaggerated by the onset of menopause. The mechanism of this female exaggeration of BMD loss is attributed to the demise of the female sex hormone oestrogen, which, in general terms, has a bone maintaining effect.

Besides the high mortality rate, most hip fracture survivors live with a drastically reduction in movement, and often a reduced quality of life. Hip fractures, the most dramatic osteoporotic fracture in the elderly, have dramatic consequences for the health service to, being both labour intensive and very expensive to treat.
Fracture rates are not even throughout different the world, North America and Scandinavia have the highest rates, whereas other more southern countries have far lower rates. In fact, the country-to-country rate in some cases, exceed the gender ratio. This observation has given investigators clues to osteoporosis being associated with our modern western way of life, risk factors mentioned are sedativity/lack of physical activity, reduced intake of vitamin D, reduced sun exposure, and many more.

Methods

For the literature review, PubMed was used as the search tool. A search for “fracture, risk and diabetes” gave 370 articles. 59 of these articles have been presented. They were selected to give information about fracture risk in diabetes, and possible pathophysiological mechanisms that were at work. The quality of the articles were defined by looking at study design, number of stydy subjects (N), adjustment for confounders and more.

The data used in the statistcal study are from the Tromsø health survey. It is a population based cohort study with five repeated health surveys since 1974. The fourth survey was done in 1994/95 and it had 27 159 participants, the part of the study that addresses bone health and fractures include 7127 of the participants, and this was the population that used for this study. The follow up time in this study was the time from date of examination which was done from 1994, and it ended 31.12.2004, which gives a follow up time of up to 10 years. The subjects of this study were examined with extra attention to bone health. Bone mineral density (BMD) was measured at examination. Fractures were registered for all participants in the follow up period. This was done by extracting the diagnose codes for different fractures from the University hospital of Tromsø (UNN). All fractures are radiographically confirmed. All types of non vertebral fractures are used in the study.

In collaboration with my mentor, Ragnar Joakimsen, it was decided that we would study $HbA1_c$ and fracture risk, based on the Tromsø 4 study. The statistical analysis that was used was COX-regression.

In the COX-regression, which is classified as a survival study, the selection criteria was one or more fracture during follow up. Those who had this criteria fulfilled were coded with fracture in SPSS and those who did not have fracture were coded differently. The survival time of those with fracture was the time period between time of fracture and date of entry in the study (start of follow up). The observation time for those without fracture was the time period in which they were observed, which was start of follow up to 31.12.2004, when the recordings stopped. Survival time and observation time were joined in one variable by using the “Transform” and “Compute” menu in SPSS.

The COX-regression was done separately for female and males. Age groups of 10 years were made, and these age groups were put into the COX-regression as a factor.
The COX- regression model calculates how much a value grows or shrinks compaired to how the value of another factor grows or shrinks. So to start with age (10 year groups), which is known to be positively correlated with fracture, we would get a value that show that fracture risk increase as age increases, this value is known as hazard ratio (HR) which is comparable to relative risk. If it ends up at 1.05, it simply means that, as our subjects get 10 years older, their chance of fracture increases by 5%. This is a value that is stipulated by the COX model, and it is the value that best fits the model, its corresponding significance value is also generated by the model. The HR and the associated significance level were calculated for $HbA1_c$ values. $HbA1_c$ values were used as a scale variable, in stead of nominal, this was recommended by Dr. Ragnar Joakimsen.
When $HbA1_c$ and age groups were put in as factors, their respecting HR and significance values are said to be adjusted for the other factor. HR is stratified for gender, which means that it was presented seperately for males and females.

The interaction term $HbA1_c$*age was evaluated by a separate COX regression analysis.

A graphical presentation of fracture risk during follow up was made for both males and females. It has age on the X-axis, and the $HbA1_c$ quartiles are clustered in the age groups. Fracture risk is represented on the Y-axis.

A confounding factor is an underlying factor that may, if not detected, reduce our ability to se the causal relationship between two variables. The confounding factor is related both to the independent variable and our dependent variable, in this case $HbA1_c$ and fracture risk. Age for example, is positively related to both $HbA1_c$ and fracture risk. So in this case, if we were to find higher fracture risk in the higher $HbA1_c$ groups, this finding would not necessarily mean that there is a causal relationship between $HbA1_c$ and fracture risk, it may just mean that we have found increasing values of both in older subjects. Such findings are called spurious relationships, and should be detected.

If there was a real connection between $Hba1_c$ values and fracture risk, without interaction with age, such an association would be consistent throughout all age groups, in both genders.

Or in more statistical terms, one could say that the relationship between $Hba1_c$ and fracture was present, even when adjusted for age and gender.

SPSS was used to do statistical analysis on this relatively big dataset. I obtained this program for free from the IT-department at the university.

Results

Literature review on relation between fracture and diabetes risk

In this section articles that cover different aspects of the topic diabetes and fracture are presented. In many of the statements that are presented below, several articles are in support or partially in support of the statements, the best articles in terms of number of participants, methods and status of the journal in which they were printed have been selected.

Fractures in diabetes

There is strong evidence that patients with type 1 diabetes have an increased fracture risk. In a cohort study with 9 years, with 35 444 participants in the Nord Trøndelag health survey Forsen L, Meyer HE, Midthjell K, Edna TH 1999[8] looked at risk (RR) of hip fracture in a population aged 50 years and older. There were 1634 new hip fractures. They found a significant elevation of RR (6,9, confidence interval CI: 2,2-22,1) for hip fracture in the diabetes type 1 female compared to non diabetic women, when other risk factors, age, impaired vision/movement and history of stoke, were controlled for. The relative risk for men was equally high, but not statistically significant.

In a large (n=9704) prospective study of osteoporotic fractures in women by Kelsey, Browner et al. 1992[9], type 1 diabetes was described as one of the independent predictors of fracture in the proximal humerus.

In type 2 diabetes most studies proclaim higher fracture rates. Schwartz, Sellmeyer et al. 2001[10] found an increased rate of fracture in “the study of osteoporotic fractures in women”, where they looked at data from 9654 women at the age of 65 years and older. Diabetes was reported in 586 of these women. In the diabetics who did not use insulin, there was a RR of hip-fracture of 1,82 (CI 1,24-1,69) and 1,94 (CI 1,24-3.02) for proximal humerus fracture, compaired to non diabetics. In the insulin dependent diabetics the RR for all fractures of the lower extremity was 2,66 (CI 1,18- 6,02). The relative risk was controlled for age, BMI, BMD and other factors associated with fracture and diabetes.

Other studies show normal fracture rates in type 2 diabetes. Gerdhem, Isaksson et al. 2005[11] found no significant difference in lifetime fracture rates in women with and without diabetes type 2, (52 vs 57%, p=0,31), when they did a retrospective study of 1132 women aged 75

years or older.

As mentioned earlier fracture is often a result of osteoporotic bone and falls. I will now discuss how diabetes affects bone quality.

Diabetes and bone quality

Johnell, O., J. A. Kanis, et al. 2005[4] showed, in a meta-analysis that comprised 39000 subjects, that BMD at the femoral neck was a strong predictor of femur fractures in both genders. At the age of 65, risk ratio of femoral fracture increased by 2,94 (CI 2,02-4,27) in men and by 2,88 (CI 2,31-3,59) in females for every step of 1 SD of BMD.

Osteoporosis is defined as having a BMD 2,5 STDs lower than that of the young population. Let us now have a look at what BMD is like in the two types of diabetes.

Most of the researce on t1dm patients show lower BMD than in the general population. The articles found were generally small in terms of study population.
Munoz-Torres, M., E. Jodar[12], et al 1996 found significantly reduced BMD in 94 t1DM patients aged 20-56 years, compaired to expected values. Z values were compaired. T-score for BMD values, measured at femoral neck (-0,99), lumbar spine (-0,89) and ward`s triangle (-1,05), all T-values were highly significant ($p<0,001$).

In type 2 diabetes patients most studies indicate that the BMD values are either slightly increased or preserved.
In a cross sectional cohort study from Canada, in 5566 women and 2187 men, at 50 years or older Hanley, Brown et al. 2003[13] found significantly higher BMD at the femoral neck and lumbar spine in t2DM women and in the lumbar spine of men, after the BMD values were adjusted for confounders.

One study by Al-Maatouq, El-Desouki et al. 2004[14] found that a lower BMD than in non diabetics. It studied two groups (104 and 101 subjects) of postmenopausal Arabic women, one group with t2DM and one without. It showed significantly higher proportions of osteoporotic and osteopenic subjects in the diabetic group, when compared to the healthy group. The study had a focus on vitamin d deficiency, both through lack of milk intake and due to sun deprivation (because of burka-wearing), and in this respect it does not apply so well to

Norwegian conditions.

To sum up what was found on bone quality, t1dm patients seem to have lower BMD values, t2dm patients show higher or normal BMD, compared to non diabetics.

The development of bone disease in t1dm

The BMD in adults is determined by two factors, peak bone mass, and subsequent loss. Peak bone mass is often achieved at the age of 30 years, and a disturbance during the skeletal growth will affect it. Subsequent loss is the gradual loss after peak bone mass is achieved. Which of these factors that are targeted in T1DM bone disease is not fully understood, but the reviewed articles point toward a disturbance of peak bone mass, which indicate a disturbance during growth.

BMD values in t1dm patients, at the time of diagnosis or shortly thereafter, showed decreased BMD values. Gunczler, Lanes et al. 2001[15] studied BMD in 23 prepubertal children and found a significant reduction in BMD, at the lumbar spine, compared to matched non diabetic controls, after adjusting for (body mass index) BMI and age.

The further development of BMD in t1dm is debated. Some studies have found no connection between BMD and duration of disease. Miazgowski, Andrysiak-Mamos et al. 1997[16], studied 54 patients with longstanding t1DM, and concluded that duration of the disease did not have a negative impact on bone quantity, determined by BMD. Bridges, Moochhala et al. 2005[17] found no deterioration of BMD related to the age of the t1DM patients either.
These findings support a hypotheses claiming that BMD loss, in previously diagnosed T1DM patients, is not a progressive complication of the disease, but rather a static condition (Kayath, Tavares et al. 1998[18]), suggesting a decrease in achieved peak bone mass and a BMD plateu-phase during most of the disease.

Some studies indicate that bone quality in t1dm deteriorates slowly during this persons grown up life, and as a consequence of this, BMD will correspond negatively with disease duration. Kayath, Dib et al. 1994[19], found a negative development of BMD values with increasing duration of disease in a study that had 90 t1DM patients aged 18 to 54 years. The same development of BMD has been reported for age, Valerio, del Puente et al. 2002[20] found a negative correlation between BMD and age in a study of 27 t1DM patients. They reported that

deterioration of BMD was correlated to diabetic control and later complications, which must be regarded as confounding factors in this article.

Biological changes

Bone disease in t1dm is of the low turnover type, meaning that there is a deficit in the bone forming cells the osteoblasts, and in the osteoclasts, which remodels the bone. The balance between bone formation and remodelling determines whether there is a loss or gain of bone mass. Bouillon et al., 1992[21] studied the BB rat model, which in short terms are rats that develop an autoimmune diabetes at a mean age of 100 days. He found low number of osteoblasts and low osteoblast activity (measured by S-osteocalcin), in both short and longstanding diabetic disease. Several metabolic dysfunctions of diabetes, hypercalcuria and low calsium uptake from the intestines worsened the bone status even more. He found low levels of testosterone and IGF-I, which are hormones that have an anabolic effect on bone, in the diabetic rats. Not surprisingly, 14 days of insulin infusion corrected, and in some cases even overcorrected the bone status of the diabetic rats.

Histological changes have been found both in diabetic patients and in animal models. The diabetic bone has in general lower mineral content, reduced thickness of trabecular bone (Bouillon 1992[21]; Botolin, Faugere et al. 2005[22]; Duarte, Ramos et al. 2005[23]) and higher fat content (Botolin, Faugere et al. 2005[22]).

We now know that the anabolic effects of insulin on bone works through different direct and indirect mecanisms. The lack of endogen insulin has been well studied in context of bone health in diabetes.

The stimulating effect of insulin on bone formation has been shown in many different models, for instance in in-vitro, Rubinacci, Boniforti et al. 1991[24] showed decresed levels of (alcalic phosphatases) ALP, which is a marker of osteoblast activity, in cultured insulin depleted osteoblast. Examination of BMD values in 27 t1dm patients revealed a decreased BMD in conjunction with lowered proinsulin values (Jehle, Jehle et al. 1998[25])

Insuline treatment revitalises bone in insulin deficient states, this has been shown in diabetic rats (Bouillon 1992[21]). In a followup study of 7 years, in 62 t1dm patients, Campos Pastor, Lopez-Ibarra et al. 2000[26] showed that an optimal insulintreatment of the disease stabilised

BMD in all measured sites.

The role of insulin as an anabolic hormone in bone, has been taken to account for the dichotomy that exists between the bone density differences found in T1DM and T2DM. In type 1 diabetes we find typically aninsulinemia, whereas in type 2 we find relative insulin resistance and varying degrees of hyperinsulinemia..
After thoroughly discussing findings of bone status, in vivo and in vitro studies in t(1/2)DM, Katryn M. Thrailkill[27] et al concluded that insulin has anabolic effects on bone.

IGF, Insulin-Like Growth Factor, interacts with a reseptor similar to the insulinreseptor in bone and many other tissues. IGF has a stimulating effect on bone formation (Bouillon 1992[21]; Jehle, Jehle et al. 1998[25]; Kemink, Hermus et al. 2000[28]), it is the principial mediator of the growth-promoting action of growth hormone GH and is produced in the liver, muscles and various other tissues (W. F. Boron, E. L. Boulpaep; Medical Physiology, 2003[29]).

Due to the similarity of the insulin and IGF reseptors, it appears that insulin and IGF have some degree of ”crosstalk” at the reseptorlevel, the hypoglycemic effect has been well studied for IGF (W. F. Boron, E. L. Boulpaep; Medical Physiology, 2003[29]), one could suspect that growth stimulation on bone could be exerted on the IGF receptor by insulin. This could explain to some degree the bone deficit seen in the t1dm patients.

Besides the ”crosstalk” between the IGF and insulin reseptors, a marked IGF deficiency has been found in t1dm patients. The deficiency of IGF is correlated to the grade of insuline deficiency in t1dm patiens (Jehle, Jehle et al. 1998[25]).

Other possible bone promoting substances have been studied in the diabetic state. Amylin is a peptide that is co-secreted with insulin from the pancreas. Adding amylin to the insulin treatment of streptozotocin induced diabetic rats improved bone indices significantly, apparently by inhibiting resorbtion and stimulating bone formation, acting on osteoclasts and osteoblasts respectively (Horcajada-Molteni, Chanteranne et al. 2001[30])

Blood glucose levels and bone quantity

High blood sugar is a common trait in uncontrolled type 1 an 2 diabetes. The following chapter will review some findings on how Hba1c relates to BMD in both diseases, and will look at how a longstanding elevated glucose level can affect bone health.

There are a lot of studies compairing blood sugar control (Hba1c) and bone quantity (BMD) in diabetics. The results of these studies are not uniform.

Several papers have studied recent and long-term blood sugar values as risk factors for osteoporosis. There was no clear relationship between present HbA_1c values and fracture risk.

When long-term (years) control was considered, based on the HbA_1c values over years, some studies pointed out a correspondence between a high Hba1c and low BMD. Kayath MJ, Dib SA Vieiaa JG 1994[19] found a relationship between long term blood glucose levels and BMD values in a study that had 90 insulin dependent diabetes patients.

In general there seems to be a closer relationship between poor glucose control I t1DM than in t2DM, but there is not a uniform understanding, and many articles had contradictive results, a few are presented in the following chapter. First some for t1DM, and then some for t2DM.

In T1DM

Valerio, del Puente et al. 2002[16]; Moyer-Mileur, Dixon et al. 2004[20] found that high $HbA1_c$ values over years, had a negative impact on bone development in adolescents. Poor blood glucose was also found to have a negative impact on BMD in a study that involved 92 adult type 1 diabetics (Olmos, Perez-Castrillon et al. 1994[31]).

Contardictary, a recent study by Bridges, Moochhala et al. 2005[17] that investigated 35 t1DM patients and bone impact, found no relation between Hba1c and BMD.

In T2DM

In a BMD screening of 145 t2dm patients, Majima, Komatsu et al. 2005[32] found a reduced BMD at the distal radius, but not at the lumbar spine or femoral neck, when they compared with similarly matched controls.

No association between BMD and HbA1c was found, when Bridges, Moochhala et al. 2005[17] studied 90 male t2dm patiens.

Based on our understanding of how poor glycemic control accelerates the development of diabetic complications, we can indirectly study the effects of years to decades of poor glycemic control on osteoporosis by comparing otherwise matching groups of diabetics with and without complications. These studies show marked effects on bone quality, and there is conformity between type 1 and 2 diabetes, more on this later.

Mecanisms of glucose induced bone disease

A few articles are present, that cover some of the theories of glucose induced bone disease, which is relevant in both type 1 and 2 diabetes.

Hyperglycemia, an obvious complication of poorly controlled diabetes, has direct and indirect effects on bone homeostasis. In vitro studies by Kumeda Y, 1998[33] show that during pure hyperglycemia, osteoblasts are generally suppressed, and they show weakened responses to PTH and vitamin D

Osmotic diuresis, is caused by a glucose load in the kidneys ultrafiltrate that exceeds the kidneys regaining capacity of glucose. The excess glucose draws a substantial amount of water and osmoles out of the body. Calcium and phosphate, which are important bone metabolites disappear with the increased urinary flow. (Thalassinos NC, Hadjiyanni P, Tzanela M, Alevizaki C, Philokiprou D, 1993[34]; Okazaki R, Totsuka Y, Hamano K, Ajima M, Miura M, Hirota Y, Hata K, Fukumoto S, Matsumoto T, 1997[35])

An elevated glucose level glycocylates many tissues including osteblasts. The level of glycosylation of red bloodcells is monitored with Hba1c, this value corresponds to the degree of glycosylation in other tissues in the body.

We know that glycosylation occurs in many other tissues like bone and cartilage, and may affect these tissues in a negative way.

Accumulation of Advanced Glycocylated Endproducts (AGE`s) could play a role in diabetic bone disease (Carnevale, Romagnoli et al. 2004[36])

AGEs (Advansed Glycocylated Endproducts) in bone matrix proteins have been shown to impeed bone formation and accelerate bone resorption, in several studies.

In vitro studies on rat bone have shown that AGE-modified collagen has reduced ability to promote differentiation of preosteoblasts to mature osteoblasts. (Katayama, Celic et al. 1997[37])

In one in-vitro study AGEs were shown to stimulate bone resorption by activating osteoclast. AGEs were also held responsible for a stimulating the monocytic/macrophage cell line and to induce their bone resorbing cytokines, causing even more bone degradation. (Miyata T, 1996[38])

Lowering glucose levels in streptozotocin induced diabetic rats, with Vanadium-based antidiabetic therapy, brought about an increase in BMD, mechanical strength, mineralization and bone formation rate.(Facchini, Yuen et al. 2005[39])

Diabetic complications, a common risk factor for fractures in both type 1 and 2 diabetes. As well as blood glucose, the two types of diabetes have another commonality, namely the diabetic complications. When considering complication as a risk factor, most studies converge in their conclusions, diabetic complications are a risk factor for fracture.

A recent cohort study which followed 24605 t1DM patients from 1975 up until 1998 found a positive correlation between fracture risk and ophtalmic, nephropathic, cardiovascular, and neurological complications. (Miao, Brismar et al. 2005[40])

These complications can affect fractures in two ways, either by causing more falls or weakening the bone of the affected patients.

Older women with diabetes have a higher risk of falls. Schwartz, Hillier et al. 2002[41], showed an increased risk of falling in a prospective study of osteoporotic fractures that had 9249 female subjects of 65 years or older. Diabetics who used insulin had an age adjusted Odds Ratio (OR) of 2,78 (CI 1,82-4,24) and OR for diabetics who did not use insulin was 1,68 (CI 1,37-2,07). Known risk factors for falling (poor vision and neuromuscular deficiencies) appeared to account for the increased fall tendency in the non insulin group, with OR falling to 1,18 (CI 0,87-1,60), when adjusted for these factors, but in the insulin dependent group adjusted OR was 2,7 (CI 1,52-5,01).

Females with a history of recurrent falling were found to have higher risk of distal radius fractures in a follow up study of 9,8 years, with 9704 female participants by Vogt, Cauley et al. 2002[43]. RR for fracture, compaired to otherwise matched controls were 1,6 (CI 1,2-2,0).

The increased risk of falls in older women with DM could be attributed to a greater risk of fall promoting risk factors in non insulin dependent diabetics, but could not fully explain by the increased risk in insulin dependent diabetics (Schwartz and Sellmeyer 2004[41]). This suggests that other factors, such as reduced bone strength must play a role in the increased risk of fracture in T1DM.

A negative association between diabetic impact scale and BMD in T1DM was shown by Valerio, del Puente et al. 2002[20].

The complications

The diabetic complications can be divided into three main categories.

The microangiopathic complications occurs in the smallest of arteries, in the glomerullary capilaryes of the kidney (diabetic nephropaty) and in the arteries supplying the retina of the eye (diabetic retinopathy)

The neuropathic complications target both the peripheral somatic and autonomous nervous system. Lesions of the somatic nervous system are the most frequent of the two, representing most often with parestesies of the feet. Autonomous lesions can affect the autonomic

reflexarch of the bladder, leading to an atonic bladder (Topical diagnosis in neurology, P. Duus et. al. 1998[43]).

Macrovascular complications. Atherosclerotic disease in the walls of the arteries is the pathophysiological explenation to the strong assosiation between diabetes and arterial diseases like stroke, heart disease disease, and peripheral artery disease.

Microangiopathic complications

Retinopathy is a form of microangiopathic complication, due to its screening appropriatenes, it is often used as a inclusion parameter when patients are grouped in microangiopathic/ non-microangiopathic groups.

Most studies conclude that rethinopathy is associated with an increased risk of fracture. Rethinopathy affects fracture rates through many mechanisms, poor visual acuity, more falls and through more direct pathways like being associated with poor bone quality, but also by being linked with other diabetic complications like paresthesies and reduced balance. This goes for both type 1 and type 2 diabetes, since both are prone to develop this complication.

Gradual loss of visual acuity, eventually resulting in blindness is associated with an increased risk of falling (Kelsey, Browner et al. 1992[9]; Vogt, Cauley et al. 2002[42])

Fracture risk was significantly elevated in older people with rethinopathy as shown by Ivers RQ, Cumming RG, Mitchell P, Peduto AJ, 2001[44]; Miao J 2005[40], in a follow up study that involved 3654 people aged 49 years or older.

Nephropathy

Although all the microangiopathic disease is charachterised by thickening of basementmenbranes, the glomerulli in nephropathic patients become more leaky with regards to protein. Albumin in the urine is used as a screening for nephropathy in diabetes (Basic pathology, Robbins et al, 2003[45]).

Chronic renal disease which is a complication of diabetes has several negative effects on bone metabolism. Diabetic nephropathy has many direct and indirect effects on bone homeostasis. Hyperphosphatemia and decreased production of 1.25 $(OH)_2D_3$ in renal failure, leads to a

decrease in ionized calcium, which triggers a surge of PTH, known as secondary hyperparathyroidism, leading to high-turnover bone disease.

In an in-vitro study, osteoblast activity was measured when cultured in serum from non-diabetic and diabetic patients with Chronic Renal Failure on hemodialysis. In the diabetic serum, there were significant decreases in terms of cell numbers, collagen formation and mineral deposition compaired to their non-diabetic controls (Serrano, Marinoso et al. 2004[46]). The findings of failing osteoblast function in diabetic CRF patients on hemodialysis suggest that the decreased osteoblast function seen in complicated diabetes is not a mere result of their impaired renal function, but probably part of a more complex patogenesis.

The trabecular bone of the femoral neck is especially sensitive to microangiopathic complications, the same complication that lead to protein leak through the capillaries of the glomerullus, in this sense, microalbinuria can indirectly be linked to the derangement of bone dynamics seen in diabetes (Goliat, Marusza et al. 1998[47]).

L. Jørgensen, et al. 2007[48], found a 71% higher risk of vertebral fractures, in women with end stage renal failure, when she compared women in the highest albumin creatinin quartile to those in the lowest quartile. Possible confounders were adjusted for. This was part of the Tromsø study, 2230 females, 55-74 years old, were included and they had a mean follow up time of 8,4 years.

Neuropathic complications

Neuropathic complications, which often are found as parestesies in the feet can have an effect on steadyness and gait, and could therefore lead to falls and fractures.

Kelsey, Browner et al. 1992[9], showed that neuromuscular deficiency was associated with an increased rate of fracture in a prospective cohort study that examined fracturerates in 9704 women aged 65 years and older.

Peripheral arterial disease

Macrovascular complications were found to be negatively associated to femoral neck BMD in a study comprising 99 women with longstanding T1DM (Lunt, Florkowski et al. 1998[49])

Decreased vascular flow to the lower extremities, which can be revealed by a decreased foot/arm-blood pressure index has been linked to an increased rate of bone loss in some localities in the the lower limb (Vogt, Cauley et al. 1997[50]), but as pointed out by later studies by Wong, Kwok et al. 2005[51] this association seems to be quite weak when possible confounders (age, sex, weight and more) were accounted for.

Discussion about complications and fractures

Patients with Impaired Glucose Tolerance (IGT) have a decreased risk HR 0,80 (0,63-1,00) of fractures in contrast to patients with established T2DM (untreated or treated) who have an increased risk of fracture HR 1,69 (1,16-2,46), this was, according to the authors, probably due to long term complications associated with DM. This was found in the Rotterdam study, which was a population based follow up study that had 6655 male and women participants[52].

The same pattern was seen in the following studies by Seeley, Kelsey et al. 1996[53]; Nicodemus and Folsom 2001[54] where treated T2DM patients had higher fracture risk compared to untreated T2DM patients. And this finding was also explained by the authors as being due to more complications in the treated group.

It remains to be elucidated whether treating diabetes and diabetic complications aggressively can alter skeletal health either directly or by preventing diabetic complications that contribute to falls and fractures. (Schwartz and Sellmeyer 2004[55])

Some medications used in the metabolic syndrome interfere with bone quality.

Diabetes type 2 is most often part of the metabolic syndrome, which is characterised by overweight, hypertension (and diabetes type 2). This condition is often treated with many drugs, and some have been shown to have a bone protecting effect, and one medication has a negative impact on bone. The effect size does not seem large in these studies they range from 1-5 % change in BMD, but the effect seems consistent because it reached significance at a 5 % level in most of the studies.

Statins have a protective effect on bone, probably mediated through their anti inflammatory properties. HMG-CoA reductase inhibitors may increase BMD in T2DM as found by Chung, Lee et al. 2000[56] in a clinical trial that had 36 patients that were treated with statines, and a control group of 33 that received placebo. In the treatment group BMD measured at the

femoral neck was significantly increased from by 5 % ($p<0,05$).

The same effect on BMD was found for ACE inhibitors and tiazides by Lynn, Kwok et al. 2005[57] in 2005 in a population based cross sectional study based on 3887 Chinese women and men, aged 65 years or older. Femoral neck BMD was increased by +0,015 g/cm^2, (which is in the order of 1-2 % change), in both women and men, and reached statistical significance by p= 0,035 and 0,017 respectively.

Sigurdsson G, Franzson L., et al., 2001[58] found that use of thiazide diuretics was associated with a 9,6 % increase in the lumbar and 5,4 % in whole body BMD, in a population based study in a group of 248 Islandic women in which 51 received tiazide treatment. After adjusting for possible confounders, they concluded that 3 % of the variability could be explained by thiazide use.

Pioglitazon in the thiazolidinediones group of anti diabetic drugs, was shown by Kahn SE, Haffner SM, Heise MA, et al., 2006[59] in the ADOPT study to have an increased risk of fracture. The study had 4360 patients with t2DM enrolled, who used pioglitazone and other comparable anti diabetic drugs were enrolled, and were followed for 4-6 years. The female patients that took pioglitazon had a fracture risk during follow up of 9,3 % compaired to 5,1 and 3,5% in the other groups. In the male group, there was no difference in fracture risk.

Summary of the literature study

As is shown over, there has been done a lot of work on the topic of diabetes and fracture. The relationship between the two is quite complex. In the first place diabetes consists of two different diseases, namely type 1 and type 2. These diseases have different pathological mechanisms, but they also have many common traits, for example high blood sugar. Diabetes type 1 was associates with a higher fracture risk, but it was not so clear for type 2 diabetes. One trait that for certain is common between the two is the development of complications. And it was in the diabetic patients with complications that one could see most agreement, in terms of an on increased fracture rate in these patients. Articles have been presented with the intent to shed light on both diseases with respect to their differences and communalities. And although the research presented is not very conclusive in terms of fracture risk, it is

worthwhile to have mentioned this theory now, before we take a look at what was found on fracture rates and HbA1c in the data from the Tromsø 4.

Result of analysis: $HbA1_c$ and fracture risk

Descriptives of the population

The study population consisted of 7127 subjects. 4040 women (56,5%) and 3087 men (43,5%). The mean age of the female participants were 56,5 years and the SD was 10,5 years, the males had a mean age of 57,9 years and a SD of 9,9 years.

The female group had a mean BMI (Body Mass Index) of 25,7 and a standard deviation (SD) of 4,3. The males had a mean BMI of 26,0 and a SD of 3,3.

32 % of the males reported daily smoking versus 31 % of women.

In total there were 799 fracture events during the follow-up, see table 1 in the appendix. In the female population there were 616 fractures (15,2 %), and in the male population there were 183 fractures (5,9 %), see table 2 in the appendix.
This also shows in the survival plot of the COX regression where there is a clear connection between fracture and gender, see figure 1 in the appendix.

Cox regression shows that there was a significant relationship between fracture rates and age in both genders. Women had an hazard ratio of 1,046, which litteraly meant that for each step up in the ten year age-ladder, there was an increase of 4,6 %, CI (3,4-5,2 %), p=0,000. For men the hazard ratio with age was 2,3%, CI (0,6-3,6 %), p=0,007.
The p-values are highly significant in both genders, in females and in males. This is shown in table 3 and 4 in the appendix.

In the study of $HbA1_c$ and fracture rates, hazard ratio for women was -14%, for each unit change of $HbA1_c$. This means that fracture rates are lower in the higher $Hba1_c$ subjects. (CI= -15.6- 0,01%), p=0.048, states that this finding is significant.
For men the hazard ratio was -6%, which also indicate lower fracture rates in the higher $HbA1_c$ subjects. (CI=-15,6-18%), p-value=0,611, which is highly insignificant. This is shown in table 3 and 4 in the appendix.

The graphical presentation of fracture rates in the different age group, in figure 2 and 3 shows an increasing fracture rate in the higher age groups in both sexes, but this tendency is

markedly stronger in women. There was no interaction between age*$HbA1_c$ and fracture risk. In females, HR 0 %, CI (-0,2-0,4%), p= 0,735. In males HR 0,1%, CI (-0,2-0,4%), p= 0,436. The figures can be found in the appendix.

Discussion

Main findings in the literature review: In the literature review of the paper one could see that t1DM patients show a higher fracture rate, but this relationship is weaker for t2DM patients. In terms of bone quality, BMD was used as the parameter. It is strongly related to bone quality, but it was shown that it did not fully serve as a parameter of bone quality and subsequently is a poor predictor of fracture risk. In the case of diabetes and bone quality, there seems to be a debate going. Results indicated that t1DM patients had a decline in bone quantity, which was shown in several studies by using BMD measurements. Type 2 diabetics, showed normal and even higher values of BMD. Both t1 and t2 diabetes patients seemed to have a plateau-phase of good BMD in their adult life, if they were well regulated. Poor blood sugar control seemed to deteriorate bone quality in both diseases. And ultimately in the complicated diabetics, the researchers were quite uniform in their statements, the two diseases had a common deterioration of bone quality, an increase of falls and more fractures. Mechanism of bone disease in diabetes was discussed, and interaction of several of the drugs that are used in diabetes were also taken into consideration.

Different ways to divide the population into a diabetic and a non diabetic group were tried. There were several variables that could be used. Self reported values of diabetes existed in the dataset. There were also blood sugar values and $HbA1_c$ values available.
There were 193 self reported diabetes subjects among the total population of 7127 subjects. One could also use blood sugar values at start of follow-up to capture some more diabetic subjects, the criteria would be BS above 11,0 for inclusion. A $HbA1_c$ of above 6,5 would also point in the direction of diabetes. When self reported diabetes, coupled with the two aforementioned criteria, the number of diabetics was no greater than 250 subjects, which in this case was to few to study fracture rates, given that fracture is not so common afterall.
In this study, $HbA1_c$ was used as the exposition, and subsequent fracture risk was analysed. The $HbA1_c$ was used as a continuous variable, which means that we have used the whole spectrum of values for the analysis.

In the female population a negative association was seen between $HbA1_c$ and fracture, in the COX regression analysis. Hazard risk was -14,6 % for each init change of $HbA1_c$, p=0,048. This finding indicates that higher $Hba1_c$ values have a protective effect with regards to

fracture in women, when gender and age were adjusted for. In men there were also a negative hazard risk of -6%, but this was not significant.

The bar chart of age and gender adjusted fracture rates showed no compelling relation between age and fracture risk in the $HbA1_c$ quartiles. By visual inspection, one could argue that there is an interaction between $HbA1_c$ and age. It looks as if $HbA1_c$ is positively related to fracture risk in the young population, and negatively in the older population. But the interaction ($HbA1_c$*age) was not statistically significant in COX analysis, at p=0,735 in females and p=0,436 in males.

Bias discussion:

The possible protective role of $HbA1_c$ in women is an interesting result. But even though the result was statistical significant, it does not mean that it represents a true association. There may be underlying factors, called bias factors that have not been adjusted for. One relevant example would be BMI (Body Mass Index), which is known to have a protective role in terms of fracture. High $HbA1_c$ is in most cases associated with a high BMI, so this may have been a confounder that has biased our results. Other bias factors that one must think of are for example, cardiovascular disease, poor neuromuscular function and other factors that can affect fracture risk.

The aim of this study is to look at the relation between the two variables under study, namely $HbA1_c$ and fracture incidence. The internal validity of the study is a measure of how well the results of the study represents the true relationship between $HbA1_c$ and fracture risk in the study population, which in this case is the Tromsø population.

Selection bias means that the selection of the participants are skewed in one or more direction. An example of this would be if one group of people came to the study and the other group did not come, then the population of the study would not be representative of the population it was meant to study. This is quite complex material. The single most important factor to determine whether the study population represents the real population is to look at the percentage of participation, that is, how many of the invited people met in the study. For this study there was 77% that met, and this is a good result for an epidemiological study. This high rate of participation protects to a great extent against selection bias.

Classification bias means if the classification of the subjects are right or not. Is the method of classification of the two variables under study precise and valid, obviously faulty measures and classifications would affect my results in a negative way. In the case of $HbA1_c$ the measurement is precise.

All fractures that are counted in the follow-up period have been confirmed by x-ray, so it is not likely that any false positive values of fracture exist. False negatives are those that have had fractures, but have not been diagnosed at UNN. Those who have had fractures diagnosed at other hospitals could represent false negatives.

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Appendix

Table 1-4

Fracture rates in boyh genders

		N	Percent
Cases available in analyses	Event (fracture)	799	11,2%
	Censored (no fracture)	6328	88,8%
	Total	7127	100,0%
Cases dropped	Cases with missing, negative or censored values	0	,0%
Total		7127	100,0%

a Dependent Variable: Observation and survival time

Table 1; Shows events (fracture), and censored (those without fracture), during the follow up time.

Male and female fracture rates

Gender	Fracture	No fracture	Fracture %
female	616	3424	15,2%
Male	183	2904	5,9%
Total	799	6328	11,2%

Table 2; Shows events and censored subjects in both genders during follow-up.

Result of COX-regression analysis in females

	B	SE	Wald	df	Sig.	HR/Exp (B)	CI
HBA1C_T42	-,149	,075	3,901	1	,048	-14%	(-26)-(-0,1)%
Age_gr_10	,042	,004	94,149	1	,000	4,3%	(3,4-5,2)%
$HbA1_c$*Age	,000	,001	,144	1	,735	,00%	((-0,2)-0,1)%

Table 3; Shows COX-regression results for the two factors, $HbA1_c$ and age, in females.

Result of COX-regression analysis in males

	B	SE	Wald	df	Sig.	HR/Exp (B)	CI
HBA1C_T42	-,060	,119	,258	1	,611	-6%	(-26)-(18)%
Age_gr_10	,020	,008	7,226	1	,007	2,1%	(0,6-3,6)%
$HbA1_c$*Age	,001	,002	,608	1	,436	0,1	((-0,2)-0,4)%

a Sex = Male

Table 4: Shows COX-regression results for the two factors $HbA1_c$ and age in females.

Figures 1-3

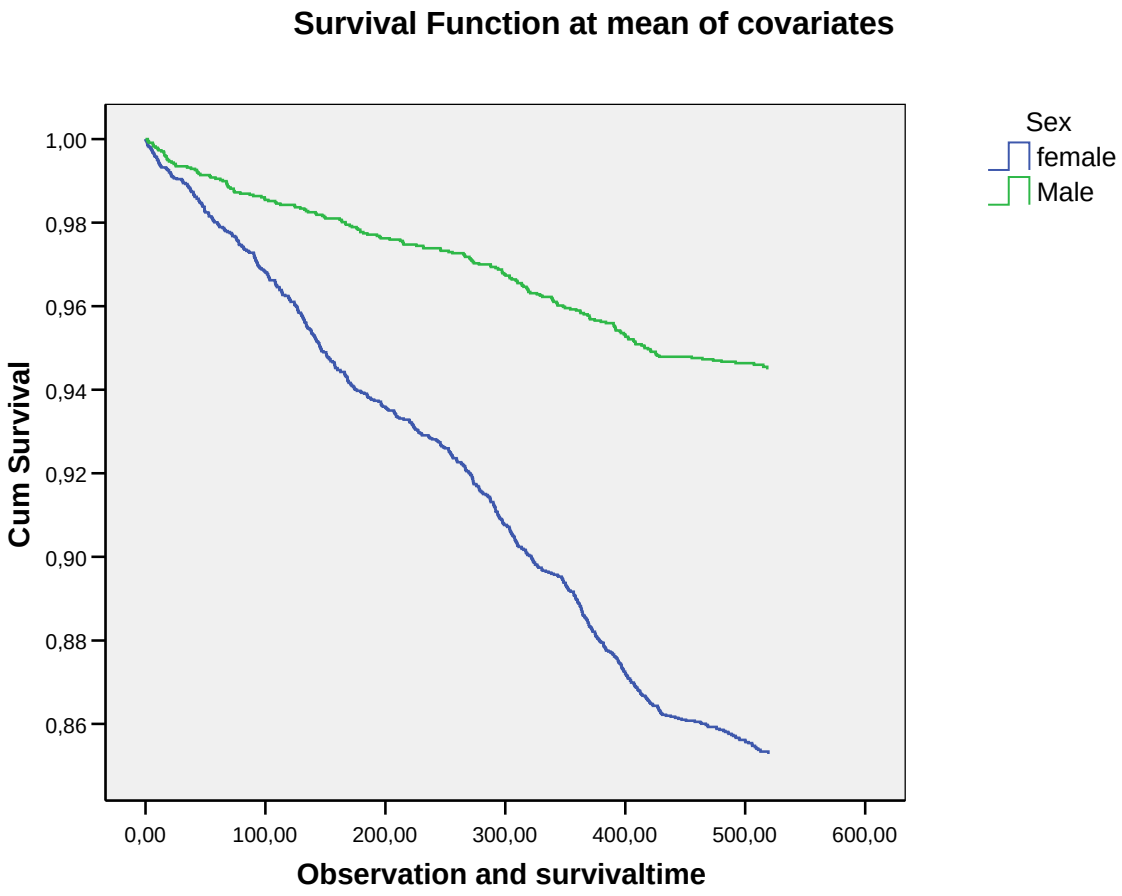

Figure 1; Survival plot of survival and observation time (in weeks) for those with and without fracture respectively.

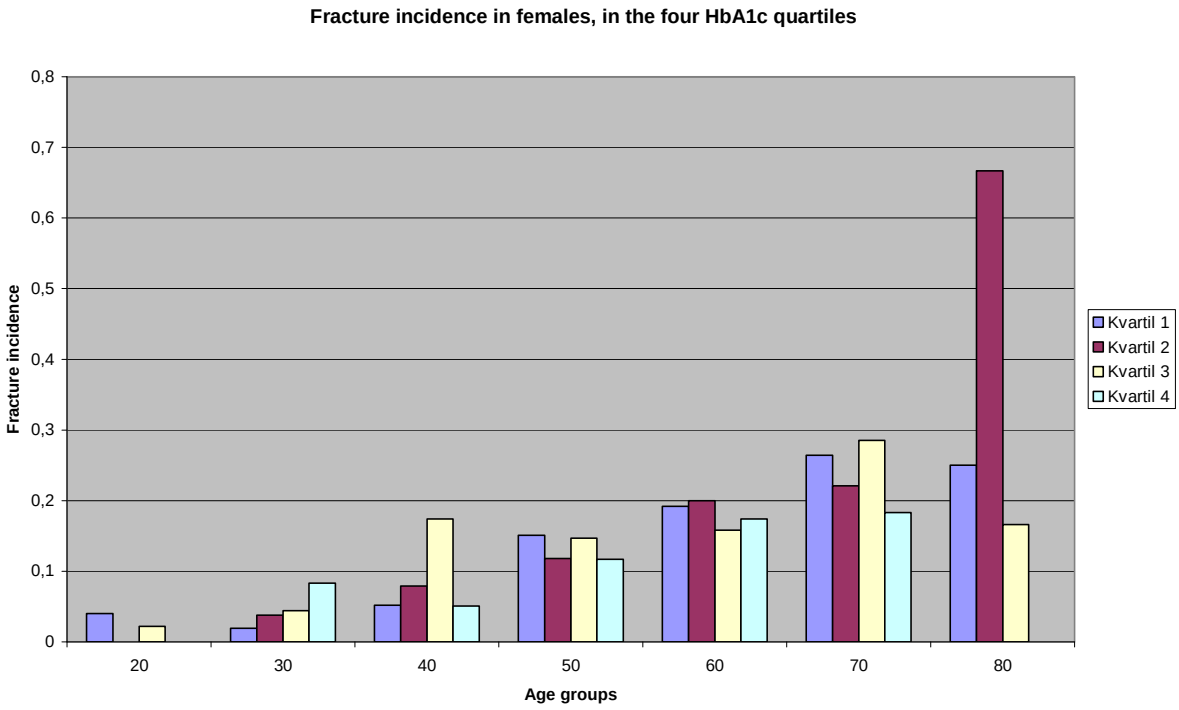

Figure 2; Fracture rate in the $HbA1_c$ quartiles, in females, 10-years age groups 20- 80 years.

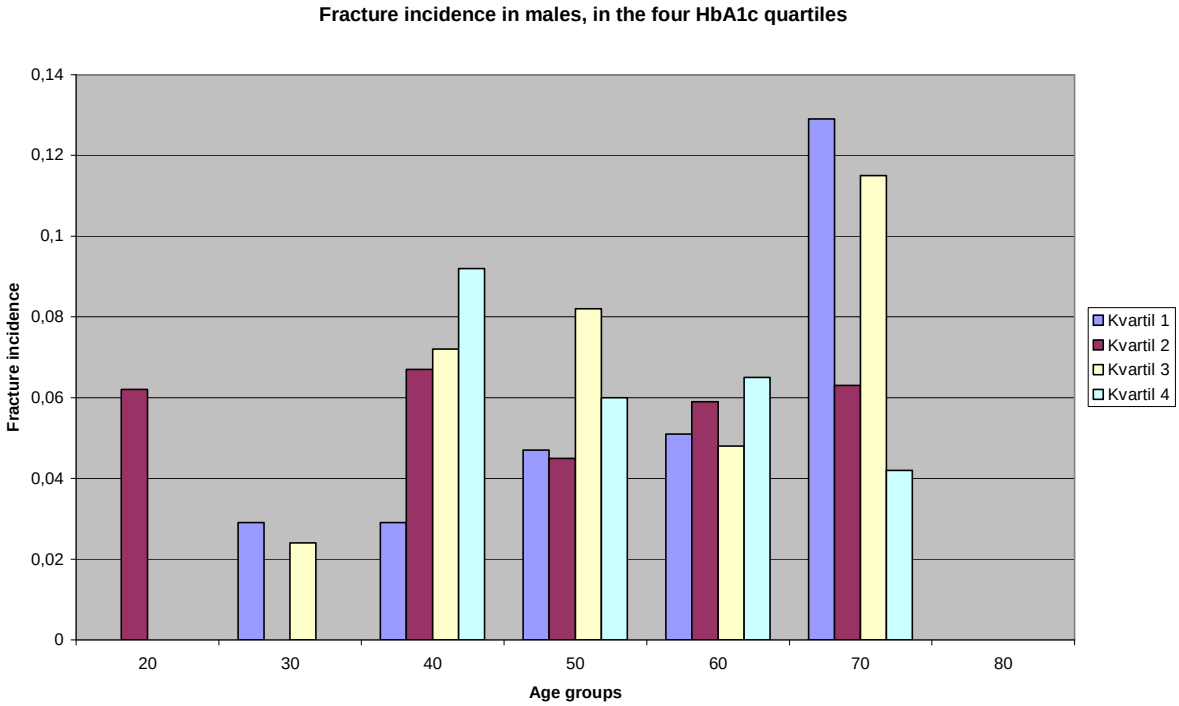

Figure 3; Fracture rates in the $HbA1_c$ quartiles, male group, 10-year age groups 20- 80 years.