Quality of municipal drinking water and the risk of osteoporotic fractures in Norway

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Contents

SUMMARY

Background: For mainly unknown reasons, the Norwegian population has among the highest incidence rates of osteoporotic fractures in the world. The risk of fracture has been shown to vary within Norway, with higher risks in the urban compared to rural areas. Norwegian drinking water is distinct; it contains less minerals and is more corrosive towards water pipes compared to water in many other countries. Drinking water quality has generally improved in recent years, but the quality still differs across Norway. The overall aim of this thesis was to examine whether the variations in quality of drinking water could be related to the risk of osteoporotic fractures, such as fractures of the hip and the forearm.

Methods: To link area information on water quality to individual fracture outcomes, a map of the waterworks supply-areas was produced using Geographic Information Systems. Water quality information was provided by the Norwegian Waterworks Register and by a survey of trace metals in water, whereas fracture information came from the Cohort of Norway (CONOR) collection of health surveys and a recently established database of hip fractures named "NORHip". Using logistic regression, variations in risk of self-reported forearm-fracture in CONOR were assessed between groups of varying water-acidity (pH). Differences in incidence of hip fractures in NORHip by levels of calcium, magnesium and three toxic metals (cadmium, lead and aluminum) were evaluated by Poisson-regression. Available background information, along with other water quality factors were taken into account, testing for confounding, mediation, effect modification and interaction.

Main Results: The risk of forearm fracture was found to be higher when the water was slightly acidic (pH<7). However, including possible intermediate factors, such as microbial indicators, showed that these could be of more importance than acidity in itself for fracture prediction. A higher magnesium concentration in the water was found to have a protective association with hip fracture, but the results for calcium were inconclusive. Although the concentration of toxic metals in the water was

generally low, men seemed to be at higher risk of hip fracture with a slightly higher level of cadmium in water. An increased risk for hip fracture in the oldest men and women (66-85 years) was also found with higher concentrations of the toxic metal lead in water. Interaction analyses indicated that collective effects of toxic metals may be stronger than singular effects.

Conclusion: Due to few studies on drinking water quality and bone health, the current thesis needs to be considered exploratory. Nevertheless, our results suggest that increasing the concentration of magnesium in drinking water could be an important protective measure against osteoporotic fractures in the population. Also, ensuring that the water is free of possible disease-causing organisms, and reducing the concentration of toxic metals such as lead and cadmium may be of benefit to bone health.

ACKNOWLEDGEMENTS

This work was carried out in the Division of Epidemiology at the Norwegian Institute of Public Health (NIPH) during the years 2010-2014. The NIPH is one of the collaborators in the Norwegian Epidemiologic Osteoporosis Studies (NOREPOS). The project was supported by the Research Council of Norway.

I started my career as an epidemiologist in a small African country, working with tropical diseases considered neglected by the global health community. Little did I know that I was later going to be studying another illness often overlooked, this time in my home country. Osteoporosis is often under prioritized because it frequently affects a population group (elderly women) that is incapable of making much noise. They fracture in silence. Unlike these patients, I can be pleased that osteoporosis came into my life. These past few years have been extremely exciting, and I have many people to thank for that.

First of all, I would like to express my sincere gratitude to my main supervisor, prof. Geir Aamodt. He never seizes to impress me with his brilliant mind and knowledge in all areas of life. Without him, this project would never have reached beyond infancy, as he was the one who introduced the linkage between the Waterworks Register and person-level health data. The most important thing Geir has taught me is that every problem has a solution, and the solution is often easier than I think. He has resolved many of my concerns the past years simply by the click of a button, and no problem is ever too small or too large for him.

My second supervisor, Anne Johanne Søgaard is an admirable honor-member of the Norwegian Epidemiology Organization (NOFE). Her original ideas and sharp eye for detail has lifted this project to its high standard of quality. Anne Johanne has taught me that in research there is never just one truth, which has made me sure to always back up my statements and to seek deeper explanations. I am very grateful to her.

My third supervisor, prof. Grethe Tell is also an honor-member of NOFE, and she is the head of the NOREPOS core research group. Although we work far apart, she has always supported me and has been very accommodating, even with her busy schedule.

She is a true professional, and I have felt honored to be working with her. I would also like to express my gratitude to prof. Haakon Meyer and prof. Emeritus Jan Falch. They have contributed greatly to this project with their knowledge in the field of osteoporosis and epidemiology. I would also like to thank prof. Trond Peder Flaten for his contributions in the field of water chemistry and epidemiology.

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Being a part of the vital environment in the Division of Epidemiology has taught me a great deal, not only through seminars and workshops, but also from listening to more experienced coworkers during coffee-breaks. Wenche Nystad, the head of this division has stood by me through administrative challenges during my PhD, and has taught me to recognize the value of my skills. Other valuable friends and colleagues that deserve to be mentioned are Tone Kristin Omsland, Kristin Holvik, Helene Devold, Caroline Fleten, Maria Magnus, Christian Madsen, Ingvild Eidem and Inger Ariansen. Thank you for all your kind words. I am also very fortunate to have been included in the NOREPOS collaboration, taking part in steering-committee meetings and workshops. In addition, I was privileged to lead the workshop “osteoporoseforum” for quite some time.

Most importantly, I would like to thank my daughter Nadia Helene and my son Felix, for teaching me what is essential in life and for giving me the rewarding task of being their mother. I would also like to thank Magne, my great love. Without your patience and support, this project would never have been completed on time and with my sanity intact. Coming home to you and our children is what makes me feel truly successful every day.

LIST OF PAPERS

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I Dahl, C., et al., *Is the quality of drinking water a risk factor for self-reported forearm fractures? Cohort of Norway.* Osteoporos Int, 2013. **24**(2): p. 541-51. PMID:23831379

II Dahl, C., et al., *Nationwide data on municipal drinking water and hip fracture: Could calcium and magnesium be protective? A NOREPOS study.* Bone, 2013. **57**(1): p. 84-91. PMID:23831379

III Dahl, C., et al., *Do Cadmium, Lead, and Aluminum in Drinking Water Increase the Risk of Hip Fractures? A NOREPOS Study.* Biol Trace Elem Res, 2013. [Epub ahead of print]. PMID:24287706

LIST OF TERMS AND ABBREVIATIONS

ALAD	Erythrocyte ALA-Dehydratase, enzyme in the heme biosynthesis.
Alkaline phosphatase	Bone-marker, high levels in blood indicating rapid bone loss
Antagonism	Opposite action, inhibition
BMD	Bone Mineral Density
Color grade	Indicator of organic content in water, measured by mg/l Pt
CONOR	Cohort of Norway
CRP	C-reactive protein
DAG	Directed Acyclic Graphs
Geocode	Placement of a point (i.e. address) onto a map according to its coordinates
Hydroxyapatite	Bone mineral, commonly written : $Ca_{10}(PO_4)_6(OH)_2$
Hematocrit	Volume percentage of red blood cells
Hemochromatosis	A disease of iron overload
IBD	Inflammatory bowel disease, inflammatory conditions of the colon and small intestine, major types: Crohn's disease and ulcerative colitis
Metallothionein (MT)	Family of proteins that bind to metals
NFSA	Norwegian Food Safety Authority, Mattilsynet
NIPH	Norwegian Institute of Public Health, Nasjonalt folkehelseinstitutt
NOK	Norwegian kroner (currency)
NOREPOS	Norwegian Epidemiologic Osteoporosis Studies
NORHip	NOREPOS Hip Fracture Database
OPG	Osteoprotegerin, inhibitor of osteoclastogenesis by binding to RANKL
Osteoblasts	Bone formation (builing up) and later bone-remodelling cell
Osteoclastogenesis	The formation of osteoclasts
Osteoclasts	Bone resorbing cell (breaks down bone)
Osteocytes	Most commonly found cell in bone, long lived
Osteomalacia	A disease characterized by softening of the bone tissue
Pathogen	Disease-causing organism
ppm	Parts per million (1 ppm= 1 mg/l in water)
PTH	Parathyroid hormone
Py	Person-years
RANKL	Receptor activator of nuclear factor kappa-B ligand, a cytokine member of the tumor necrosis factor family
Rickets	Softening of the bones due to impaired vitamin D metabolism
Synergism	The effect of the interaction is greater than the sum of the individual effects
VREG	Norwegian Waterworks Register
Waterborne	Transmitted by water
WHO	World Health Organization

LIST OF FIGURES AND TABLES

1 Introduction

For mainly unknown reasons Norway has among the highest incidence rates of osteoporotic fractures in the world [1-5]. The incidence of osteoporotic fractures varies between countries and ethnicities, by regions within a country, and by urbanization degree [1, 3, 6-14]. Differences in bone mineral density (BMD), body mass index (BMI) and height may explain some of the variation in risk [5, 14-17], but the underlying cause(s) are not fully understood.

Because studies including traditional risk factors have not been able to fully explain the above mentioned variations in fracture risk, our aim was to explore areas where there is limited knowledge, in this case exposure to various components in drinking water and their possible effect on bone health. Due to cold climate, distinctive geological conditions, and an extensive use of surface water, Norwegian drinking water has a different chemical composition than the water in many other countries [18-20]. Most Norwegians are exposed to drinking water from municipal sources, but to a varying degree and quality. The overall aim of this thesis was therefore to study a possible association between municipal drinking water quality and the risk of osteoporotic fractures in Norway.

1.1 Norwegian Epidemiologic Osteoporosis Studies (NOREPOS)

The Norwegian Epidemiologic Osteoporosis Studies (NOREPOS) research collaboration was established in 1997, with the purpose of increasing knowledge on osteoporosis, osteoporotic fractures and their risk factors in Norway. NOREPOS is a national research collaboration network of researchers from five different scientific institutions across Norway: University of Bergen, UiT The Arctic University of Norway, Norwegian University of Science and Technology, University of Oslo, and the Norwegian Institute of Public Health (NIPH). In 2008 NOREPOS investigators received a large grant from the Research Council of Norway. This new project was named “Hip fractures: Predictors, Incidence and Survival”, and had three main focus areas: 1) to assess the incidence of, and mortality after hip fracture in the Norwegian population 1994-2008 ; 2) to assess whether drinking water is related to osteoporosis

and hip fracture and 3) to prospectively investigate the impact of the combination of vitamins D and A status in serum on the risk of hip fracture. The project has contributed to the establishment of the NOREPOS Hip Fracture Database (NORHip), a national database of all hip fractures in Norway from 1994-2008. More can be read about NOREPOS and the ongoing work at: www.norepos.no

1.2 Osteoporotic fractures

1.2.1 Definitions

Osteoporosis is defined as "a systemic disorder, characterized by low bone mass and micro-architectural deterioration of bone leading to an increase in bone fragility and susceptibility to fractures" [21]. Persons with osteoporosis are at high risk of sustaining osteoporotic fractures, also called fragility fractures, which are the clinical manifestations of this disease.

Osteoporotic fractures are fractures that occur from a relatively low trauma, such as a fall from standing height or lower. The three main types of fracture commonly referred to as osteoporotic fractures are distal forearm fractures, hip fractures and vertebral fractures [22]. This thesis will only discuss forearm and hip fractures. For more information on vertebral fractures, see: Waterloo (2013) [23]

1.2.2 The epidemiology of osteoporotic fractures worldwide and in Norway

1.2.2.1 Incidence, mortality and consequences of osteoporotic fractures

Distal forearm fractures are fractures of the wrist (distal radius and/or distal ulna). When including all ages, this is the most common type of fracture in both men and women, although worldwide 80 percent are sustained in women [24]. The current literature suggests that the incidence of forearm fractures in women increases steadily into old age, both in Norway [25],and in other countries[10, 26]. The incidence in men is relatively stable between the ages of 20 and 80 years [10, 25]. An overall prevalence of forearm fracture of 14% has been found in the Norwegian population 30 years and older, with a similar proportion in men and women [14].

Hip fractures account for 18.2 % of osteoporotic fractures worldwide [24], and are considered to be the most serious of the osteoporotic fractures. The vast majority of hip fractures come to clinical attention and is treated surgically in the hospital. Suffering this type of fracture dramatically increases the risk of dying, as approximately one in five women and one in three men die during the first year after the fracture (Omsland TK 2013 personal communication). Of those who survive, most do not regain the level of physical function they had prior to their fracture [27]. Very few hip fractures occur before the age of 50, but the incidence increases exponentially after this age [28, 29]. In Norway, 71% of the hip fracture patients are women [28].

1.2.2.2 Time trend in incidence.

Until the 1980s a rise in hip fracture incidences were reported, but then a plateau was reached, and now many western countries, including Norway, are reporting a decline[4, 10, 11, 28, 30-32]. For example, in the United States the age-adjusted incidence of hip fracture increased from 1986 to 1995 and then steadily declined from 1995 to 2005 [30]. In Norway, the trend in incidence of total hip fracture declined by 13.4 percent in women and 4.8 percent in men between the years 1999-2008 [28]. Whether the decline is due to period or cohort effect is under discussion. The most recent studies indicate a lower incidence in later birth cohorts (especially in women), possibly combined with a downward trend with period [31, 33]. The worldwide trends in the incidence of distal forearm fracture seem to be similar to that of hip fracture [10], although no significant change was found in Oslo between 1979 and 1998/99 [25] . In contrast to the pattern seen in Western countries, the rates of hip fractures in Asia and Mexico seem to be increasing [34, 35]. It has been estimated that by 2050 as much as 50% of hip fractures will occur in Asia [9].

No single factor has been found to fully explain neither the rise nor the fall in fracture incidence. Most likely the causes for both trends are multifactorial, comprising several underlying risk factors (more information on risk factors in chapter 1.2.3).

1.2.2.3 Regional differences in incidence

Between countries. Country specific rates of hip fracture can be difficult to compare due to different methods of assessment. Some examples are:

- Whole country-based study vs. based on only a small geographic area
- Differences in accuracy and coverage of the registers
- Difference in methods used to separate incident hip fractures from re-hospitalizations, i.e. counting patients only once every calendar year, versus allowing several hospitalizations in a year (but adding information about surgical procedure codes and fracture site to avoid counting the same incidence several times).
- Counting total fracture numbers versus only first fracture
- Different background populations used for age-standardization of the rates

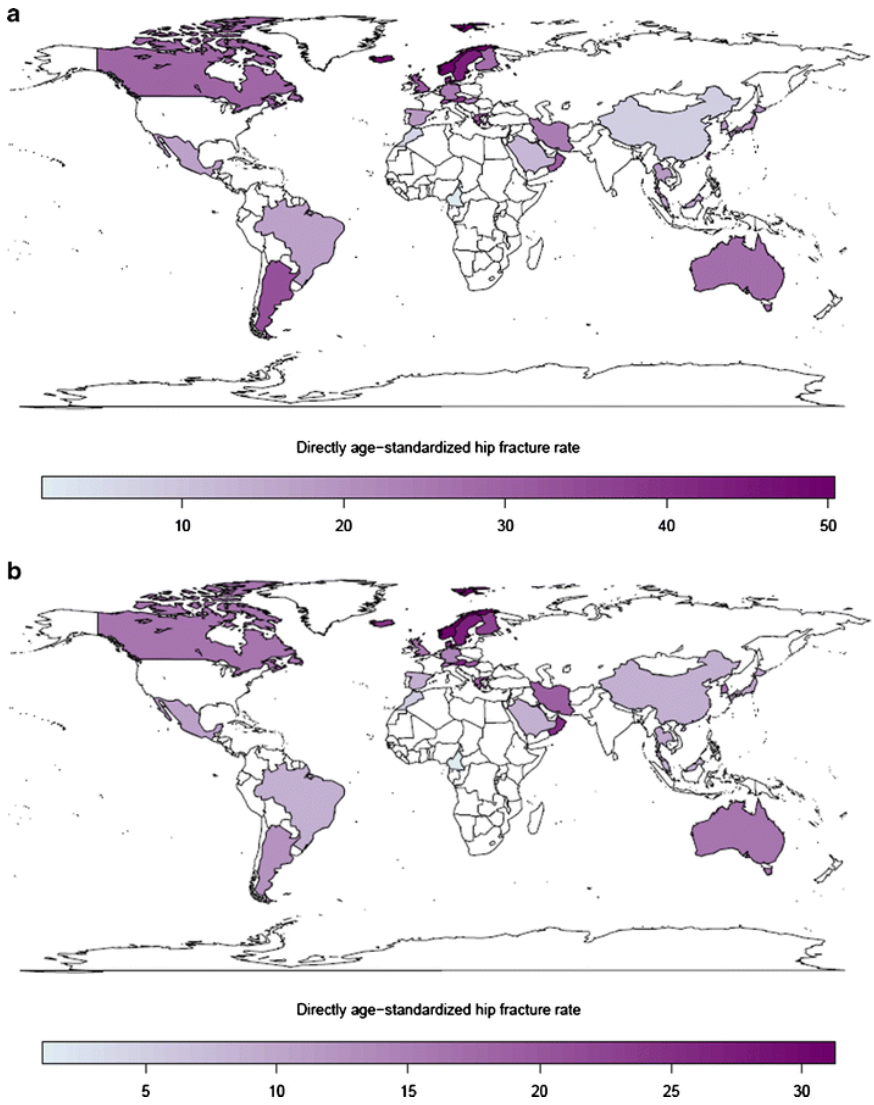
Nonetheless, these discrepancies in estimation of incidence have not been found to undermine that there are still substantial regional differences across the world [3]. Incidence rates of osteoporotic fractures are generally reported to be higher in Scandinavia, whereas southern European, Latin American and African populations experience lower rates [1, 3, 10], see figure 1. In 2008 the hip fracture incidences (age group 65+ years, men and women combined) in Norway and the Netherlands were 126 and 67 per 10 000 persons, respectively (Holvik K and Omsland TK 2013 work in progress), hence almost twice as high in Norway as in the Netherlands.

Within Norway. Comparisons of local and regional studies have since the beginning of the 1990s identified the highest incidence of hip fracture in the East of Norway, with lower rates in the West [11] and the North [8]. Currently, there is no published comparison that includes all regions in Norway;but recent national data on county-differences show that the hip fracture incidence is still highest in Oslo for both men and women (Omsland TK 2013 personal communication). The counties of Sør-Trøndelag (middle region) and Telemark (eastern region) follow close behind, whereas the lowest incidence for both genders combined is found in Finnmak county (northern region) (Omsland TK 2013 personal communication).

By urbanization degree. The risk of osteoporotic fractures varies by degree of urbanization in Norway, and also in other western countries. Urban areas seem to have a higher incidence of both forearm fractures and hip fractures compared to the rural areas [1, 6, 7, 13, 14, 36]. The incidence of hip fracture has also been found to vary within the capital city of Oslo, with a higher incidence in the east compared to the west, but still the western part of the city had a higher incidence than the rural areas of the country [12].

Possible explanations. The cause(s) of the regional differences between and within countries have not been identified, although variations in BMD and BMI may explain parts of the differences [5, 15, 17, 36, 37]. Across the world, a north-south gradient has been proposed, with more fractures occurring in the north, mainly due to snowy and icy conditions, and to lower cutaneous vitamin D production [38, 39]. However, within Norway the south has been found to have a higher incidence than the north. A difference in hip fracture incidence between summer and winter months has been found in some [40, 41], but not in all studies [4].

Figure 1. Age-adjusted hip fracture incidences in a) women and b) men[1]



[1] Fig. 1a and b are reproduced (with permission) from Cheng SY 2011(figure 2)

a Directly age-standardized hip fracture rates (per 10,000 population) for **women** aged 50 and older by country. In the case that multiple studies were included for a single country/region, the average age-standardized rate was used in the figure.

b Directly age-standardized hip fracture rates (per 10,000 population) for men aged 50 and older by country. In the case that multiple studies were included for a single country/region, the average age-standardized rate was used in the figure

Burden of fractures. In Norway there are each year around 9000 hip fractures and 15,000 forearm fractures [2, 28]. For women over the age of 45, osteoporotic fractures cause more days in hospital than any other disease, including diabetes and myocardial infarction [42]. In Denmark (year 2011), the economic burden of osteoporotic fractures for the population 50 years and above was estimated to 1.56 billion Euros. If we use these figures on the Norwegian population, anticipating the same fracture incidence, the economic burden would be around 10 billion NOK (10 milliard in Norwegian). Hip fractures account for the majority of these expenses [43]. Rates of hip fracture increase with age, and the number of Norwegian residents 65 years and older are expected to double towards year 2100 [44]. Thus, osteoporotic fractures will continue to pose a national and international public health challenge.

1.2.3 Risk factors for osteoporotic fractures

1.2.3.1 Established risk factors

Having suffered a previous fracture and having low bone mineral density (BMD) are the most established risk factors for fracture. The amount of bone mineral can be measured using radiological methods, most often dual energy x-ray absorption (DXA), and is frequently expressed as bone mineral density (BMD g/cm^2).An individual's BMD is expressed relative to a reference population (young adults) in standard deviation units, called t-scores. An individual with a t-score between -1.0 to -2.5 has osteopenia, whereas an individual with a t-score $\leq$-2.5 is osteoporotic. The risk of fracture increases linearly with decreasing BMD, approximately one standard deviation decrease in hip-BMD is equivalent to 2.5 times increased risk of hip fracture [45].

Some other established risk factors for osteoporosis and fractures are: higher age and female gender, taller stature (only hip fracture), Scandinavian (Caucasian) ethnicity, early menopause, genetic factors (e.g. mother suffered a hip fracture), being prone to falling, being underweight, losing weight, little sun exposure, being physically inactive, high alcohol consumption, and smoking. A low dietary intake of

vitamin D and calcium and possibly other nutritional factors could increase the risk. Additionally, there are conditions (e.g. Celiac disease and Crohns disease) that may cause malabsorption of necessary dietary factors, leading to increased risk. Other inflammatory conditions outside the gut (e.g. rheumatoid arthritis), and medications taken to alleviate these conditions (e.g. cortisone) are of significance. More information on risk factors for osteoporotic fractures can be found in: Nasjonale faglige retningslinjer [46].

Calcium: The most important sources of calcium in the Norwegian diet are dairy products like milk and cheese [47].Norwegian men consume on average 1038 mg dietary calcium per day, and Norwegian women on average 811 mg per day, which is above or around the recommended limit of 800 mg/day [47]. The intake of vitamin D is also sufficient [47]. More than 99 percent of body calcium is stored in the skeleton, and a high calcium intake has been found to be beneficial for bone mass [48]. Randomised controlled trials have shown that combined supplements with calcium and vitamin D can prevent all types of osteoporotic fractures [49]. On the other hand, the effect of high calcium on the risk of osteoporotic fractures in the absence of vitamin D is under debate, and may depend on the fracture site [50-54]. A concentration threshold, above which calcium does not have a further beneficial effect on fractures has been suggested [48, 52, 55], and increasing calcium above this threshold may even have a harmful effect on the risk of hip fractures [51, 53, 55].

Factors affecting calcium absorption and excretion could obscure the effect of calcium on bone. A high protein intake has been associated with increased calcium excretion (see chapter 1.2.3.2 “Acids”), and excess sodium intake may increase calcium excretion and decrease BMD [56]. A low calcium status could also cause increased uptake of heavy metals such as lead [57, 58], and body burden of lead has been reported to be negatively correlated with calcium in blood [59].

1.2.3.2 Less established risk factors

Some of the less established risk factors for osteoporotic fractures, with emphasis on dietary and environmental factors that can also be found in drinking water, are discussed below.

Acids:

From food: Intake of certain foods may produce acids in the organic form or in the inorganic form. Organic acids in the body are by and large mitigated by the elimination of carbon dioxide from the lungs, and the rest is excreted by the kidneys in the form of ammonium and other acids (containing hydrogen ions) [60]. However, protein from animal sources is rich in the sulfur-containing amino acids cysteine and methionine. These are being oxidized to inorganic sulfate, which is not easily eliminated [60]. Studies show that a high intake of protein from animal sources may have a negative effect on bone, mainly by increasing calcium excretion [61-66]. On the other hand, protein is necessary for bone formation, and may also *increase* calcium absorption from the intestine, reducing its own acidifying effect [61, 64]. Fruits and vegetables have also been found to reduce acid load, which could counteract the negative effect of a high protein diet, and act positively on BMD [66, 67].

From other sources: Acids in the *in*organic form (which is the form not easily eliminated), can also be ingested through polluted water (acid rain contains sulphuric acid) and cola beverages (containing phosphoric acid). Intake of colas has been studied and found to be associated with a lower BMD at the hip in women [68, 69]. Heaney and Rafferty (2001) concluded that the displacement of milk and other nutrients necessary for good bone health may be more important than the intake of phosphoric acid in itself [68], but the association with impaired bone health has been found only for beverages containing phosphoric acid, and not for other soft drinks [69, 70]. In a Norwegian cross-sectional study, an index reflecting frequent intake of soft drinks and rare intake of fruit and vegetables was inversely related to distal forearm bone mineral density [67].

Magnesium: The major sources of magnesium in the Norwegian diet are bread, milk, fruits and coffee [47]. Magnesium intake across Europe varies geographically, but is generally lower than the recommended intake (300 mg/day), especially in women and the elderly [71]. Magnesium deficiency is common in the elderly and in alcoholics, and is associated with chronic gastrointestinal and renal diseases (among other diseases) [72, 73]. Low-magnesium may also increase the risk of developing and dying from cardiovascular disease and stroke [74]. In Norway, the recommended limits are 350 mg/day for men and 280 mg/ day for women, and the dietary intakes are generally higher than this (on average 439 mg/day in men and 346 mg/day in women), but somewhat lower in the older age groups [47]. Magnesium is commonly stored in bone, which contains 60-65% of the body's magnesium. Most studies on magnesium and bone have considered only BMD, and it is still uncertain what influence magnesium could have on osteoporotic fractures [72, 75]. Although magnesium deficiency is more common than excess [71], research indicates that both too low *and* too high magnesium intake may be harmful to bone health [72, 76-78]. A high rate of wrist fracture has been found in postmenopausal women with high intake of magnesium in a Women's Health Initiative Study [79]. The calcium/magnesium ratio could also be of importance [72].

Fluoride: Exposure to fluoride is primarily from the water supply and from water-based beverages, but also from food and air pollution. It is well known that high doses of fluoride are harmful to bones and teeth; however the effect of fluoride on bone in lower doses is debated. A U-shaped pattern has been suggested for the relation between the prevalence of overall bone fractures and water fluoride level [80]. If the concentration of fluoride becomes excessively large, skeletal- and dental fluorosis may occur, and bones and teeth become brittle and fragile [64, 81]. On the other hand, research has shown that optimal drinking water fluoridation (at or around 1 mg/l) clearly prevents dental caries [64]. In a meta-analysis of fluoride treatment, the authors reported no overall effect of fluoride on hip or spine fractures, but that low fluoride doses($\leq$20 mg/day of fluoride equivalents, e.g. sodium fluoride or monofluorophosphate) were associated with a significant reduction in fracture risk

[82]. This finding of a positive effect of certain low doses of fluoride on bone was also supported in more recent reviews [81, 83].

Phosphorus: Phosphorus is widely distributed in foods including meat, poultry, fish, eggs, dairy products, nuts, legumes, cereals and grains. It is also found in cola beverages, as phosphate-salts (food additives) in processed foods, and in medications [64, 84]. Only very low amounts are found in drinking water. About 85% of the body's phosphorous resides in the skeleton, mainly in the inorganic phase in combination with calcium (hydroxyapatite). Acute phosphorus deficiency is associated with the release of calcium from the skeleton, resulting in low bone mineralization (Rickets or osteomalacia) [70]. However in American and European populations dietary phosphorus intake has been reported to be well above the current recommendations (approximately 700 mg/day) due to the relatively high intake of processed foods [64, 70, 84]. Phosphorous intake above the recommended level has been reported to reduce the production of 1,25-hydroxy vitamin D, increase PTH in serum , and reduce the absorption of calcium from the intestine [70, 85]. High intake has also been found to be associated with an increased risk of fracture in a cross-sectional dietary survey [86].

Cadmium: The majority of cadmium comes through food (especially rice, potatoes, cabbage and other plants) and to a lesser degree through drinking water. Smokers have a high absorption of cadmium in the lungs, and are therefore exposed to the same amount of cadmium from the tobacco plant as from food [87]. Smoking has been found to have a strong, positive dose-dependent relation with both hip fracture and overall fracture risk [88]. Cadmium accumulates in the body and can be toxic to the kidneys and to bone, among others [89]. Collectively, there is a great deal of evidence indicating that cadmium has unfavorable effects on bone, even at low doses. Reduced bone strength and an increased risk of osteoporosis have also been seen with a low-level (1mg/l) long-term exposure of rats to cadmium, which could reflect the lifetime general population exposure to cadmium in some areas [90]. In Japan 50 years ago, the cause of Itai-itai ("ouch-ouch") disease, a debilitating bone disease leading to multiple fractures, was discovered to be caused by consumption of rice growing in

cadmium-contaminated water [91]. Cadmium intake from the diet has been associated with an increased rate of any type of fracture and a lower BMD, independently of tobacco smoking [92, 93].Increasing urinary cadmium concentration, a marker of long-term exposure has also been associated with an increase in fractures and a decline in BMD, also in non-smoking women [94]. Cadmium in water is further discussed under "Water quality indicators" (chapter1.3.6.3).

Lead: Lead is primarily taken up through the diet, but also through air and drinking water. Lead has been found to both have a direct effect on bone and an indirect effect on fracture risk by increasing the possibility of falling. It accumulates in the body, especially in bone where it has a half-life of 10 years, and it is toxic to the nervous system, the kidneys and to blood formation [95-97]. Lead toxicity is a vicious cycle, not only does high lead increase the risk of bone-breakdown, but the breakdown of bone can also cause a release of lead stored in the skeleton [98, 99], affecting other parts of the body such as the nervous system, and thereby leading to an even greater fall-risk [100]. Lower bone mass, decreased mechanical strength and an increased bone concentration of lead were found in moose with fractures in the southern part of Norway [101]. Lead in water is further discussed under "Water quality indicators" (chapter1.3.6.3).

Aluminum: The main source of aluminum is the diet (including food additives). Other sources are drinking water, cooking utensils, and pharmaceuticals such as phosphate binders [102, 103]. Its absorption in the gut is pH dependent, with a lower absorption at neutral pH compared to acidic and alkaline, however only about 0.1% of the aluminum intake is absorbed in healthy persons, the rest is excreted [104]. Bone is the major accumulation tissue for aluminum [105]. Aluminum has been found to increase the risk of fractures in kidney patients on dialysis [81, 104, 106]. However, the effect of aluminum on bone in healthy people is still under debate [107]. Aluminum in water is further discussed under "Water quality indicators"(chapter 1.3.6.3).

Iron: The most common sources of iron in the Norwegian diet are bread and red meat [47]. Iron intake has been found to be lower in the north of Europe than in the south, and lower in women than in men, but is generally above the recommended limits in Norway [47, 71]. Iron is also found in the water supply in varying amounts [108]. It acts as a cofactor for enzymes involved in collagen synthesis [64, 109], and is involved in the activation of vitamin D, thereby affecting calcium absorption [109]. Low iron-availability has been reported to be associated with osteoporosis and low BMD in postmenopausal women [110]. Too high concentrations of iron may however act as a toxin to bone cells and contribute to osteoporosis or other bone diseases in people with impaired iron metabolism and iron overload [64, 111, 112], such as in hemochromatosis. Hemochromatosis is relatively common condition in Caucasian populations [111, 113], but the occurrence varies within Norway (lower prevalence in multiethnic populations in the north) [113]. It is not clear whether the harmful effect on bone is due to iron itself or from other mechanisms [64], such as oxidative stress in the osteoblast [114].

Zinc and copper: Zinc and copper are primarily found in animal protein foods and grains, among others [64, 115]. The elderly often have reduced serum levels of zinc, either due to insufficient uptake, to chronic inflammations in the body, or to medication use [109, 116, 117]. Both zinc and copper are essential nutrients for healthy bones, and low intakes have been linked to worse bone health [81, 118-120]. Zinc is needed for osteoblastic activity (collagen synthesis and alkaline phosphatase activity)[109], and has been shown to inhibit osteoclastic bone resorption [121]. Zinc supplementation may also protect against femoral neck fractures during chronic cadmium exposure [122, 123]. Lysyl oxidase, a copper-containing enzyme, is essential for cross-linking of collagen fibrils, thereby increasing the mechanical strength of bone [109].The amount of zinc and copper in natural water is generally low, but can be greatly increased in drinking water due to corrosion of water pipes [124]. Too high concentrations of copper and zinc may be harmful to bone in individuals with already sufficient intakes [119].

Other less established risk factors include inflammatory agents that could cause chronic inflammation [125, 126], and air pollution [127]. Also, boron [115, 128], manganese [115] and gallium [81, 129] may play a role in bone health, but little information is currently available. Reviews of lesser known risk factors in osteoporosis can be found in: Palacious 2007 [109]; Price et al 2012 [115] and Aaseth et al 2012 [81].

1.3 Drinking Water

1.3.1 Drinking water management

"Water is essential to sustain life, and satisfactory (adequate, safe and accessible) supply must be available to all"(World Health Organization (WHO) [130]). The management of the water supply in Norway is under the responsibility of the Norwegian Food Safety Authority (NFSA), the municipalities, and the waterworks themselves [131, 132]. The large majority of the 1547 waterworks reporting to the Waterworks Register are municipally owned, whereas 548 are privately owned, and one is owned by the state [133].The Drinking Water Regulation sets the norms for drinking water parameters in accordance with the EU directives on water [131, 132]. Alternative recommendations are sometimes made by the NIPH, for example in the case of pH where the norm is 6.5-9.5, but the NIPH recommends a pH between 7.5 and 8.5 [124]. The Drinking Water Regulation has been revised several times, and the last major revision was in 2001[132].

1.3.2 Norwegian geology

Besides management, the quality of the water supply is also influenced by Norwegian geology. In short, about two-thirds of Norway is covered by metamorphic rocks (such as gneiss and shale) formed during the Caledonian Orogeny [134]. Sulphide ore mining and processing (mainly for copper) in the Caledonian edge has been an important industry in Norway, and the influence on the freshwater quality (acidification and very high metal levels) is substantial at several locations [135]. Southern and North-western Norway has generally `acidic' lithology, dominated by

gneissic bedrock of Precambrian age, except in the Oslo area where the bedrock is transected by the Oslo graben (deep valley) spanning from north to south [134, 135]. The Oslo graben was formed around 200 million years ago during volcanic activity and consists of both volcanic rocks and sedimentary layers rich in fossils [134]. Several large drinking water sources (e.g. Maridalsvannet, one of the largest sources in the Oslo area) are situated in areas with volcanic rocks, which contribute in making the water more acidic [134, 135].

1.3.3 Water sources

Surface water. About 90 percent of the Norwegian population receives water from surface sources (i.e. streams, rivers, ponds and lakes) [20]. Surface water is vulnerable to the surrounding environment (e.g. atmospheric pollution and human activity, vegetation, bedrock), which often makes it acidic (pH less than 7), soft (low concentration of minerals and small buffering capacity), and of a high color grade (a high content of organic material). Acidic water with low buffering capacity is corrosive to several materials in the water supply system, and a high content of organic material can make it more difficult to properly disinfect the water [20, 130]. Surface water has also been reported to contain three times more mold than groundwater [136].

Groundwater is used by approximately 10 percent of the population in Norway [20]. For comparison, in Sweden, Denmark and Finland about 50 percent or more of the population receive water from ground sources, whereas in the UK the fraction is approximately 30 percent [20]. This type of water comes in two different categories: groundwater from unconsolidated deposits (sand, gravel or clay), and groundwater from solid bedrock. Groundwater is usually well protected against pollution on the surface, which means it has less need for disinfection. It also has higher pH than surface water. However some problems, such as too much iron and manganese can arise from the surrounding geological environment [19, 20, 137]. Problems with water hardness (too hard or too soft) and too high concentrations of fluorides and/or radon could also occur [19, 20, 137].

1.3.4 Water hygiene

Drinking water treatment is one of the most important measures for avoiding sickness and death in a population. Water treatment should be customized according to the water-quality at the source, but a minimum of two hygienic barriers is required by the Norwegian Drinking Water Regulation [131, 138]. The most common types of water treatment in Norway are **chlorination** and **coagulation**, but **UV-irradiation** is also becoming more common [139]. The effect of **chlorine** depends on the dosage, and the contact time with the water. The effect is also dependent on the content of organic material in the water, the water's temperature, and the pH of the water (less efficient at pH> 8). During **coagulation** one or more chemicals (e.g. iron or aluminum based coagulant) are added to the water so that unwanted particles aggregate and can be filtered out. Coagulation could remove a large portion of parasites and bacterial spores, along with organic material. Although there are more people drinking chlorinated water in Norway, there are three times as many waterworks that use **UV-disinfection**, but these waterworks are mostly small [133]. UV-disinfection is efficient at eliminating spores from bacteria such as *C. perfringens*, and also for killing parasites such as *Giardia* and *Cryptosporidium*. Other water treatment processes used in Norway include membrane filtration, aeration, oxidation, ion exchange and absorption [138]. Due to the natural softness of Norwegian drinking water, it is also important to prevent corrosion, i.e. adding chemicals to raise the water's pH. More information on corrosion follows in chapters 1.3.5 and 1.3.6.1.

1.3.5 Water supply

The water distribution system must supply a sufficient amount of drinking water of satisfactory quality at all times [140]. The largest challenge is to uphold the quality standards of the water during transportation from the waterworks to the consumers. The water supply system in Norway has been estimated to have between 34 and 50% leakage, which is substantial compared to other countries: Sweden has 14 % leakage, Denmark around 6%, and Finland and England around 16% [140]. If the pressure in the supply system drops, it can lead to pollution from the outside (e.g. sewage) seeping

into the pipes, contaminating the drinking water before it reaches the consumer. Other problems in the supply system are establishment of biofilms (a type of sludge consisting of organic material and bacteria that are resistant to disinfection) and corrosion of the water pipes leading to higher concentrations of metals in the water. Water that has been stagnant for some time can contain increased concentrations of metals such as lead and cadmium originating from the piping material and from faucets made of brass [140]. Aluminumions can get into the water from cement-based piping material [140].

1.3.6 Water quality indicators

The quality of the drinking water is influenced both by natural and anthropogenic processes [124]. Examples of natural influences are climate, minerals and organic components released from bedrock, soil and vegetation, or aerosols blown from seawater in coastal areas. Anthropogenic influences include agricultural runoffs, impacts from settlements, landfills or industry (local and long-range). The water treatment process and the water distribution system also affect the finished drinking water that reaches the consumer. Although the term "drinking water quality" includes many aspects of the drinking water, the current work has primarily focused on three main areas: acidity level (pH) and the concentration of calcium and magnesium (hardness); bacterial indicators and color grade; and toxic metal concentrations.

1.3.6.1 Acidity and hardness (calcium and magnesium)

Acidity. The pH is a measure of the water's acidity level, and it regulates several chemical conditions in the water, such as the mineral concentration. When the pH is below 7, the water is acidic and usually low in calcium, magnesium and carbonate (i.e. soft). Norwegian water is generally soft. This is due to geological conditions, but also to the cold climate and to most water sources being located on the surface with a high input of precipitation (which is both naturally acidic and could be further acidified from long-range air pollution) [18, 141]. The regions that have been most severely affected by acid rain pollution are the Agder counties (southern region), Telemark (south-eastern region) and Rogaland (south-western region). These areas have received

substantial amounts of long-range air pollution from other European countries, and the geological conditions in these areas have a low capacity to withstand acidification [141]. It is important to keep the water's pH under control to minimize corrosion.

Hardness. Hard water is primarily due to a high concentration of calcium and magnesium in the water. Water is usually considered "hard" when the calcium concentration is higher than 35 mg/l. Hardness, calcium and magnesium are not regulated in Norway, but to limit corrosiveness, it is recommended that calcium in drinking water is not below 15 mg/l [124]. No upper limit of calcium concentration has been recommended by the NIPH due to research on the conceivable positive health effects of hard water (many reviewed in [142] and [143]).

Calcium. Calcium in drinking water sources comes from bedrock rich in lime (such as limestone and marble). It could also come from lime being added during the water treatment process, or being released from cement-based water pipes. There is a marked regional difference in calcium concentration across Norway; however, the calcium concentration of the drinking water is generally below 15 mg/l [124]. For comparison, the median calcium concentration in U.S. drinking water is 26 mg/l [144]. A concentration minimum of 20 to 30 mg/l calcium has been suggested by the WHO [143].

Magnesium. Magnesium usually comes from magnesium-rich bedrock, or from precipitation originating from seawater in coastal areas. Magnesium can also be added by filtering the water through (or adding) dolomite ($CaMg(CO_3)_2$) during water treatment. To control hardness, a recommended upper limit of 10 mg/l has been set for magnesium in Norwegian drinking water, but the natural magnesium concentration is rarely above 10 mg/l [124]. On the contrary, a concentration minimum of 10 mg/l magnesium in drinking water has been suggested by the WHO [143].

1.3.6.2 Bacterial indicators

The majority of water-transmittable microorganisms that are pathogenic originate from human or animal feces [124], and most are not able to grow or multiply in water [130]. The spectrum of species is broad, but four groups of bacteria are commonly used as

indicators in Norwegian drinking water: Coliform bacteria, *E.coli*, intestinal enterococci and *C. perfringens*. The presence of these indicators is not necessarily harmful to humans, and it is not certain that they originate from fecal contamination (e.g. coliform bacteria could also come from decaying plant material), but they are pointers to whether the water could be contaminated with pathogens. *E.coli* is an inhabitant of normal fecal flora, and it is used to determine whether the fecal contamination is fresh [130]. Intestinal enterococci live longer in water than the coliform bacteria and *E.coli*, and could therefore serve as an indicator of intestinal virus from humans [130]. *C.perfringens* is an anaerobe bacterium; it grows best with little oxygen in the water. When the conditions are not optimal for growth, it forms spores that can survive most disinfection methods, except UV-radiation[130]. Spores could indicate the presence of intestinal virus and cysts from possibly harmful parasites, such as *Giardia* and *Cryptosporidium*. *C.perfringens* itself could also cause disease if permitted to multiply in food. According to the Norwegian Drinking Water Regulation, no bacterial indicators and/or spores should be present in treated drinking water [131]. On the other hand, the absence of indicator bacteria does not guarantee that the water is free of contamination.

1.3.6.3 Toxic metals in drinking water

Metals that have no essential function in the body and that could be harmful are often termed “toxic metals”. Considerable amounts of several metals are supplied to Norwegian lakes (including drinking water sources) through long-range atmospheric transport from other European countries [145]. Lake sediments are polluted with these metals to a varying degree, with the highest concentration in the south of Norway [145]. Some toxic metals in drinking water that may be of importance to bone are mentioned below.

Cadmium concentrations in Norwegian drinking water are very low, usually less than 1 µg/l [124], whereas the limit for cadmium set by the authorities is 5 µg/l [131]. However, with low water-pH, cadmium can be dissolved from the surrounding soil and bedrock. Old faucets and other parts of the water distribution network can contain

cadmium, but it is no longer permitted to use cadmium in new pipelines in the distribution network in Norway [124].

Lead concentrations in Norwegian drinking water are generally (but not everywhere) well below the Norwegian and international limit for lead in drinking water (10 µg/l) [124, 131]. Lead levels in lake sediments in Norway has declined during the last decades, primarily due to a reduction in leaded petrol, but lead is still found in high concentrations compared to many other metals [145]. Lead can also enter drinking water from alloys, from old solders and from faucets in the water distribution network. It is no longer permitted to use lead in the water distribution network in Norway, but also new plastic pipes can leach lead [124].

Aluminum is a very common metal found in the environment, the earth's crust consists of 8% aluminum by weight. It is usually not very soluble in water, but with a lower pH (<5.5), aluminum can be extracted from soil and other surroundings [18]. Aluminum salts are widely used as coagulants in water treatment [130, 138], which could lead to increased levels of aluminum in the water supply. The Norwegian (and international) limit for aluminum in drinking water is 0.2 mg/l. Aluminum concentrations as high as several mg/l has been found in finished Norwegian drinking water [124].

2 Rationale and aims of the study

2.1 Rationale

The lack of reliable data on individual exposures to drinking water implies that few previous studies of drinking water exposure and bone health exist (none in Norway). Only fluoride has previously been studied on a population level, however most of these studies were of ecological design. Reasons for exploring the potential association between drinking water quality and osteoporotic fractures were:

- The incidence of hip fractures increased in Norway during the period 1970 to 1990, and then leveled off. Over the past 10 years a slight decline in the

incidence has been observed [28]. Before 1990 the quality of the Norwegian drinking water was far from optimal. New regulations for better water treatment in accordance with European declarations were put in place in 1995 and 2001 [132]. In addition, acid rain pollution and leaded petrol has been reduced over the last 20 years [141, 145]. Thus, there has been an improvement in drinking water quality in Norway in the same period that we have observed a change in the incidence of hip fractures.

- The incidence of osteoporotic fractures seems to vary between countries, with Norway at the world-peak in incidence. The quality of municipal drinking water also differs between countries [20, 138]. Compared with populations in other countries, Norwegians receive lower amounts of minerals such as calcium, magnesium and fluoride through drinking water [124, 144]. The main source of drinking water in Norway is surface water [20]. Even though groundwater often has higher concentrations of minerals, Norwegian groundwater is also generally mineral-poor [20].
- The fracture incidence within Norway varies by urbanization degree and by region of residence [14, 17]. Likewise, the quality of Norwegian drinking water varies across the country of Norway, depending on downfall of long-range air pollution, geological differences, and proximity to agriculture, mining and other industry [18, 124, 134, 135, 141].

2.2 Aims and objectives

The overall aim was to explore a possible association between municipal drinking water quality and osteoporotic fractures in Norway.

Objectives:

For causal diagrams, see appendix I

1. To investigate relations between acidity level (pH) in drinking water and osteoporotic fractures, adjusting for potential confounding factors and taking possible intermediate factors in the water into account (paper I).

2. To examine the possible relations between concentrations of calcium and magnesium ("hardness") in drinking water and risk of hip fracture, after considering the effects of urbanization degree, acidity level (pH) and other potential confounders (paper II).

3. To study the relations between the drinking water's toxic metal concentration (cadmium, lead and aluminum) and hip fracture risk, and investigate possible effect modifications and interactions by other factors related to bone health (paper III)

3 Materials and Methods

3.1 Data sources

3.1.1 Cohort of Norway (CONOR), paper I

COhort of NORway (CONOR) is a national database containing regional data from 10 health surveys during 1994-2003. All surveys contained 1) a short physical examination with measurements of blood pressure, heart rate, weight, height, waist- and hip circumference, 2) one or more questionnaires, 3) a non-fasting blood sample drawn for analyses of lipids and a sample of EDTA blood stored at – 80 degrees C for later analyses and extraction of DNA.

The surveys used 50 common questions including self-reported health and selected diseases, various risk factors, socio-demographic factors, use of medications and reproductive history in women. Some of the questions were asked differently in different health surveys, but these have been recoded to a common scale. The location of the study sites and information on each study, along with a description of the participants has been published previously [146], and more information may also be seen at: www.fhi.no/conor

3.1.2 The Norwegian Waterworks Register (VREG), paper I

Information on the quality of drinking water was provided by the Norwegian Waterworks Register (VREG). This is a national register of waterworks supplying more than 50 persons or 20 households. The water quality of 87% (ca. 4.3 mill.) of the population is reported to this register, however not all waterworks report every year. The database includes information on the number of persons/ households supplied, transport system (pipelines), water sources, water-treatment processes, and quality for a large set of components. Samples are taken from both raw water (before treatment) and tap water (after treatment). Data are available in electronic format and has been collected in 1994 and 1996, and yearly since 1998. In 2010 waterworks started reporting to the NFSA, and information has been transferred from NFSA to VREG on a regular basis. More information on VREG is publicly available in a recent report with English summary [133] and at www.fhi.no/vreg (in Norwegian).

3.1.3 NOREPOS Hip Fracture Database (NORHip), papers II and III

A database consisting of all hip fractures treated in Norwegian hospitals from 1994 through 2008 has been established by the Norwegian Epidemiologic Osteoporosis Studies (NOREPOS) research collaboration. This database has been named "NORHip" and includes almost 140,000 incident hip fractures (a maximum of two fractures per person). Hip fractures were electronically retrieved by a system developed by the Norwegian Knowledge Center for the Health Services. This system was linked to the Patient Administration System (PAS) in 48 hospitals/health trusts performing hip fracture surgery in Norway. The validity of the database has been assessed by comparisons to local hip fracture registries (verified by hospital records and radiographic archives) in the cities of Oslo and Tromsø, and the combined Cohen's kappa was 0.95 [28]. More information on the data quality assurance can be found under Research>>Documentation at: http://www.norepos.no/

3.1.4 Trace Metal Survey, papers II and III

During 1986-1991 a survey of 30 physical-chemical parameters was conducted in selected waterworks throughout Norway. The selection of waterworks was based on

the Norwegian Waterworks Register. Waterworks were chosen to ensure geographical spread and a variation in waterworks-sizes, however all the largest waterworks were represented. Samples were collected four times (spring, summer, fall and winter) in the two southern most counties (Aust-Agder, Vest-Agder), and two times (spring and fall) in the remaining counties in Norway. Personnel at the waterworks were asked to collect samples of raw and treated water into polyethylene bottles and send them to the Norwegian Institute of Public Health for analysis. Water samples were collected from the source (raw water) and at an early point in the distribution network (treated water), ensuring minimum influence from collection devices and pipelines. The analyses were performed by conventional standardized methods. A total of 566 waterworks, supplying 64% of the Norwegian population, provided samples. Maps of the approximate sampling areas are found in section the result section (chapter 4.2.5). More on the sampling and analysis procedures are described elsewhere in Norwegian [147].

3.2 Data linkage

Databases containing exposure (water quality on area level) and outcome (fracture occurrence on individual level) were linked using Geographic Information Systems (ArcGIS 9.3, ESRI 2008). This linkage was carried out in several steps:

1. **Identification of the waterworks supply areas (map layer 1).**
    a. Largest cities: Supply-areas (maps) were provided by the city-administrations (such as Oslo, Bergen and Tromsø).
    b. Municipalities that jointly operate one waterworks (such as the inter-municipal waterworks in Rogaland (IVAR)) were merged into one supply area.
    c. Suburban and rural areas: Commonly, each municipality operate their own waterworks, and for these the municipality borders (from year 2006) were taken as supply-boundaries. However, in some areas

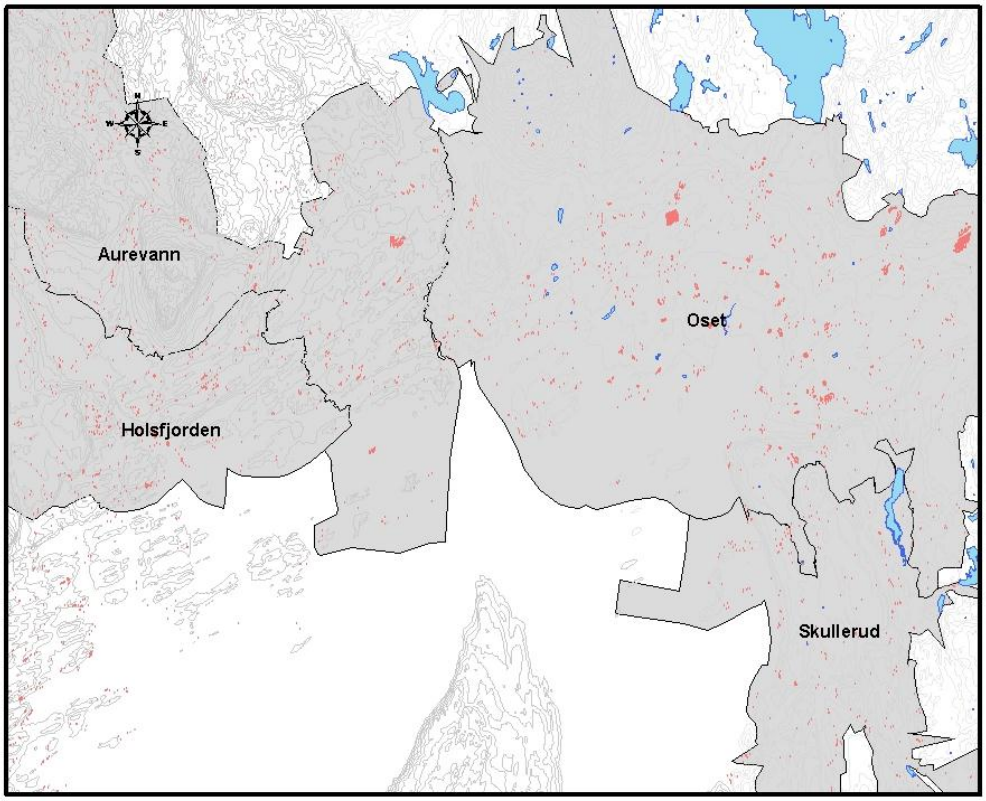
the boundaries had to be defined so that each of them was being served by only one waterworks (ensuring unique exposure):

This process was performed using Voronoi triangulation; polygons were created in such a way that the approximate boundary of each polygon was closer to its generating point than to any other point. The "points" or coordinates in this circumstance being the geographical locations of the waterworks. Coordinates of the individual waterworks were provided by the Norwegian Geological Society (NGU) or the Norwegian Water Resources and Energy Directorate (NVE). Existing maps and municipal administrations were also consulted in this process.

2. **Geocoding of participants (map layer 2).**
   The coordinates/address points of the entire Norwegian population included in the population and housing census from the years 1960, 1970, 1980, 1990 and 2001 (representing approximately 6.5 mill individuals) were placed on the map ("geocoded") in their respective waterworks-supply areas. Using census-data from several years ensured that also deceased or emigrated participants were counted.

3. **Joining of map layers 1 and 2.**
   The two map layers were subsequently joined, so that waterworks information and data on drinking water quality could be assigned to each individual and their health information. Figure 2 shows an excerpt of the finished waterworks map, including a *subset* of the geocoded recipients.

Figure 2. Excerpt of the waterworks-supply map (Oslo-area). Waterworks areas are outlined in black and a subset of the water-recipients (indicated by red dots) have been geographically placed (geocoded).



3.3 Study population

3.3.1 Paper I

From 1994-2003 173,236 subjects 20 years and older (mean age 50.2 years) participated in CONOR. The analyses were conducted among 127,272 geocoded CONOR participants with complete information on forearm fractures, pH in tap water, and background variables (age, gender, BMI, and degree of urbanization). The exclusion of participants and the number of observations used are depicted in appendix II.

3.3.2 Papers II and III

In papers II and III we restricted the sample to men and women 50-85 years old in year 1994-2000, for whom there was complete information on either calcium and magnesium (paper II) or cadmium, lead and aluminum (paper III) in their drinking water. The follow-up period was between 3 and 14 years.

Calculation of person-years: To calculate the population at-risk or number of person-years (py) we used the population numbers in 1-year age classes per calendar year. Within waterworks and age groups we then added the time contribution per calendar year, weighting the terms according to population size using a formulae from Carstensen (2007) [148]. In short, within each one-year age group (50-85 years) we added the total amount of individuals served by each waterworks for every single year in the time period 1994-2000 (a total of 2,125,381 person-years in men and 2,414,261 person-years in women).

3.4 Variables

3.4.1 Fracture outcomes

Forearm fractures: Information on prevalent forearm fractures were ascertained from participants' self-reports to the following question: "Have you ever broken (fractured) your wrist/forearm? (yes/no)".

Hip fractures: Incident hip fractures were identified and retrieved from all hospitalization records with an International Classification of Disease (ICD) diagnosis code for hip fracture, ICD-9: 820 (Fracture of neck of femur) with all subgroups, ICD-10: S72.0 (Fracture of neck of femur), S72.1 (Pertrochanteric fracture) and S72.2 (Subtrochanteric fracture). Surgical procedure codes and additional diagnosis codes were used for fracture assessment. Up to two hip fractures were included per person.

3.4.2 Exposures

pH measurements (paper I): pH in treated water was measured throughout the year by the individual waterworks using a pH meter, and reported to VREG on an annual basis. Due to some years of non-reporting, an average exposure indicator was calculated using the years 1994-2008. Before merging, the pH-measurements were converted back to their hydrogen ion (H+) concentrations. This was done to avoid a distortion of the averaged values toward the center of the pH-range, which might occur when calculating an arithmetic mean from measurements made on a logarithmic scale. The pH-variable was categorized into four groups, and also dichotomized: <7 and ≥7

Calcium and magnesium (paper II): Water concentrations of calcium and magnesium in treated water were measured by electrothermal atomic absorption spectrometry. The average of the mineral concentrations in the two or four samples (collected at different seasons) provided by each waterworks was used in the analyses. We categorized the exposures into tertiles (based on the concentrations at waterworks) in the statistical analyses. Tertiles: Calcium 0.22-1.50 mg/l; 1.51-3.67 mg/l; 3.68-112 mg/l. Magnesium 0.08-0.44 mg/l; 0.45-0.78 mg/l; 0.79-31.5 mg/l.

Toxic metals (paper III): Water concentrations of cadmium, lead and aluminum in treated water were measured by electrothermal atomic absorption spectrometry. The average of the metal concentrations in the two or more samples (taken at different seasons) provided by each waterworks was used in the analyses. The variables were dichotomized in the study, with a cut-off based on the waterworks-average: Cadmium ≤0.1µg/l, >0.1µg/l; lead ≤1.16 µg/l, >1.16 µg/l; aluminum ≤0.11 mg/l, >0.11 mg/l.

3.4.3 Covariates

3.4.3.1 Demographic and anthropometric variables

Age and gender of individuals was provided by the Norwegian Person Registry. Age was included as a continuous variable to adjust for confounding in all analyses. In paper III, age was also divided into two groups (50-65; 66-85) to check for effect modification. All analyses were stratified on gender to take into account differences in outcome and exposure by gender. Body mass index (paper I) was calculated as weight in kg/height in meters^2, and continuous BMI was used for adjustment. Marital status (paper I) was dichotomized into married vs. not married (i.e. cohabitants, widow/widowers, separated, divorced), and smoking (paper I) was classified as current daily smoker vs. previous/never. The participants were asked about years of education (paper I), and this variable was dichotomized into "12 years and less" and "more than 12 years".

3.4.3.2 Geographic variables

Urbanization degree: Degree of urbanization was determined based on the participant's home address. In paper I participants were divided into three population density groups (information from Statistics Norway): rural (municipalities with less than 10,000 inhabitants), suburbia (municipalities with 10,000-19,999 inhabitants), or cities (municipalities with 20,000 or more inhabitants). In papers II and III the urbanization degree of each municipality was provided by Statistics Norway and represents the fraction of inhabitants in each municipality living in a cluster of houses with at least 200 people, and where the distance between the buildings does not exceed 50 meters. The scale of urbanization for each individual was specified by a number on a continuous scale between 0 and 1, where close to 0 indicated no urbanization (all households located more than 50 meters apart), and close to 1 indicated cities [149]. For waterworks that supplied more than one municipality, a population-weighted average of the urbanization degree was calculated. To ease interpretation, urbanization degree was divided into tertiles based on the municipal urbanization degrees (low or

"rural": 0-0.33, medium or "suburbia": 0.34-0.62 and high or "cities": 0.63-1.00), but continuous urbanization degree was also used for adjustment in paper II.

Geographic region (papers II and III): To take into account variations in sampling and underlying geographic differences, the waterworks were divided into five regions based on their county of location (see figure 1 in papers II and III).

Water source (papers II and III): Water source was registered in the Trace Metal Survey as either "surface" or "ground" source.

3.4.3.3 Other water quality variables

In paper I, all available water quality variables were included to explore whether there was an association with forearm fracture. They were divided in to 4 groups: metals, bacterial indicators, sensory indicators (color grade and turbidity) and others. The variables that showed an association ($p<0.25$) with fracture, and with pH, were carried forward in multivariate models. The included variables were "Intestinal enterococci", "*C. perfringens*" and "Color grade". Possible interactions with the available metals (iron, aluminum and manganese) were also tested. In papers II and III the potential bone-related factors included were based on existing literature. In paper II pH was grouped into tertiles based on waterworks concentrations: 4.52-6.30; 6.31-7.08; 7.09-10.20. Table 1 (chapter 4.2) shows the water quality variables included in paper III. They were measured (using standardized methods, see [147]) in treated water, except for fluoride, phosphorus and ammonium which had substantially less observations in treated water compared to raw. For these three variables the measurements from raw water were used. Drinking water in Norway is not fluoridated; therefore the mean concentration in raw water reflects the concentration in treated water, which was also observed in our data. The mean concentrations of phosphorus and ammonium in raw and treated water were also found not to differ much. All variables in paper III were dichotomized in the analyses.

3.5 Ethics and approvals

Waterworks serving less than 100 people were not included to protect the identity of fracture patients. All participants in the regional health surveys comprising CONOR (paper I) provided written informed consent. The study, including the linkages between waterworks (Norwegian Waterworks Register and the Trace metal survey), and the databases (CONOR and NORHip)was approved by the Norwegian Data Protection Authority, the Regional Committees for Medical and Health Research Ethics, the Norwegian Directorate of Health and the owners of the registers and databases, all in compliance with the Declaration of Helsinki.

3.6 Statistical Methods

3.6.1 Standard statistical analyses

The statistical analyses were done in STATA version 11 (StataCorp LP, Texas) and SPSS version 17 (SPSS Inc, Chicago). Additionally, R version 2.15.0. was used to create figures of non-parametric functions of fracture-risk across the spectrum of pH (paper I), and the ranges of calcium and magnesium (paper II). All tests were two-sided, and the level of significance was set to 0.05. Mean, median, range and standard deviations were examined. Water quality variables were not normally distributed; therefore we used Spearman correlation (pairwise) to investigate linear relations between the water-variables. In paper I, univariate and multivariate logistic models gave odds ratios (OR) of forearm fracture with 95% confidence intervals (CI). In papers II and III, Poisson regression was used to model the rate of hip fracture (IRR, 95% CI). Adjusted effects were compared to unadjusted effects with the same number of observations in all three papers. The attributable fraction for two statistical models of combined risk factors (pH with its covariates in paper I, and cadmium with its covariates in paper III) were calculated as: (Risk ratio-1)/Risk ratio.

3.6.2 Effect modification and interaction

According to Hernan and Robins [150] a distinction should be made between variables that cannot (hypothetically) be intervened upon, e.g. background variables such as age,

and modifiable variables, such as water quality variables. Modification of causal effects by the former is called effect modification, whereas the modification by the latter is called interaction. Statistical testing is an aid in elucidating the relations between variables, and identification of effect modification may result in identification of interaction [150]. For example, a difference in the effect of cadmium exposure between men and women could lead to the identification of components (e.g. iron) interacting with cadmium on the effect of fracture. Effect modifications and interactions were examined by stratification, and by including multiplicative terms (variables dichotomized).

3.6.3 False discovery rate

In paper III, the interaction analyses included a large number of statistical tests (33 for each gender); therefore p-values were adjusted using false discovery rate (FDR). The FDR attempts to control the amount of false positives among the reported statistically significant results (rejected H_0), and is less conservative than the Bonferroni method. The p-values from the analyses were arranged sequentially from the largest to the smallest. A q-value was calculated: $q_i=kp_i/i$, where i is the rank of the p-value among the k number of tests (k=33). The FDR_i was then equal to the smallest q-value of the rank i or above (i.e. a q=0.04 at i=2 would be set to FDR=0.025 if q at i=3 was equal to 0.025). Only FDRs <0.05 were included in the final results.

3.6.4 Principal components (additional analyses)

Water quality factors are often correlated; therefore the factors are frequently assembled in "principal components" to reduce the number of variables in the analysis. In this method, weights based on the variance in the dataset are assigned to individual variables, and components of properly weighted variables are constructed. The component with the highest eigenvalue explains the largest amount of variance in the dataset, and this component is listed first. The components (which are uncorrelated to each other) may then be used in further analyses, i.e. in regression models. In the current thesis, we used principal components in additional analyses not included in the papers.

4 Results

4.1 Synopsis of the papers

4.1.1 Paper I

Title: Is the quality of drinking water a risk factor for self-reported forearm fractures? Cohort of Norway.

Forearm fracture is one of the strongest predictors for later hip fractures, and the prevalence has been found to be higher in cities compared to rural areas. pH regulates most chemical reactions in water, and is related to the water's mineral (e.g. calcium) concentration, the concentration of toxic metals (e.g. cadmium and lead), and to the concentration of microorganisms (e.g. indirectly by modifying the effectiveness of disinfectants such as chloride). The pH of drinking water has been seen to vary across the country. The aim of this study was to investigate whether self-reported forearm fracture was related to pH in drinking water, and to explore whether other indicators of water quality may influence this possible association. pH was categorized into four groups. We found that forearm-fracture had an inverted u-shaped relation with pH, a higher risk of fracture around pH 6.0-6.9 compared with pH 7.0-7.5. This association was somewhat stronger in men, but could not be entirely explained by background factors such as age, BMI and urbanization. The alkaline category showed a slight protective effect, however this association was attenuated when adjusting for urbanization level. When studying possible intermediate factors, we found that three variables partially explained the variation in fracture risk by pH. The participants being supplied water that contained the bacterial indicators intestinal enterococci and/or *C.perfringens*, and/or had a relatively high color grade (much organic material in the water) were at higher risk or forearm fracture. The relation between pH and forearm fracture was also diminished at higher concentrations of metals (iron, aluminum and manganese).

Conclusion: pH of the drinking water seems to be inversely associated with forearm fracture, with a higher risk of fracture at slightly acidic pH-levels. However, this

association may not be directly due to the acidity as such, but rather to the variation in concentrations of microorganisms and/or metals in drinking water at different levels of pH.

4.1.2 Paper II

Title: Nationwide data on municipal drinking water and hip fracture: Could calcium and magnesium be protective? A NOREPOS study

To further explore the geographic variations in fracture risk, we investigated the relations of calcium and magnesium in municipal water to the incidence of hip fracture in men and women 50-85 years. We also calculated the contribution of drinking water to the total amount of calcium and magnesium taken in from dietary sources, based on the findings in a national dietary survey (Norkost 3). Concentrations of calcium and magnesium in water were low, but still an inverse association between magnesium and hip fracture risk was found in both genders: a 20 % lower risk in men and a 10 % lower risk in women with high magnesium. No associations were seen between calcium and hip fracture, but when considering calcium and magnesium together, a positive association was found between calcium and hip fracture in women (IRR=1.11). The increased risk of hip fractures in cities compared to rural areas was confirmed (IRR_{men}=1.23, IRR_{women}=1.24), however calcium and magnesium did not explain the association between urbanization and hip fracture. The contribution of drinking water to the total intake of calcium and magnesium was generally low.

Conclusion: Magnesium in drinking water has a possible protective effect on hip fracture risk, but the role of calcium is less clear. The increasing risk of hip fracture in the cities could not be explained by variations in calcium and magnesium in the water.

4.1.3 Paper III

Title: Do cadmium, lead and aluminum in drinking water increase the risk of hip fractures? A NOREPOS study

Cadmium, lead and aluminum are some of the toxic metals in drinking water that may have harmful effects on bone health. Cadmium from the diet has shown to be

associated with worse bone health and fracture in several studies. Lead has also been tied to an increased risk of fracture, but apart from osteomalacia in dialysis depended patients, the role of aluminum is less clear. The exposure by these metals have declined in the past years and their concentrations may be higher in urban than in rural areas, possibly corresponding with the variations in hip fracture risk. We investigated the risk of hip fractures in men and women 50-85 years being supplied drinking water with low (below the waterworks average) and high (above the waterworks average) concentrations of these metals. We also considered effect modification by background variables (age, gender, urbanization degree, water source) and interactions between the toxic metals and other water components. Generally, low concentrations of these three metals were found in Norwegian drinking water, although 12 percent of waterworks in the south exceeded the permissible limit of aluminum. The risk of hip fracture was significantly increased in men being supplied relatively high concentrations of cadmium in their drinking water (IRR= 1.10), also when adjusting for background variables such as urbanization. The effect of lead was found to vary by age, with a higher risk of fracture with relatively high lead in the oldest age group (66-85 years). The statistical effect of all three metals also varied by urbanization degree, but only in men. Several interactions were found, the most prominent one between lead and aluminum in both men and women.

Conclusion: A relatively high concentration of cadmium in drinking water could increase the risk of hip fracture in men. High concentrations of lead and aluminum also seemed to increase the risk, but their effects were dependent on other factors such as age, urbanization degree and other components in drinking water.

4.2 Additional results not published in the papers

4.2.1 Relations of other water quality factors with hip fracture

Table 1 shows statistical effects of other components in drinking water mentioned in paper III. Dichotomized calcium and magnesium had an inverse association with hip fracture in both men and women. In men only, inverse associations were seen with pH

and manganese, whereas a higher risk was found with higher concentrations of iron and copper. No association between fluoride and hip fracture was found when dichotomizing at the average level in water (table 1).

TABLE 1. Incidence rate ratios, 95 percent confidence intervals (IRR, 95% CI) of hip fractures according to concentrations of potentially bone-related factors in drinking water (dichotomized using the waterworks mean as cut-off, where lower than mean is reference category). Ages 50-85 years. A NOREPOS study

		MEN		**WOMEN**	
Water-quality factor, range	Category: >mean	N hip fractures[a] (N category >mean)	IRR (95% CI), adjusted for age, region, urbanization degree and water source type	N hip fractures [a] (N category >mean)	IRR (95% CI), adjusted for age, region, urbanization degree and water source type
pH, 4.5-10-2	>6.7	5,483 (3,609)	0.92 (0.87, 0.98)**	13,629 (9,089)	0.98 (0.94, 1.01)
Calcium, 0.22-112 mg/l	>5.39 mg/l	5,469 (1,507)	0.84 (0.78, 0 .90)***	13,588 (3,865)	0.94 (0.90, 0.98)**
Magnesium, 0.08-31.5 mg/l	>1.03 mg/l	5,469 (929)	0.88 (0.82, 0.95)**	13,590 (2,227)	0.89 (0.84, 0.93)***
Fluoride[b], 0.1-2.60 mg/l	>0.14 mg/l	5,407 (492)	1.02 (0.92,1.13)	13,460 (1,313)	1.00 (0.94,1.06)
Phosphorus[b] , 1.0-41.0 µg/l	>4.39 µg/l	5,388 (2,167)	0.95 (0.90,1.01)	13,425 (6,784)	0.98 (0.94,1.01)
Zinc, 1.0-1,137 ug/l	>30.7 µg/l	5,483 (590)	0.95 (0.87,1.05)	13,629 (1,441)	0.96 (0.90, 1.02)
Iron, 0.01-1.73 mg/l	>0.07 mg/l	5,483 (1,697)	1.07 (1.00,1.14)*	13,629 (4,192)	1.03 (0.98,1.07)
Manganese, 0.001-0.73 mg/l	>0.02 mg/l	5,483 (1,661)	0.93 (0.87,0.99)*	13,629 (4,075)	0.97 (0.93,1.00)
Ammonium[b], 0.005-0.19 mg/l	>0.02 mg/l	5,364 (2,875)	1.05 (0.99,1.11)	13,354 (7,236)	1.02 (0.98, 1.06)
Copper, 1.00-1500 µg/l	>56.0 µg/l	5,483 (899)	1.10 (1.0, 1.18)*	13,629 (2,190)	1.05 (1.00, 1.10)

*p≤0.05,**p≤0.01, ***p≤0.001, statistical significance, NOTE: Reference (Category < mean) =1 not depicted

[a] Total number of hip fractures with complete observations of cadmium, lead, aluminum and the listed water-quality factor.

[b] Measured in raw water before treatment. Not enough observations in treated water.

4.2.2 Water composition: correlation between water components and principal component analysis of variables in paper III

Table 2 shows correlations between water-quality variables included in paper III. Strong ($r \geq 0.4$) positive correlations were found between pH and calcium, calcium and magnesium, zinc and copper, and iron and aluminum. A strong negative ($r \leq -0.4$) correlation was found between pH and copper. Several moderately strong ($|r| \geq 0.30$) correlations were also found (table 2). Figure 3 shows the score for the two principal components (PC) with the highest eigenvalues (explaining the largest amount of variance in the dataset). PC1 had a high positive score of metals (cadmium, lead, aluminum, zinc and iron), but a high negative score of calcium, magnesium and pH. PC2 had a high positive score of calcium, magnesium and pH, in addition to a high positive score of ammonium, cadmium and aluminum.

An inverse (protective) association was found between PC2 and hip fracture risk, but only in women (table 3).

Table 2. Pairwise Spearman correlations between potentially bone-related water quality factors

	pH	Calcium	Magnesium	Fluoride	Phosphorus	Zinc	Iron	Manganese	Ammonium	Copper	Cadmium	Lead
pH	1											
Calcium	0.60	1										
Magnesium	0.32	0.55	1									
Fluoride	0.13	0.23	0.08	1								
Phosphorus	0.03	0.15	0.25	0.06	1							
Zinc	-0.31	-0.03	0.05	0.06	0.12	1						
Iron	-0.25	-0.22	-0.14	0.04	0.25	0.35	1					
Manganese	-0.20	0.046	0.07	0.20	0.17	0.31	0.36	1				
Ammonium	0.01	-0.23	0.10	-0.14	0.16	0.01	0.30	0.31	1			
Copper	-0.44	-0.13	-0.06	-0.03	0.03	0.42	0.23	0.11	-0.09	1		
Cadmium	-0.03	-0.07	-0.07	0.09	-0.08	0.06	0.10	0.05	0.07	0.05	1	
Lead	-0.27	-0.18	-0.14	-0.10	-0.03	0.33	0.25	0.08	0.13	0.31	0.05	1
Aluminum	-0.18	-0.17	-0.12	0.04	0.16	0.28	0.41	0.39	0.35	0.06	0.10	0.13

Figure 2. Scores of individual water variables in the two principal components (PC1 and PC2) explaining the largest amount of variance

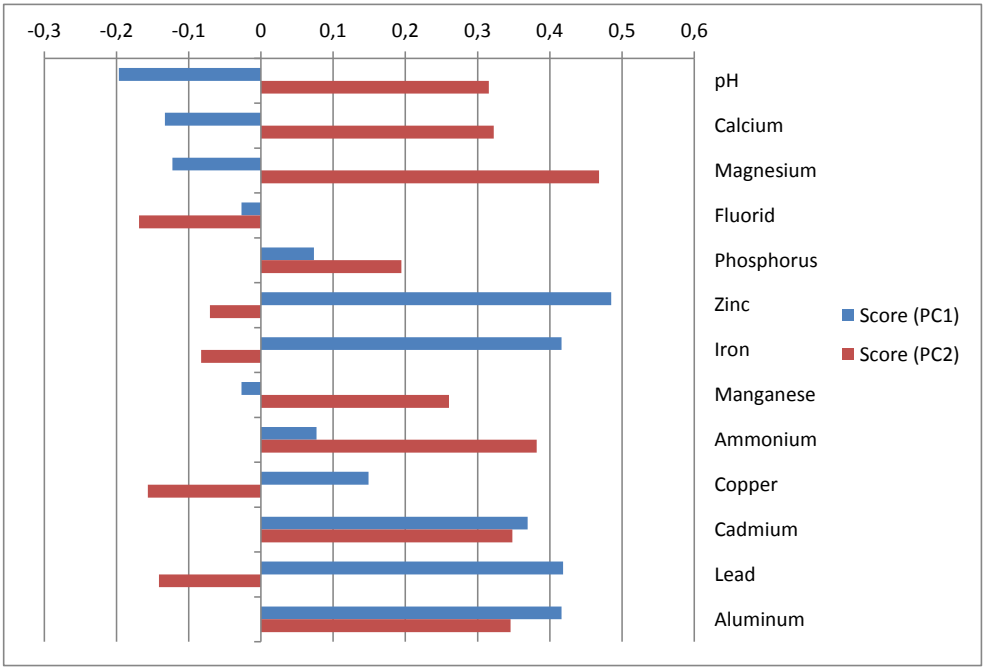


Table 3. Incidence rate ratios, 95 percent confidence intervals (IRR, 95% CI) of hip fractures according to principal components (PC) in drinking water, adjusted for age, region, urbanization degree and water source type. Ages 50-85 years. A NOREPOS study

	Men	Women
	IRR (95% CI)	**IRR (95% CI)**
PCA1	1.02 (0.96,1.08)	1.02 (0.98, 1.06)
PCA2	0.99 (0.93,1.05)	0.95 (0.92, 1.00)*

*Significant at $p<0.05$

4.2.3 Calcium/magnesium ratio

Calcium/magnesium (Ca/Mg) ratio was found to have a positive association with hip fractures in women (p_{trend}=0.01). An 8% increased risk was found in the third vs. the first tertile of the ratio (IRR=1.08, 95% CI: 1.02, 1.14). A tendency for an increased

hip fracture risk with increasing Ca/Mg ratio was also seen in men, but was not statistically significant.

4.2.4 Attributable risk, magnesium

In paper II, an IRR of 0.80 was found in men, and an IRR of 0.90 was found in women in the highest compared to the lowest tertile of magnesium. Assuming no bias present, this indicates that men drinking water with magnesium concentration in the lowest tertile (0.08-0.44 mg/l) have a 25% increased risk of hip fracture compared to those drinking water with magnesium concentration in the highest tertile (0.79-31.5 mg/l), an IRR of 1.25. The attributable risk (AR) is: (1.25-1)/1.25=0.20. The corresponding AR in women with a risk increase of 11% (IRR=1.11) is 0.10.

4.2.5 Hip fracture incidence in waterworks areas

Figure 3a and 3b show the selected waterworks areas in papers II and III (which includes 64% of the Norwegian population), and the incidence of hip fracture in these areas.

Figure 3a. Incidence of hip fractures (adjusted for age) in **men** for waterworks sampled in papers II and III (includes 64% of the Norwegian population). Average from years 1994-2000.

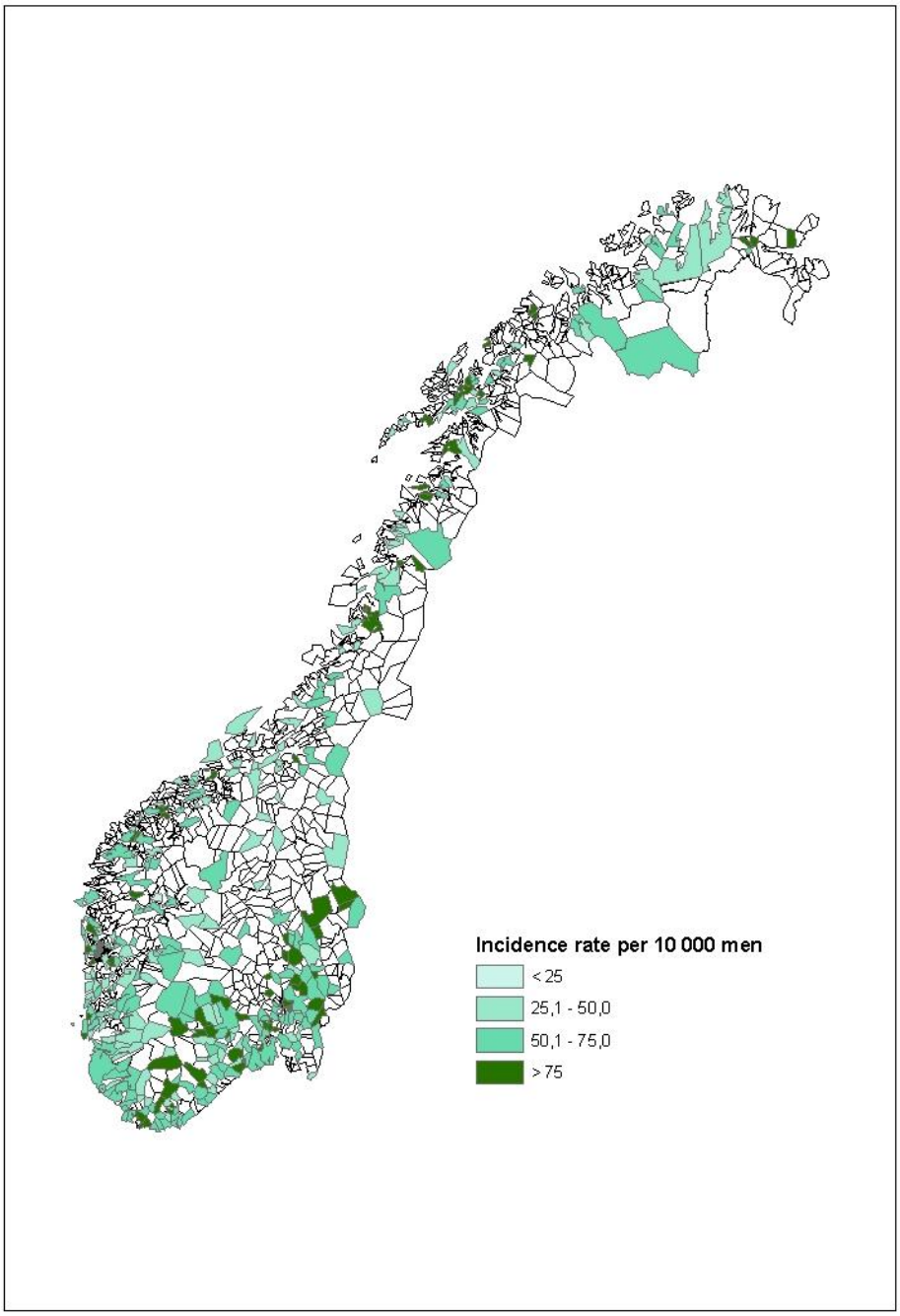
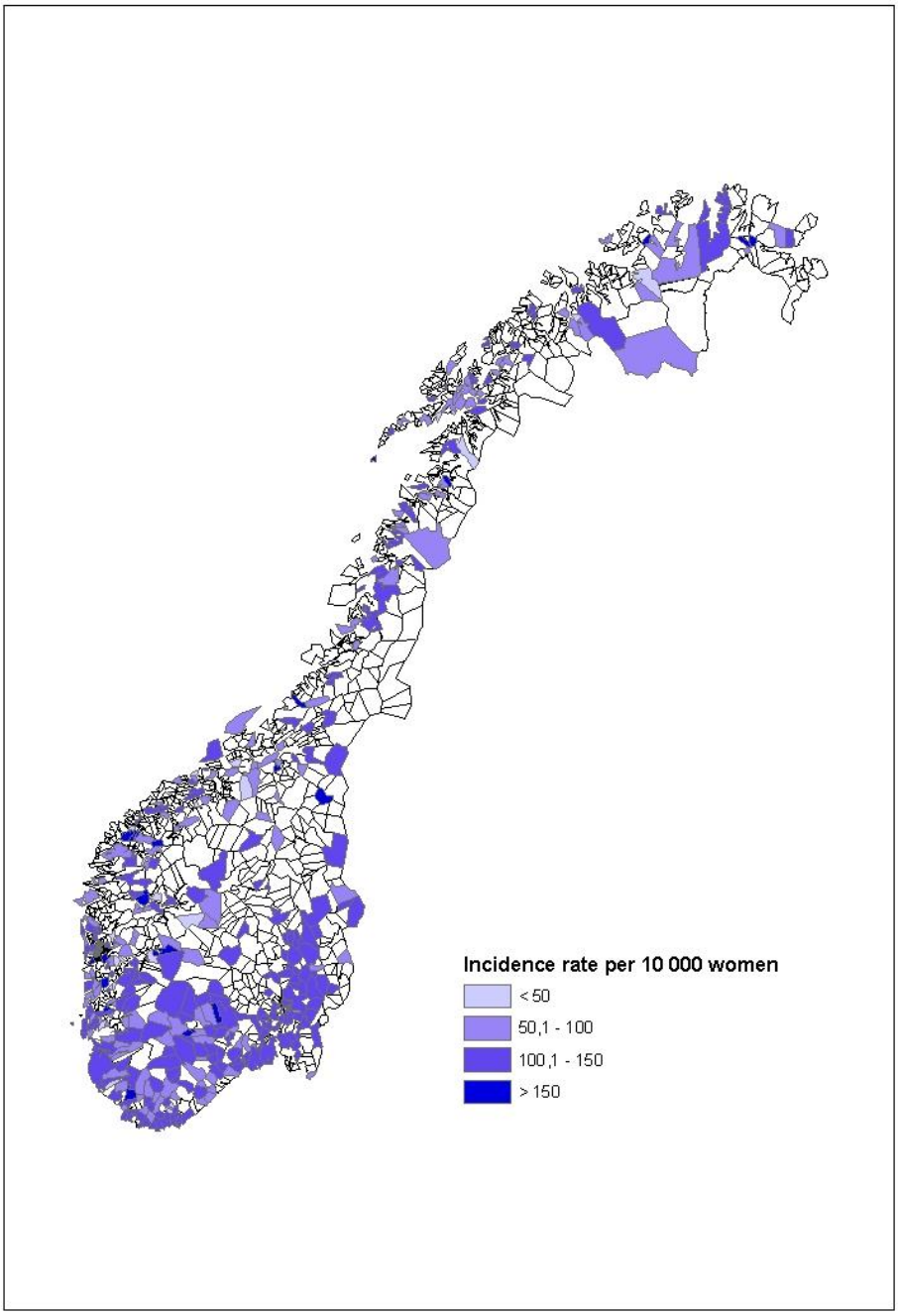


Figure 3b. Incidence of hip fractures (adjusted for age) in **women** for waterworks sampled in papers II and III (includes 64% of the Norwegian population). Average from years 1994-2000.



5 Methodological considerations

5.1 Design

5.1.1 Cross sectional study

The cross sectional design used in paper I can be used for studying prevalence of a condition or disease regardless of time. Two problems often associated with cross sectional studies are reverse causation and underrepresentation of diseases of high mortality. For many participants the forearm fracture occurred before the water-exposure was measured. Still, having experienced a forearm fracture is unlikely to cause anyone to drink water of a certain quality, eliminating the problem of reverse causation. Although acute in character, the mortality from forearm fracture is low and underrepresentation is not likely to be a problem. Thus, lifetime prevalence gives a fairly accurate picture of the true occurrence.

5.1.2 Cohort study

In papers II and III an open cohort design was used to determine the incidence of hip fracture. Hip fracture is a comparatively rare outcome; however the size of the cohort (based on the entire country) was large enough so that a sufficient amount of cases were obtained (see study size below). Loss of subjects due to competing risks (death, migration) was taken into account by calculating person-years and incidence estimates within groups of waterworks and age (one-year age groups).

5.2 Random error and precision

Random errors for some water quality variables may exist, such as when different instruments are used to measure water quality indicators. In papers II and III, all water-samples were sent to the same laboratory at the NIPH for analysis with standardized methods, giving more precise exposure-values. Size may influence precision in a study. In larger studies the variance for the effect measure is reduced and gives more precise estimates with narrow confidence intervals. In paper I the analyses were done among 127,272 participants with information on forearm fracture, pH and background

variables (see appendix II), but was somewhat reduced when including other water variables (smallest sample size: 70,341). In papers II and III the number of hip fractures (N) and personyears (py) are given in the tables. The smallest N for hip fractures was 796 (men, cadmium >0.10 µg/l), indicating good precision in all studies.

5.3 Systematic error and internal validity

5.3.1 Selection bias

If selection of participants is an effect of both exposure and outcome, selection bias occurs and could result in a distorted association between exposure and outcome.

Response rate. Overall, the response the CONOR health surveys was approximately 56%. Usually, only the healthiest participate in health surveys, which could have led to an underestimation of the prevalence of forearm fracture in the CONOR areas. However, in the Oslo Health survey (part of CONOR) non-response has been discussed, and the authors concluded that "self-selection according to sociodemographic variables had little impact on prevalence estimates" [151].

Geocoding. Some participants could not be geocoded because they did not have a numerical address registered in Statistics Norway. In CONOR this was approximately 13% (see appendix II).Addresses were predominantly lacking in less-populated areas, and it is not possible to find out if the effect measures are over-or underestimated due to this.

Missing observations. Not all waterworks are required to report on all quality-measures every year, consequently there were several missing observations for some of the variables. For example, in VREG calcium was only reported by the largest waterworks, possibly leading to an attenuation of its effect on forearm fracture. On the other hand, all included waterworks in the trace metal survey were analyzed for calcium, but still no consistent effect of calcium on hip fracture was found. We did not perform multiple imputation analyses in this thesis.

5.3.2 Information bias (misclassification)

Information bias can be present if the information from included subjects is incorrectly measured. For discrete variables, this is also called misclassification. Differential misclassification may occur when error in the exposure depends on the outcome (or vice versa), whereas nondifferential misclassification implies independence in errors. Bias caused by differential misclassification can either exaggerate or underestimate an effect, whereas bias caused by nondifferential misclassification usually distorts the effect towards the null (attenuation) [152].

5.3.2.1 Possible differential misclassification

Actual water intake. In the current thesis, the exposure status was determined by geographic location, and not by actual individual intakes. The actual water-intake varies between individuals, and some get most of their hydration from alternative sources, such as bottled water, soft-drinks, or milk. According to a dietary survey of 2,672 Norwegian men and women ages 16-79 in 1997(Norkost 1997 [153]), men and women drank on average 378 gram of water per day (approximately 0.4 liters), and 22 percent of this was bottled mineral water. In the same survey an average of 301 grams of soft-drinks and 401 grams of milk were also consumed daily. Poor quality drinking water may cause recipients to seeking alternative sources for hydration. In paper I, a strong effect on forearm fracture was found with color grade 15-20 mg/l Pt, whereas >20 mg/l Pt showed no significant association with fracture. The lack of association in the highest category may be due the yellow or brown appearance of water with a color-grade around 25 mg/l Pt, leading recipients to seek other drinking-sources. If the alternative source (e.g. soft drinks or milk) had a strong association with fracture, it may have led to differential misclassification, either distorting the effect toward the null (if the alternative source was protective), or further away from the null (if the alternative source was harmful). On the other hand, drinking is not the only means of exposure to tap water [143]; it is used in preparation of foods, and in tea and coffee (intake of 204 grams and 449 grams per day, respectively, in Norkost 1997). Therefore, it is likely to assume that everyone is exposed to tap water to a certain degree, alleviating some of the potential errors caused by differential misclassification.

5.3.2.2 Disease misclassification (nondifferential)

Self-report. In paper I forearm fractures were ascertained from participants' recall. About 10,000 participants did not report on whether they had experienced a fracture in their lifetime. Some of the self-reported forearm fractures in the CONOR-database have been compared to a radiographic archive, and 90% were found to accurately state that they had experienced a fracture [154]. It is unlikely that the recall of fracture is related to the exposure status (drinking water quality), however this slight underreporting may have led to an attenuation of effects in paper I. On the other hand, recall may depend on age, and including age at survey time in our statistical analyses could have lessened some of this potential bias.

5.3.2.3 Exposure misclassification (nondifferential)

Waterworks inclusion. For ethical reasons we did not include waterworks supplying less than 100 people and VREG also does not include waterworks supplying less than 50 persons. The persons belonging to these waterworks were attributed exposure status from their neighboring waterworks. The fraction of waterworks serving <100 persons accounted for less than 0.5% (in 2009) [133], which means that this error probably did not have large effects on our results.

Time. We were not able to examine whether changes in water composition over time may have influenced our results. In papers II and III, waterworks information was measured only in a short time window before the follow-up period started. With a long follow-up period, the water quality within waterworks may have changed over time, which could have led to misclassification of exposure, i.e. distorting the effects toward the null when the follow-up period was long. For this reason, the follow-up period was limited to year 2001.

Migration. Moving between waterworks could potentially misclassify exposure, especially in paper I where participants were tied to only one waterworks (in which they were located at the time of the CONOR-survey). In papers II and III, moving was accounted for by including the participants in the new waterworks the following year after moving. The occurrence of hip fracture may still have depended on exposure

from previous waterworks, but unless migration was large and systematic, this would not have posed a big problem. According to Statistics Norway, only around 4% of Norwegians moved in 2005, and around half of these relocations were within counties [155]. The largest migration occurred within the biggest municipalities (e.g. Oslo and Tromsø). Since neighboring water districts generally supply water of the same quality (due to similarities in the geology), the change in exposure is expected to be very small. In papers II and III, people over the age of 85 were excluded because these elderly frequently move to nursing homes.

5.3.3 Confounding, effect modification, interaction and internal validity

Confounding. To be considered a confounder, an extraneous factor needs to both be associated with both drinking water quality, and the occurrence of fracture (Fig.4a). If confounding is not controlled for in statistical analyses, it could pose a threat to the internal validity of a study. We found that urbanization degree was related to drinking water quality (i.e. pH), and it was associated with fractures of both the forearm and the hip. Urbanization degree was adjusted for in all analyses in the current thesis. pH may also have been an important confounder, as it was found to have a substantial effect on the association between calcium in water and hip fracture risk in men. Even when including important covariates, residual confounding could occur if confounders are misclassified. In paper I urbanization degree was based on the number of inhabitants in the municipalities (supplied by Statistic Norway), whereas in papers II and III urbanization degree was measured on a set scale based on the distance between dwellings (according to figures from Statistics Norway [149]). Improvement in measurement could have reduced confounding by urbanization degree in the later papers.

Effect modification. With effect modification, the exposure (drinking water quality) is randomly assigned and independent of the effect modifier (hence, no arrow between the effect modifier and drinking water quality in Fig. 4b). Failing to include an effect modifier in statistical analyses is not a threat to the internal validity of a study; however, crucial variations in effects may be overlooked. An important effect

modifier in the current thesis was gender. All analyses were stratified on gender, and effects of drinking water quality were often found to differ by gender.

Interaction. Hernan and Robins [150] consider interaction an association due to common causes ("conditioning on common effects), similar to selection bias. When conditioning on a common effect (i.e. placing a box around the interacting variable in Fig 4c), it opens the path through the interaction term. Crucial interactions between water components and dietary factors (e.g. calcium intake) in paper II, and between toxic metals (especially cadmium) and smoking or BMI in in paper III could not be included in our current studies (because of lack of information on these variables). Results in future studies may therefore differ within strata of these variables. However, not including these possible interactions does not discredit the internal validity of the obtained results in the current thesis.

Figure 4a-c) depicts the causal consequence of confounding, effect modification and interaction on the association between exposure (drinking water quality) and outcome (fracture).[2]

Fig. 4a)

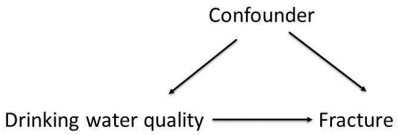


Fig. 4b)

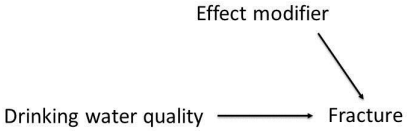
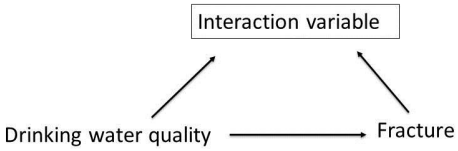


Fig. 4c)



[2] NOTE: Variables may not fit exclusively into one of the above categories, e.g. urbanization degree is a confounder of the association between drinking water and fracture, but it may also be considered an effect modifier.

5.4 Generalizability

This thesis utilizes nationwide data on forearm fracture, hip fracture and drinking water. Still, some areas (i.e. the south and southwest) were not included in CONOR (paper I), and 36% of the Norwegian population was not included in the trace metal survey (papers II and III). Whether the results from this study are generalizable to the entire country (and to other countries) depends on the distribution of confounders and effect modifiers in the population. However, Rothman and Greenland argue that statistical generalization is not equivalent to scientific generalization, and if internally valid comparisons between subject groups are made, the results should be universal [152].

6 Discussion of the results

6.1 Discussion of main results in relations to current knowledge

The current thesis provides some of the first insight into the field of drinking water quality and bone health in populations. A lower risk of hip fracture was found in men and women with a higher drinking water concentration of magnesium, and a higher risk from relatively high cadmium was found in men. When considering effect modification by age, a possibly harmful effect from lead was seen in the oldest age group (66-85 years) of both men and women. Acidic water (low pH) was seen to increase the risk of forearm fracture; however most of this effect may have been due to a higher microbial content when the water was slightly acidic.

6.1.1 Magnesium

We found a consistent inverse relation between magnesium in drinking water and hip fracture risk in both men and women. To our knowledge, only one previous study [156] dealing primarily with the relation between magnesium in water and bone health has been performed. No effects on biomarkers of bone metabolism from spring water supplemented with magnesium was observed, however the study was small (67 postmenopausal women) with a short follow-up period (12 weeks). In reviews

considering magnesium from other sources than drinking water, magnesium has been reported to have protective effects on both bone formation, bone strength and bone mineral density [72, 157]. When stratifying on calcium concentration level in paper II, the effect of magnesium in the low-calcium group was attenuated, indicating that a certain amount of calcium may be needed for magnesium to be protective towards fracture.

6.1.2 Calcium

No consistent effect of calcium was seen in our studies. Protective effects on bone health in postmenopausal women have been found in both experimental trials [158-160] and in observational studies [161, 162] of calcium in drinking water, and the bioavailability of calcium from water compared to dairy products has been reported to be high [163]. However, these studies (most of them performed in France) may not be generalizable to the Norwegian population, due to different dietary intakes of calcium. In Norway the intake of calcium from food sources in postmenopausal women has been found to be higher than in France, approximately 800 mg on average per day in Norway [153] vs. 700 mg in France [161]. The populations may also differ on other factors, such as vitamin D intake. A larger effect of calcium from drinking water may have been seen if Norwegians had a lower habitual calcium intake, as suggested for dietary calcium by Meyer HE (2004) [52]. In paper II a higher risk of hip fracture in the third tertile of calcium (when adjusting for magnesium) was found in women, and hip fracture risk in women was also seen to be higher with a larger calcium/magnesium ratio (additional analyses). This may signify that, although a certain amount of calcium may be essential, too much calcium compared to magnesium may be unfavourable towards bone [72, 143].

6.1.3 Cadmium

Cadmium in drinking water (from unpolluted areas) contributes little to the total cadmium intake [164]. Still, an increased risk of hip fracture was seen in the current thesis, primarily in men. Cadmium at general population-level intakes (no excess pollution) from the diet has been positively linked to hip fractures in men [93], and

lower BMD values have been found in men with occupational exposure to cadmium [165].

6.1.4 Acidity

In the current thesis we found an increased risk of forearm fracture in those drinking acidic water. Acidity from inorganic acids in the water has been proposed to affect bone health by disturbing the mineral homeostasis in the body [166, 167]. The current study on pH in Norwegian municipal water is, to our knowledge, the first population-based investigation of drinking water-acidity levels and bone health. In a small study of 39 women, ingesting 1 liter of bicarbonate-rich water per day (as opposed to water rich in sulfide and thus acidic) gave a beneficial effect on bone metabolism [168]. Another small study including 30 women comparing alkaline mineral water to acidic mineral water found a lower PTH level and reduced bone resorption in those drinking alkaline water [169]. Although we observed associations between pH and fracture, our results suggested that factors associated with the pH level, such as the microbial content of the water, could be of more importance than the acidity per se.

6.1.5 Bacterial indicators and color degree

We found strong positive associations between the bacterial indicators intestinal enterococci, *Clostridium perfringens* and fractures of the forearm. The indicator bacteria in themselves may not be harmful, but they could point to the presence of bacteria or other organisms that may trigger chronic inflammation in the gut. A high color degree, often signifying a high iron-concentration and high organic content in the water, was also of importance. High iron concentration in Norwegian drinking water has previously been linked to increased incidence of inflammatory bowel disease [108]. A high amount of organic material could reduce the effect of the disinfection process, increasing the amount of microorganisms in the water [124]. Other consequences of a high organic content are possibly harmful bi-products from the disinfection (i.e. with chloride) and increased corrosion of the water distribution network [124]. The Norwegian limit for color in drinking water is 20 mg/l Pt [131], but our results show a harmful association with forearm fracture at color grade as low

as 15-20 mg/l Pt. As previously mentioned, the highest category of color degree may be subject to misclassification (chapter 5.3.2.1). It could therefore be reasonable to assume that also water with color grade >20 mg/l Pt may be harmful to bone health.

6.1.6 Lead

No association between lead in water and hip fracture was found in the general population (50-85 years) in the current thesis, however age seems to be an important factor in lead exposure (chapter 6.2.1). Associations between lead in blood and bone health have previously been reported in several studies [96, 100, 101, 170, 171].

6.1.7 Aluminum

Many waterworks were found to exceed the permissible limit for aluminum in drinking water; however no effect on hip fracture in the general population was observed. Aluminum has previously been linked to increased risk of fracture in kidney patients [81, 104, 106], and aluminum has been shown to accumulate exponentially in bone with increasing age [172]. But, analogous with our results, no consistent evidence of an association between aluminum and hip fractures has been documented [107, 173]. We did however observe a strong interaction with lead (especially in men, IRR= 1.36 with high lead-high aluminum compared to high lead-low aluminum). Synergistic relations, where the effects are stronger together than individually, are common with toxic metals [174]. Our studies confirm that interactions are important to consider in environmental exposures and bone health.

6.1.8 Fluoride

Contrary to other drinking water studies, we found no association between hip fracture and fluoride. The current permitted level of fluoride in drinking water in Norway is 1.5 mg/l [131], but Norway does not fluoridate its water supplies, and the natural levels are typically much lower. We found an average fluoride level of only 0.14 mg/l with little variation (range: 0.1-2.60 mg/l), which may be the reason why we did not see an effect. There exists a great deal of evidence that fluoride may be beneficial for bone health [64, 80-83], but the protective range may be narrow.

6.2 Discussion of other potentially important findings

6.2.1 Gender and age

We found no effect of cadmium from drinking water in the general population of women, although cadmium uptake in postmenopausal women has been shown to be the same as in men [175-177]. Men may be more sensitive to inflammation induced by cadmium (chapter 6.3.3.2).

The harmful effects of lead on bone have previously been reported to be stronger in men due to a higher (occupational) exposure and to higher hematocrit levels [175]. We did not see evidence of this in our study, but a strong effect of high lead on hip fracture in men was found in the high-iron category. This suggests that iron concentrations in the body may be of importance for the effect of lead on bone, as men generally have higher background iron-levels than women [175].

In accordance with our results, there are reports of statistically significant age-related increases in the concentration of toxic metals in bones (particularly lead in the hip bone), which may be one of the explanations for the higher fracture risk in the older age groups [96, 97, 100, 178]. The reason for increased concentrations in the elderly can be higher exposure in early life (e.g. to leaded petrol), life-course accumulation in bone (due to a long half-life of lead), or to a disrupted calcium metabolism in the elderly that could increase the uptake of lead [97]. Increased bone loss with age may also increase the endogenous (within the body) lead exposure [99, 171, 175], causing a vicious cycle.

6.2.2 Geographic variation

The variations we found in forearm and hip fractures by level of urbanization could not be explained by drinking water components studied in this thesis. Urbanization degree was the strongest background factor in all analyses. Geographic region was only associated with hip fracture risk in women. When including the water variables pH, magnesium or lead, only region west had a lower risk compared to region east, whereas the other regions showed no difference.

6.2.3 Collective effects of drinking water parameters

Based on principal component analyses, a protective effect was seen with a water type high in calcium, magnesium, pH, ammonium, cadmium and aluminum. Grouping water-factors into components or "types" could mask singular effects on bone, i.e. cadmium (harmful association) was included in a protective water type. This shows that water-effects may vary greatly depending on the context; effects may act antagonistically and even cancel each other out. Important singular factors in drinking water may therefore be overlooked when it comes to their effect on health. In the current thesis principal component analyses appeared to be less valuable than ordinary regression analyses (including single factors) in explaining hip fracture risk.

6.3 Suggested biological mechanisms

Very few studies have been performed on the association between drinking water quality and bone health, therefore the current studies need to be considered exploratory. Although not completely understood, mechanisms of action for the studied factors have been suggested in the literature, and we summarize some of them below. It is important to note that all of the suggested mechanisms are connected and may together exert effects on bone.

6.3.1 Uptake and absorption

Uptake of components from drinking water may depend on water chemistry, e.g. lead may be taken up more easily from soft water than from hard water [58, 179, 180]. Skeletal integrity has been closely linked to proper absorption of calcium, vitamins and trace elements from the diet [181]. Vitamin D is important for the absorption of calcium. Inhibition of vitamin D may cause calcium release from bone due to lower intestinal absorption [181]. Exposure to lead and cadmium has been found to counteract the absorption of protective factors such as calcium and vitamin D, and others from the diet [98, 170]. High concentrations of aluminum may inhibit the uptake and absorption of phosphate needed for normal bone mineralization [182, 183]. Absorption from drinking water may be higher than from food [156, 163], and sometimes increased when taken in with food, as in the case of magnesium [184].

6.3.2 Direct biological effect on bone

6.3.2.1 Bone matrix and mineral

The majority of bone is made of the bone matrix, primarily carbonated hydroxyapatite mineral ($Ca_{10}(PO_4)_6(OH)_2$), and collagen with growth factors. Calcium in the matrix provides bone with its stiffness and much of its strength, but it may also act as a rapid supply when the calcium concentration in blood drops. Additionally, calcium could be released from bone during chronic acidosis, because calcium carbonate leaves bone to act as a buffer in acidified blood [167, 185, 186]. It has been theorized that a lower pH in blood could occur with a prolonged intake of inorganic acids, such as with polluted water [167]. A high cadmium intake may contribute to acidosis and the release of calcium [187], whereas sufficient magnesium may mitigate it [72]. As bone ages, the hydroxyapatite crystals become larger, causing reduced bone strength. Magnesium (smaller than calcium) can replace calcium in bone, thereby controlling the size of the hydroxyapatite crystals and improving the quality of bone [72, 185]. Cadmium and lead compete with calcium, and lead has its primary site of accumulation in cancellous bone [97]. With age-related dissolution of bone mineral, lead may enter the blood and exert its influences in the body (including bone) through the calcium metabolism [188]. Bone types may also differ with regard to their sensitivity, e.g. aluminum is primarily deposited in trabecular bone (which is more metabolically active), possibly resulting in less mineralization (osteomalacia) [183].

6.3.2.2 Bone cells

Bone matrix development is governed by four different types of cells: Osteoblasts, osteoclasts, osteocytes and lining cells [187, 188]. Matrix formation and mineralization is the primary responsibility of osteoblasts, whereas osteoclasts are responsible for bone resorption (breakdown). Osteocytes coordinate the function of osteoblasts and osteoclasts [189]. Low pH in blood and in extracellular media has been found to increase the activity of osteoclasts, causing a higher rate of bone resorption [186, 190]. Deficiency of magnesium has been shown to reduce the number of osteoblasts and also increase osteoclast formation [72, 157, 191]. Osteoblasts and their

collagen synthesis have also been reported to be affected by heavy metals such as cadmium [192], and cadmium could make osteoblasts secrete a substance that stimulates the formation of osteoclasts [193]. Similar to cadmium, exposure to lead has been found to impair collagen synthesis [188], and to affect osteoblasts and osteocytes through the Wnt/β-catenin pathway, reducing bone formation [194].

6.3.3 Indirect biological effect on bone

6.3.3.1 Vitamin D and parathyroid hormone (PTH)

Vitamin D and PTH are important regulators of the calcium concentration in blood. Magnesium deficiency has been found to impair the secretion and the activity of PTH, thereby lowering the vitamin D-levels and calcium absorption, reducing bone formation [64, 72, 157]. Lead levels in bone have been shown to vary by vitamin D-receptor type [195]. Lead has been reported to interact with calcium and PTH, and impair the function of vitamin D [98, 174, 177, 195, 196]. Aluminum may reduce the osteoblastic response to PTH, leading to lower bone formation [102, 183].

6.3.3.2 Inflammation and oxidative stress

Chronic inflammation, e.g. inflammatory bowel disease (IBD), is a strong trigger of bone loss through the production of pro-inflammatory cytokines [125, 197, 198].Cytokines up regulate the formation of osteoclasts through the RANK-RANKL/osteoprotegerin (OPG) system, thereby increasing bone resorption [197, 198]. RANKL has been found to be expressed in osteoblasts, but recently especially in osteocytes [198, 199]. Oxidative stress has also been reported to increase bone resorption through the RANK-RANKL/OPG pathway [200].

Intestinal enterococci and *C. perfringens* may not be harmful, but they may signify a presence of pathogenic virus (e.g. Norovirus, Adenovirus), siderophoric (iron-loving) bacteria (*Yersinia enterocolitica* and others), in addition to spore-forming bacteria (e.g. *Clostridium difficile*) and parasites such as *Giardia lamblia* and C*ryptosporidium*, which may trigger or exacerbate chronic inflammation in the gut [126].

Magnesium-deficiency promotes low-grade inflammation, releasing pro-inflammatory cytokines[72, 157, 201-204]. Magnesium also has anti-oxidant defences and may reduce free radicals (oxidative stress) in the body [72]. The anti-inflammatory effect of magnesium may be the reason why we found a protective effect of magnesium, but not calcium on hip fractures in the current thesis.

Cadmium exposure increases C-reactive protein (CRP) and may have an effect on several inflammatory mediators [205]. Although cadmium cannot directly produce free radicals [206], it still induces oxidative stress, which may be mitigated by the antioxidant properties of zinc [207]. Contrary to this, we observed a greater harmful effect of cadmium with high zinc in water (paper III), and the reason for this is unclear. The pro-inflammatory properties of cadmium could account for the gender differences we found in paper III, as cadmium administration in male rats has been shown to induce higher levels of cytokines (especially IL-6) in males than in females [208]. Lead exposure, even at low levels, has also been shown to produce immune-changes and alter cytokine-expression [209], and it promotes oxidative stress through several pathways [210]. The effects of aluminum on the immune system are not clear [211], but aluminum may induce oxidative stress [105].

6.3.3.3 Other indirect effects

Aging has been shown to reduce skeletal blood flow in rats, which may increase bone loss and the risk of fracture [212]. Adequate magnesium levels could possibly counteract this effect [72, 213].

Cadmium causes renal damage, leading to a lower activation of vitamin D (less calcium absorption), proteinuria, and higher excretion of calcium [174, 187, 214, 215]. Bone-effects through renal damage happen in addition to, and after, the direct effects on bone discussed above. Metallothionein (MT) is a group of metal-binding proteins (enzymes) that normally bind to zinc and copper, but also to cadmium, and is induced during cadmium exposure [174, 216]. Inflammation (especially the cytokine IL-6) and oxidative stress can induce MT-expression [174, 216]. Cadmium bound to intracellular MT protects against cadmium toxicity, but extracellular cadmium-MT transported

from the liver has been shown to be toxic in the kidneys [174]. Other toxic metals, such as lead also induces MT expression [209].

Lead blocks calcium entry into nerve terminals [188], thereby compromises neuromuscular, neurological, coordination and sensory characteristics, predisposing elderly to falls [100]. Lead also inhibits the important heme-synthesis enzyme Erythrocyte ALA-Dehydratase (ALAD) [196]. Caucasians more frequently than other ethnicities have an ALAD allele type that leads to higher blood levels of lead, and could therefore have an increased risk from lead exposure [196]. There is also an overrepresentation of hemochromatosis (a disease of iron overload) in Scandinavian populations [111, 113]. Individuals with hemochromatosis have been found to have higher blood levels of lead compared to healthy individuals [196].

6.4 Total intake of calcium and magnesium from drinking water

In our studies an average contribution of calcium from drinking water to total calcium intake was found to be between 0.5-0.6 %, and the average contribution from magnesium was 0.3% (paper II). This small contribution was primarily due to the low concentration of minerals in Norwegian water, which were 5.39 mg/l calcium and 1.03 mg/l magnesium. In a study by Wynn et al [217], 150 (bottled) mineral waters were sampled randomly from countries in Europe (not including Norway) giving an average of 179 mg/l for calcium, and 39 mg/l for magnesium. Using these concentrations instead of the actual study-concentrations would give a potential contribution of 14% (men) and 20% (women) for drinking water-calcium, and 8% (men) and 12% (women) for drinking water magnesium. This extensive divergence in contribution shows that there may be a large unexploited opportunity for improving bone health in the Norwegian population by increasing the mineral concentration in drinking water.

6.5 Possible public health implications

In 1997, Norwegian men and women reported to drink approximately 0.4 liters of (municipal and bottled) water per day [153], but in 2010 this had increased to approximately 1.1 liters [47]. Drinking water is now a more important source for

hydration than milk and soft drinks. This could indicate that the quality of drinking water has become better, or that Norwegians' health awareness has increased. The influence of any constituent in water to increasing or decreasing the incidence of osteoporotic fracture was found to be modest compared to established risk factors. The benefit can nevertheless be substantial, because of the large populations affected over long periods of time. If assumed causal, the current thesis shows that by increasing the hardness of Norwegian drinking water (raising pH and increasing the mineral content), 1 in 5 cases of hip fracture could be avoided purely due to the higher magnesium concentration. Based on the current thesis, we recommend some simple measures to improve the quality of the water being ingested:

a) Central regulatory changes (Drinking Water Regulation):

i) Include regulations on calcium and magnesium to promote higher levels of these essential minerals.

ii) Raise the lower limit for pH to 7.5 (currently recommended by the NIPH).

iii) Lower the highest permissible limit for color grade to 15(currently 20 mg/l Pt)

iv) Possibly introduce indicators that better reflect the presence of parasites and other pathogens (e.g. mold). This topic is currently being debated.

b) Measures to be taken by the waterworks:

i) Raise pH by adding dolomite flour that contains calcium and magnesium, instead of using calcium carbonate that does not contain magnesium.

ii) Continue to introduce UV-irradiation for disinfection.

c) Measures to be taken by the public:

i) Use only cold water for drinking and cooking to reduce heavy metals

ii) Before consumption, flush out water that has been sitting for several hours (overnight water could contain a higher concentration of metals)

7 Conclusions and future perspectives

Although not explaining the entire geographic variation in fracture risk, results presented in the current thesis shows that certain components in drinking water could potentially increase, or reduce, the risk of chronic diseases like osteoporosis. Of particular interest to public health is the possibility of magnesium in drinking water to protect against hip fractures. There are only few studies on the relations between drinking water and fracture, therefore the current thesis needs to be considered exploratory and our results hypotheses-generating. The credibility of our finding would be strengthened if systematic differences in bodily levels of minerals and metals (e.g. in bone, serum, urine) were shown in participants being supplied water with low or high levels. A possible collective pathway for the associations seen in this thesis is activation of the immune system leading to increased bone resorption. Verifying a higher inflammatory state in persons exposed to drinking water containing bacterial indicators, low magnesium, or higher concentrations of toxic metals would further support our findings. Future studies will therefore aim towards strengthening the biological link between water components and bone health.

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9 Appendices

Appendix I: Causal diagrams for perceived relations between variables in objectives 1-3.

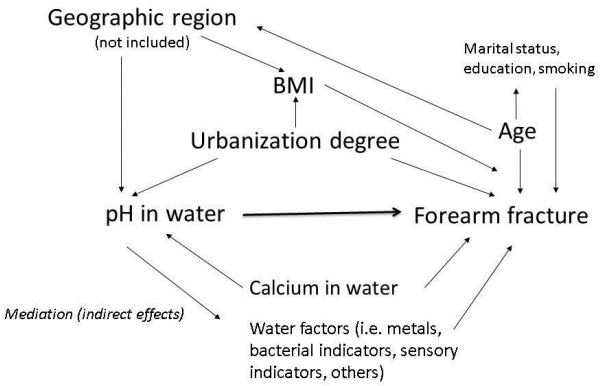
Appendix II: Depiction of the number of participants included in paper I (also submitted as online resource to paper I)

Appendix III: Amendments to the papers

Appendix I

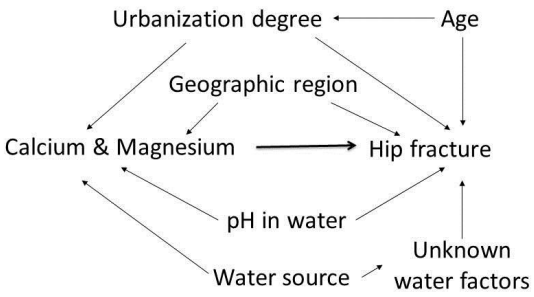
For further explanation of the arrows in the causal diagrams below, see chapter 6.1.3.3 (Confounding, effect modification, interaction and internal validity). The causal diagrams do not include gender, because all analysis in the current thesis are stratified on gender

Causal diagram for paper I- Also, see figure 1, paper I

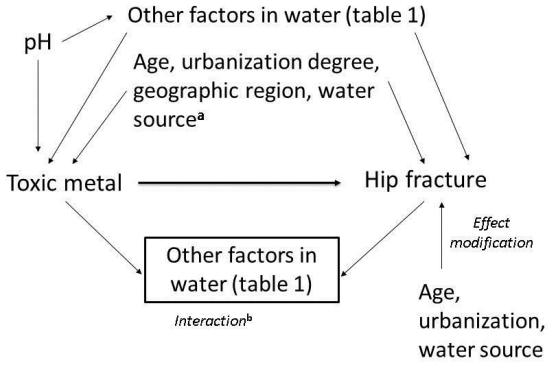


NOTE: an arrow between urbanization degree and BMI has been included (not previously included in figure I, paper I), indicating that BMI may also vary by urbanization degree.

Causal diagram for paper II



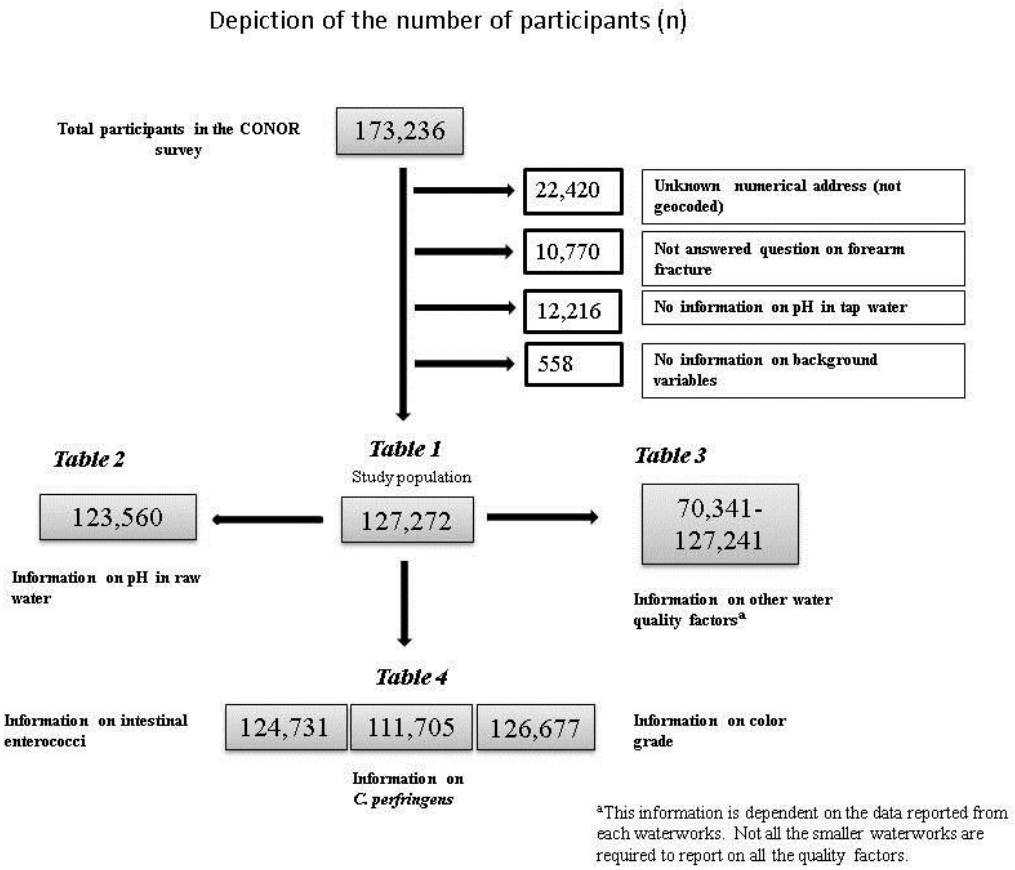
Causal diagram for paper III



[a] The arrows are further specified in the causal diagram for paper II (above)

[b]The dialogue-box has been outlined to show that conditioning on common effects opens the path for interaction between toxic metals and other water quality factors.

Appendix II


Depiction of the number of participants (n)
Total participants in the CONOR survey
173,236
22,420
Unknown numerical address (not geocoded)
10,770
Not answered question on forearm fracture
12,216
No information on pH in tap water
558
No information on background variables
Table 2
123,560
Information on pH in raw water
Table 1
Study population
127,272
Table 3
70,341-127,241
Information on other water quality factors[a]
Table 4
Information on intestinal enterococci
124,731
111,705
126,677
Information on color grade
Information on C. perfringens
[a]This information is dependent on the data reported from each waterworks. Not all the smaller waterworks are required to report on all the quality factors.

Appendix III

Amendments to paper I

Page 546 (first sentence): […] compared with men and women consuming water of pH 7.0-7.5 (Table 2).

References

Reference	Old reference	New reference
10	Norwegian Institute of Public Health (2004) [Watersupply ABC: chapter C, watersources and catchment]. Drinking water quality and hygiene [in Norwegian]. Available at http://www.fhi.no/eway/default.aspx?pid=233&trg=MainArea_5661&MainArea_5661=5631:0:15,3030:1:0:0:::0:0. Accessed 5 May 2011	Norwegian Institute of Public Health. *Vannforsyningens ABC: Kap C. Vannkilder og nedbørfelt* [Report in Norwegian] 2011; Available from: http://www.fhi.no/tema/drikkevann/vannforsyningens-abc.
11	Norwegian Institute of Public Health (2006) [Watersupply ABC: chapter E, water distribution system]. Drinking water quality and hygiene [in Norwegian]. Available at http://www.fhi.no/eway/default.aspx?pid=233&trg=MainArea_5661&MainArea_5661=5631:0:15,3030:1:0:0:::0:0. Accessed 13 May 2011	Norwegian Institute of Public Health. Vannforsyningens ABC: Kap E. Vannforsyningsnett [Report in Norwegian] 2010; Available from: http://www.fhi.no/tema/drikkevann/vannforsyningens-abc.
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24	Norwegian Institute of Public Health (2011) [Waterworks register]. Drinking water quality and hygiene [in Norwegian]. Available at http://www.fhi.no/eway/default.aspx?pid=233&trg=MainArea_5661&MainArea_5661=5631:0:15,2873:1:0:0:::0:0. Accessed 24 March 2011	Norwegian Institute of Public Health. *Vannverksregisteret [in Norwegian]*. 2011 [cited 2013 Dec 1]; Available from: http://www.fhi.no/vreg

Amendment to paper II

Abstract: 566 waterworks (not 556)