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Kruse, Christian

DOI (link to publication from Publisher):
10.5278/vbn.phd.med.00101

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

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BY
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DISSERTATION SUBMITTED 2017

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Dissertation submitted:	September 2017
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Department:	Department of Clinical Medicine

ISSN (online): 2246-1302
ISBN (online): 978-87-7210-068-5

Published by:
Aalborg University Press
Skjernvej 4A, 2nd floor
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2017

CV

Dr. Christian Kruse was born in Odense, Denmark in 1986 and graduated from Aarhus University in 2012 as a licensed physician (M.D.). Dr. Kruse completed his medical residency at Randers Regional Hospital, followed by a one-year introductory position in internal medicine & endocrinology at the Department of Endocrinology at Aalborg University Hospital. Dr. Kruse subsequently enrolled in the PhD programme at the Department of Clinical Medicine at Aalborg University in December 2014.
Dr. Kruse has authored nine scientific publications, among them six as the corresponding author. Dr. Kruse is the primary investigator of the ”BONATHIAD – Bone Association with Thiazide Diuretics” clinical trial at the Department of Endocrinology at Aalborg University. Dr. Kruse has supervised several pre-graduate projects involving predictive modelling and machine learning at the medical school of Aalborg University and has taught internal medicine at the bachelor’s level. He is a board member of the Danish Society for Bone Mineral Research and the Jutland Medical Society.

ENGLISH SUMMARY

Hyponatremia, a condition of low serum concentrations of sodium, shares an intertwined and often paradoxical relationship with thiazide diuretics and osteoporosis. In retrospective studies, thiazides have been shown to protect against osteoporosis-related fractures, but also to cause hyponatremia which is associated with a higher risk of falling. In recent years, evidence has been found of an association between hyponatremia and osteoporosis in epidemiological and basal *in vitro* studies. Further research is needed to determine three aspects; who will benefit from thiazides in terms of fracture risk, why this is the case, and who are running an unnecessary risk of thiazide-induced hyponatremia when commencing therapy, predisposing to fractures.

The aim of this thesis was to investigate the role of hyponatremia in Danish osteoporosis patients, to investigate possible age groups that may benefit from thiazide therapy on fracture risk, and to examine the effect of hyponatremia on mortality, hospitalization burden and readmission risk.

This thesis is based on six retrospective epidemiological studies using Danish and Belgian data of regional and national origins, and one systematic review and meta-analysis reviewing clinical trials of thiazide use on bone mineral density and electrolyte metabolism.

Hyponatremia was found to be associated with a higher risk of WHO-defined osteoporosis in the hip region, with a dose-response relationship between lower serum sodium and lower BMC and BMD in all regions of interest in the hip region. In fracture-prone areas of the hip, chronic mild hyponatremia ([Na^+] 130-137 mmol/L) was associated with worse progression of osteoporosis compared to normonatremia, despite a worse offset with lower BMD at baseline. In the meta-analysis, thiazide therapy was found to provide borderline significantly positive effects on BMD and a potentially beneficial pattern of altered electrolyte metabolism, i.e. lower urinary calcium, higher serum calcium and borderline significantly lower PTH. Compared to non-users, commencing thiazide therapy was associated with an increased weekly fracture risk beyond an originally higher pre-commencement risk during the first 42 weeks of therapy, but then progressively lower weekly risk from week 43 onwards. Between ages 50 and 63 years, commencing thiazide therapy was found to have 10-year risks of fractures comparable to non-users, while commencing therapy hereafter was associated with increased 10-year risks. Discontinuing therapy after age 73 was found to be beneficial to 10-year fracture risk. Lower serum sodium during hospitalizations was associated with longer LoS and greater CoS, but with comparable readmission risk. Neither serum sodium nor thiazide use was found to be a relatively strong predictor of 5-year mortality or 3-year incident immobility in older men.

Prospective studies of hyponatremia in relation to bone metabolism, i.e. by observing biochemical markers of bone metabolism, are warranted to further investigate the causal mechanisms. More clinical trials on the effects of thiazides on bone metabolism and postural balance are needed to segment users who may benefit versus those who may suffer from this therapeutic approach.

DANISH SUMMARY

Hyponatriæmi er en tilstand med lav serumkoncentration af natrium i kroppen. Tilstanden kan være indbyrdes forbundet med både vanddrivende medicin af typen thiazid, og osteoporose, på en ofte paradoksal måde. Thiazid er vist at beskytte mod frakturer i retrospektive studier, men kan samtidig forårsage hyponatriæmi, som er associeret med højere risiko for fald. I de senere år er der også fundet evidens for en association mellem hyponatriæmi og osteoporose i både epidemiologiske og cellulære studier. Yderligere forskning er nødvendig for at adskille dem, der kan drage fordel af thiazid-behandling på frakturrisiko, og dem, som løber en risiko for thiazid-relateret hyponatriæmi og de mulige konsekvenser heraf.

Formålet med denne afhandling var at undersøge hyponatriæmis rolle blandt danske osteoporosepatienter, at undersøge hvilke aldersgrupper, der muligvis kan drage fordel af thiazid-behandling på frakturrisiko, og at undersøge hyponatriæmis rolle i indlæggelses- og genindlæggelsesbyrden i Danmark.

Denne afhandling udgøres af seks retrospektive epidemiologiske studier af danske data af både regional og national karakter, tillige af belgiske regionale data, samt ét systematisk review med meta-analyse, som gennemgår kliniske studier om thiazid-medicins rolle i knogle- og elektrolytmetabolisme.

Hyponatriæmi blev fundet at være associeret med en højere risiko for WHO-defineret osteoporose i hoften, med et dosis-respons forhold mellem lavere natrium og lavere knoglemasse og –tæthed i hofteregionen. Kronisk mild hyponatriæmi ([Na+] 130-137 mmol/L) blev fundet at være associeret med værre forløb af osteoporose i frakturrelaterede regioner i hoften sammenlignet med normal natriumkoncentration, trods et værre udgangspunkt med lavere knogletæthed. I meta-analysen blev thiazidbehandling fundet at have en nær-signifikant positiv effekt på knogletæthed, og at forårsage et muligt gunstigt mønster af ændret elektrolytmetabolisme via lavere urinudskillelse af kalk, højere kalk i blodet og nærsignifikant lavere niveauer af parathyroideahormon i blodet. Påbegyndelse af thiazid-behandling var associeret med en øget ugentlig risiko for frakturer fra et i forvejen forøget risikoleje fra uge 0-42 i forhold til ikke-brugere, hvorefter der fra uge 43 observeredes en tiltagende lavere risiko for frakturer. Mellem 50- og 63-års alderen blev påbegyndelse af thiazid-diuretika fundet at have sammenlignelig 10-års risiko for frakturpådragelse sammenlignet med ikke-brugere, hvorimod påbegyndelse efter 63-års alder var associeret med en tiltagende øget risiko. At seponere behandlingen efter 73-års alderen var associeret med lavere 10-års risiko for frakturpådragelse sammenlignet med ikke-brugere. Lavere natriumkoncentrationer under indlæggelse var associeret med længere indlæggelsestider og højere samlede omkostninger, men med sammenlignelig genindlæggelsesrisiko. Hverken natriumkoncentrationer eller thiazidforbrug blev fundet at være stærke forudsigende faktorer for 5-års mortalitet eller 3-års udvikling af immobilitet hos ældre mænd.

Prospektive studier er nødvendige for yderligere at belyse forholdet mellem hyponatriæmi og osteoporose, eksempelvis ved at måle biokemiske markører for knoglemetabolisme ved intervention fra hyponatriæmi til normonatriæmi. Herudover er flere kliniske studier af thiazid-vanddrivendes indflydelse på knoglemetabolisme og balance nødvendige for at skelne mellem hvem, der kan drage fordel af behandlingen, versus hvem, der kan lide unødvendig overlast af behandlingen.

ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ALP	Alkaline Phosphatase
AQP	Aquaporins
ARB	Angiotensin II receptor
ATC	Anatomical Therapeutic Chemical Classification
AUC	Area under the curve
BIA	Bioimpedance
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CaSR	Calcium-sensing receptor
CCI	Charlson Comorbidity Index Scores
CI	Confidence Interval
CK	Christian Kruse
CoS	Costs of stay
CPR	Danish social security number
CTX	C-terminal telopeptide
CV	Coefficient of Variance
DANAK	Den Danske Akkrediteringsfond (“The Danish Certification Foundation”)
DDD	Defined daily dose (WHO-defined)
DKK	Danish kroner
DPP-4	Dipeptidyl peptidase 4
DXA	Dual-energy X-ray absorptiometry
EmBASE	Excerpta Medica Database
ENaC	Epithelial sodium channel
FGF-23	Fibroblast growth factor 23
FRAX	Fracture Risk Assessment Tool
GDS	Geriatric Depression Scale
GP	General Practitioner
HbA1c	Hemoglobin A1c
HR	Hazard ratios
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Edition
ICP	Intracranial pressure
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IPSS-35:	International Prostate Symptom Score
IQR	Interquartile range

ISCD	International Society of Clinical Densitometry
Kg	Kilograms
L1-L4	Lumbar Spine 1 – Lumbar Spine 4
LoS	Lengths of stay
LSC	Least significant change
Mmol	Millimole
Mosm	Milliosmoles
NCC	Sodium-chloride symporter
NCX1	Sodium-calcium exchanger
NSTEMI	Non-ST-Segment Myocardial Infarction
OR	Odds ratio
P1NP	Procollagen type I N-terminal propeptide
PHPT	Primary hyperparathyroidism
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT	Physical Therapy
PTH	Parathyroid hormone
PV	Peter Vestergaard
QC	Quality Control
QQ-plot	Quantile-Quantile plot
RANK	Receptor activator of nuclear factor kappa-B
RDRS-2	Rapid Disability Rating Scale
Runx2	Runt-related transcription factor 2
RAAS	Renin–angiotensin–aldosterone system
SD	Standard Deviation
Se-Ca	Serum Calcium
SF-36	The Short Form (36) Health Survey
SHBG	sex hormone binding globulin
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
SMD	Standardized Mean Difference
SMI	Skeletal Muscle Mass Index
SSRI	Selective serotonin reuptake inhibitors
STEMI	ST-Segment Myocardial Infarction
TARP	Tartrate-resistant acid phosphatase
TGUG	Timed Get-Up-and-Go
TRPM6	Transient receptor potential cation channel subfamily M member 6
TRPV5	Transient receptor potential cation channel subfamily V member 5

U-Ca	Urinary Calcium Excretion
WHO	World Health Organization

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ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Hans-Henrik Lervang, PhD, former Chief of Endocrinology at Aalborg University Hospital. I assume that you looked past academic achievements and saw other qualities when I was offered a temporary position at the Department of Endocrinology at Aalborg University Hospital in early 2012. From there, one thing has led to another, with this thesis as the most recent step, and I hope to continue my clinical and scientific career as a legacy to the opportunity you provided me with. I also hope to inspire others to provide their peers with opportunities, big or small, as they can easily compound throughout the years.

To my supervisor and mentor, professor Peter Vestergaard: your pool of knowledge, work ethic and patience towards your students, patients and peers is already well-known, so I will to speak to the other qualities I have witnessed throughout four years of working together with you. Thank you for embracing every idea I have put forward; some have materialized, some have not, and you have treated either outcome as experiences to learn from. Thank you for instilling values of productivity, frugality and self-improvement, and thank you for allowing me to balance work and family in my own peculiar ways.

To Dr. Pia Eiken; your ability to revise and structure scientific papers is second-to-none and it has been a profound joy to walk through each suggestion, comment and split infinitive. I have happy memories of talks with you at conferences domestic and abroad.

To my colleagues at the Department of Endocrinology; doctors, nurses, lab workers, secretaries. Thank you for your friendly banter and helping hands with issues big and small. To the Obel Family Foundation and Dept. of Clinical Medicine for their financial support of my PhD thesis.

To my immediate family; my father Jesper, mother Inger Margrethe, sister Vibeke and grandmother Grethe. My childhood and adolescence were privileged in a decade known for alienation and family breakdown. I am grateful that you introduced me to computer science very early in life and allowed me to develop this interest, but also appreciative that Wilhelm will know a way of life similar to when it was less present.

And finally, to my beloved Anne Sofie. I will never understand the scope of your strength and your ability to help others carry their burdens, even when you faced life's greatest difficulties. Watching our young Wilhelm grow (even) bigger, discovering the world and curiously learning is the greatest joy to experience.

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1. INTRODUCTION

1.1 - THIAZIDES, HYPONATREMIA AND OSTEOPOROSIS

The relationship between osteoporosis, thiazide diuretics and hyponatremia is one of intertwined effects that are both beneficial and harmful, often in paradoxical ways.

All three matters are currently affecting Danish patients to a great extent. The Danish incidence of osteoporosis is increasing despite a large estimated prevalence of 500-600,000 people[1]. According to data available to our group, thiazide diuretics are among the ten most frequently prescribed pharmaceuticals in Denmark in the full 7-digit ATC system hierarchy[2]. Hyponatremia is a common electrolyte disturbance among hospitalized older patients, affecting almost 6-10% of older, hospitalized Danish patients[3].

While thiazide diuretics are known to cause hyponatremia[4–7], known to be harmful to gait stability and postural balance[8], retrospective studies have shown a protective effect of thiazides on fracture rates in both Europe and North America[9–14]. Similarly, recent studies have documented an association between hyponatremia itself and greater risks of osteoporosis[15–20]. For several decades, randomized clinical trials have investigated the direct effect of thiazides on bone mineral density[21–25], but have not lead to a summarized conclusion about the role of thiazides in managing osteoporosis.

This thesis examines the effect of hyponatremia and thiazides on osteoporosis, fractures and similar fracture-related aspects, i.e. hospitalization burden and future mobility.

1.2 – OSTEOPOROSIS

1.2.1 - OSTEOPOROSIS AND FRACTURE RISK

Osteoporosis is defined by the WHO as "*a systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility*"[26].

In clinical practice, the disease is diagnosed either by the occurrence of a low-energy fracture (hip or lumbar spine) or by BMD lower than certain thresholds. For the latter criteria, an estimation of BMD can be performed using DXA to define BMD in the lumbar spine and hip. This estimation of BMD in the femoral neck of the hip, the total hip and total lumbar spine region is then compared to peak bone mass of individuals of the same sex[26]. This can be used to assess the number of

standard deviations below or above peak bone mass and results in the statistical T-score. The T-score and knowledge about concurrent fractures is then translated clinically to four categories (Table 1)[27].

Table 1: WHO-defined categories of BMD[28]

Normal	A value of BMD within 1 standard deviation of the young adult reference mean (T-score ≥ -1).
Low bone mass (osteopenia).	A value of BMD more than 1 standard deviation below the young adult mean, but less than 2 standard deviations below this value (T-score < -1 and > -2.5).
Osteoporosis.	A value of BMD 2.5 standard deviations or more below the young adult mean (T-score < -2.5).
Severe osteoporosis (established osteoporosis).	A value of BMD 2.5 standard deviations or more below the young adult mean in the presence of one or more fragility fractures.

In a population of western women, the prevalence of osteoporosis increases with age, as the T-score is a component of age and BMD. At ages 50-59, 14.8% are expected to have osteoporosis, while the prevalence increases to 21.6% at ages 60-69, 38.5% at 70-79 and 70.0% at ages above 80 years[26].

Osteoporosis can be defined as primary osteoporosis, i.e. age-related loss of bone mineral due to menopause in women or andropause in men, respectively, or secondary osteoporosis caused by comorbidities and/or medication that cause increased bone loss. This increased bone loss can be caused directly by recruiting and stimulating pro-resorptive osteoclasts, or by limiting anabolic osteoblast activities due to lower 25-hydroxycholecalciferol levels or osteoblast apoptosis[1].

Absolute decreases in T-score increase individual fracture rates exponentially [29], but the greatest proportion of affected individuals in the total population resides in the osteopenic group[30]. This can be explained by other risk factors for fracture, e.g. gait instability[31], peripheral neuropathy[32,33], poor postural balance[34,35] and poor eyesight[36]. Fractures due to risk-prone behavior, e.g. specific types of physical activity, are also likely causes of this discrepancy. Fracture risk can be estimated by commercially available tools such as FRAX® which estimates 10-year risk of major osteoporotic fractures[37]. Among predictors of fractures, BMD explains the main part

of individual fracture risk, but prediction is further improved by adding knowledge of clinical risk factors[38].

Osteoporosis is a widespread disease in Denmark, estimated to affect 500-600,000 individuals[1]. In one older study, the lifetime risk of hip fractures in Western women was estimated to be 16%[39]. Hip fractures are associated with great burdens on the individual patient and on society in terms of hospitalization, convalescence and lost productivity[40]. Ageing populations in Western societies place a great need for identifying patients at risk of fractures by diagnosing osteoporosis, and alleviating factors that make individuals prone to falls.

1.2.2 – BONE METABOLISM AND ELECTROLYTES

Bone tissue undergoes a continual process of resorption and anabolism to ensure an optimal balance between bone strength and elasticity. This process requires an intricate relationship between bone-resorbing cells, *osteoclasts*, and bone-forming cells, *osteoblasts*. The balance between resorption and anabolism changes throughout life due to age-related and hormonal effects[41]. BMD increases from birth until the age of 25-30 years, followed by a gradual decrease year by year with accelerated loss during the menopausal transition in women[42].

Human bone tissue is comprised of calcified tissue of organic matrix and calcium-phosphate matrix tissue, hydroxyapatite[43], that ensure both mechanical strength and elasticity to withstand the effects of gravity. Pro-resorptive factors increase the size and activity of osteoclasts which adhere to the trabecular surface and resorb bone through acidic protease release. The resulting bone resorption recruits bone-forming pre-osteoblasts to the bone tissue site. Osteoblasts form osteoid out of collagen and proteins[44], which mineralizes to calcified tissue. Osteoblasts regulate osteoclast resorption through stimulation by nuclear factors (i.e. RANK ligand[45]) and inhibition through the decoy OPG[46], which inhibits RANK ligand. This relative activity between osteoblasts and osteoclasts is highly dependent on hormonal aspects, particularly age-related aspects. As such, progressively increased anabolism continues until age 25-30 years where peak bone mass is attained[47], following by progressively increased resorption that further accelerates during the menopausal transition due to estrogen depletion[48,49].

Several specific hormonal agents are involved in bone metabolism, but especially the effects of vitamin D (25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol), PTH and FGF-23 are important for post-menopausal osteoporosis and calcium metabolism[50,51]. In the event of low or high extracellular calcium, the CaSRs of the parathyroid glands undergo conformational changes to secrete or cease secretion of PTH from the parathyroid glads[52]. In the kidneys, the CaSR exerts several effects across the nephron, i.e. sodium secretion in hypercalcemia in thick ascending limb and distal tubule. [53]. In hypocalcemia specifically, this facilitates an increase in serum calcium due to increased bone

resorption, and also decreases serum phosphate due to increased renal secretion. Further, the increase in PTH accelerates conversion of 25-hydroxycholecalciferol to active 1,25-dihydroxycholecalciferol, which induces RANK-ligand production[41] in osteoblastic cells and thereby promotes osteoclastic bone remodeling to mobilize calcium from the skeleton. 1,25-dihydroxycholecalciferol also increases gastrointestinal absorption of calcium and phosphate[54]. However, the pharmacological form of 1,25-dihydroxycholecalciferol, calcitriol, improves BMD in post-menopausal osteoporosis[55]. PTH directly and indirectly alters renal excretion of most electrolytes. For calcium and phosphate specifically, the hormone increases renal excretion[56]. FGF-23 is produced in response to increased 1,25-dihydroxycholecalciferol and hyperphosphatemia. The growth factor increases renal excretion of phosphate and inactivates 1,25-dihydroxycholecalciferol[57].

1.3 – HYPONATREMIA

Hyponatremia is defined as low serum concentrations of sodium. Depending on geography and assay specification, reference values of serum sodium range between 135-145 mmol/L or 137-147 mmol/L[58,59].

Regulation of serum sodium is dependent both on renal reabsorption of sodium regulated by RAAS[60–62] and renal water retention by vasopressin and aquaporins[63–66]. Of total body water, approximately 60% resides as intracellular (ICF) and 40% as extracellular fluid (ECF). Water flows between the ICF and ECF to maintain osmolality which is comprised of ions, e.g. sodium, potassium, calcium, magnesium and phosphate, and ineffective substances, e.g. glucose and urea. Sodium is the predominant extracellular ion alongside the anion chloride.

Table 2: Formulas for calculated plasma osmolality

P-Osmolality = 2 x [Sodium] + 2 x [Potassium] + [Glucose] + [Urea] or P-Osmolality = 2 x [Sodium] + [Glucose] + [Urea] [67,68]

1.3.1 - SERUM SODIUM IN RESPONSE TO HYPOVOLEMIA

Sodium forms a critical part of vascular volume. In the event of hypovolemia in the intravascular compartment, jugular baroreceptor and renal juxtaglomerular responses stimulate juxtaglomerular cells to increase conversion of pro-renin to renin. When released into circulation, renin converts angiotensinogen to angiotensin-I, which is then converted to angiotensin-2 by ACE[69,70]. The resulting increase in angiotensin-2 both increases arterial blood pressure through vasoconstriction and promotes production of aldosterone in the adrenal glands[71,72].

Aldosterone increases renal reabsorption of sodium through three primary mechanisms; 1) increased activity of the basolateral Na^+/K^+-ATPase of the distal tubule and collecting ducts of the nephron[73], 2) by increasing activity of the ENaC channels of the renal collecting ducts[74,75], and 3) by increasing NCC activity in the distal tubule[76]. The resulting increase in intravascular sodium content, coupled with increased water retention, increases intravascular volume and lowers baroreceptor response progressively as euvolemia is attained. The increased angiotensin activity augments the effect of AQP in the renal collecting ducts[77].

1.3.2 - SERUM SODIUM IN RESPONSE TO INCREASED PLASMA OSMOLALITY

The major component of body water regulation is the water-electrolyte balance inherent in plasma osmolality. With greater loss of free water, i.e. by perspiration, renal or gastroenterological excretion, the serum concentration of sodium increases with an even greater increase in plasma osmolality, per the formula of osmolality listed above[78–81]. At plasma osmolalities above 275-295 mosm/kg, hypothalamic osmoreceptors are activated to increase hypothalamic production of vasopressin preprohormone, followed by increased pituitary release of vasopressin[82,83]. The relation between osmolality and vasopressin production is exponential from an individual set-point of increased production. Vasopressin acts on the renal collecting ducts by increasing AQP (mainly AQP-2[84]) insertion in the apical membrane[85,86] to facilitate free-water retention from the dilute urine made available in this portion of the nephron[87]. The resulting dilution of serum by free water results in progressively lower plasma osmolality towards the normal reference, whereby the vasopressin response decreases.

1.3.3 - HYPONATREMIA AS A DISORDER OF SERUM SODIUM REGULATION

Hyponatremia has traditionally been categorized by two categories; 1) acute versus chronic hyponatremia, and 2) hypovolemic, euvolemic, and hypervolemic hyponatremia[88–90].

1.3.3.1 – ACUTE VERSUS CHRONIC HYPONATREMIA

The differentiation between *acute hyponatremia* and *chronic hyponatremia* relates to the urgency of which the condition has emerged, and has substantial effects on the bodily ability to adapt to changes in osmolality.

With *acute hyponatremia*, commonly defined as occurring within a 48-hour onset (e.g. by acute polydipsia), the rapid decrease in serum osmolality results in sudden, large shifts in free-water between the vascular and cerebral space, leading to brain edema, increased ICP and a clinical spectrum from somnolence to seizures, coma and respiratory arrest. This condition warrants a prompt elevation of serum sodium typically through hypertonic saline[91,92].

In *chronic hyponatremia*, less-rapid decreases in serum sodium allow the cerebral space to adapt to chronically lower plasma osmolality, resulting in decreased cerebral volume. This condition has been referred to as "asymptomatic hyponatremia"[93], but is increasingly recognized to affect cognitive performance[8,94], gait stability and physical performance negatively[94]. Due to the chronic adaptations of the cerebral space, the therapeutic approach to chronic hyponatremia is not one of rapid correction as in acute hyponatremia, but of slowly increasing serum sodium, i.e. by discontinuing causal pharmaceuticals, treating comorbidities or restoring sodium content through isotonic saline[95]. Suggestions of how quickly to raise serum sodium vary in European and North American treatment guidelines. Raising serum sodium too abruptly in chronic hyponatremia runs a substantial risk of osmotic demyelination syndrome with permanent neurological disability[96].

1.3.3.2 – HYPOVOLEMIC, EUVOLEMIC AND HYPERVOLEMIC HYPONATREMIA

A further traditional segmentation of hyponatremia has involved the presence of concurrent hypovolemia, euvolemia or hypervolemia. The volume state relates to the relative loss, maintenance or abundance of free-water and sodium in the extracellular space[89].

In *hypovolemic hyponatremia*, loss of free-water with greater simultaneous loss of sodium results in decreased intravascular volume and hyponatremia. This condition can be caused by gastroenterological expulsion (e.g. gastroenteritis, chronic inflammatory bowel disease, malignancy-related vomiting), skin burns, diuretics use (e.g. furosemide, thiazides and potassium-sparing diuretics) and cortico- or mineralocorticoid deficiency (e.g. Addison's disease).[88] This condition is therapeutically managed by discontinuing aggravating pharmaceutical agents, replenishing extracellular volume through saline and/or treating any underlying pathological condition[90].

In *euvolemic hyponatremia*, increased free-water in the presence of normal, physiologic sodium content in the ICF dilutes serum sodium with no effect on volume status. This can be caused by increased oral water intake (e.g. polydipsia[97,98]) or renal retention of water inappropriate to plasma osmolality. The latter condition has historically been referred to as the Schwartz-Bartter syndrome[99],

but has in recent decades been rephrased as SIADH, per the original author's reference. This condition involves plasma levels of vasopressin that are elevated inappropriately to current plasma osmolality, e.g. high vasopressin concentrations at plasma osmolalities where vasopressin production is normally suppressed to immeasurable levels (for instance, 260 mmol/L). Increased vasopressin levels at these osmolalities allow for water retention and thereby dilution of serum sodium. SIADH can be caused by pharmaceutical agents (e.g. selective serotonin reuptake inhibitors[100–102] or antiepileptics[103]) and/or pathological conditions (e.g. malignancy[104105], nausea[106], inflammation and acute infections[107]). Therapeutic management involves restrictions of oral water intake[108], discontinuation or replacement of aggravating agents (e.g. selective serotonin reuptake inhibitors to serotonin–norepinephrine reuptake inhibitor), curing causal conditions (e.g. tumor extirpation[109]) or by competitively inhibiting vasopressin actions (i.e. vasopressin antagonists; vaptans[110]).

In *hypervolemic hypernatremia*, increased sodium retention with even greater water retention results in expanded extracellular volume with concurrent hyponatremia due to dilution[111]. In conditions of reduced effective vascular volume, i.e. chronic renal injury[112], congestive heart failure and hepatic cirrhosis[113], inappropriately active baroreceptor and juxtaglomerular responses increase RAAS activity and facilitate increased renal sodium retention. The resulting increase in plasma osmolality increases hypothalamic stimulation of pituitary vasopressin release and thereby increases renal water retention, but the mechanism itself has also been shown to augment the relative effect of vasopressin of aquaporin synthesis in the renal collecting ducts. Clinical edema is commonly observed in hypervolemic hyponatremia[88]. In a similar condition of increased sodium retention by increased aldosterone production, Conn's syndrome, effective vascular volume is not reduced and atrial natriuretic peptides prevents formation of edema[114]. Therapeutic management of hypervolemic hyponatremia involves managing the underlying condition pharmaceutically (i.e. ACE inhibitors, beta-receptor antagonists, spironolactone, diuretics), by assisting devices (i.e. pacemaker therapy, dialysis), by surgery (i.e. transplantation) and/or by a symptomatic approach (i.e. water restriction, diuretics)[115].

1.4 – HYPONATREMIA AND OSTEOPOROSIS

In several population studies, a possible relationship between hyponatremia and osteoporosis has been investigated during the past decade. A large North American study revealed an association across all age-groups between lower serum sodium and higher risk of osteoporosis[116]. In Europe, a Danish study showed a significant relationship between lower serum sodium and lower total hip BMD, but no significant association to lumbar spine BMD[16]. A smaller South Korean study showed an association between osteoporosis and hyponatremia[17]. In specific patient

groups, patients with anorexia nervosa between ages 17 and 54 showed lower BMD values with decreases in serum sodium in a cross-sectional study[117,118]. Some studies have also shown a more neutral association between serum sodium and BMD[119], while there are no studies showing evidence of a protective association between hyponatremia and osteoporosis. In a systematic review and meta-analysis by Upala et al.[120], some of the mentioned papers were combined and showed a relationship between hyponatremia and higher risks of both osteoporosis and fractures.

In a rat model study of the possible cellular explanations for this relationship, Verbalis et al. compared the effect on bone metabolism of chronic hyponatremia induced by free water infusion versus normonatremia maintained by a solid diet. Both rat models received injections of 25-hydroxycholecalciferol. After three months, the hyponatremic rat model showed greater loss of cortical and trabecular bone volume and worse trabecular number, indicating that hyponatremia can induce BMD loss and structural bone deterioration[121]. From the same group, a further cellular study by Barsony et al.[20] induced different severities of hyponatremia to colonies of bone stem cells and adjusted for the resulting lower osmolality through insoluble mannitol. Murine macrophages increased in both size and resorptive capability when exposed to hyponatremia, following a dose-response association with greater resorptive ability and greater resorption of dentin with lower concentrations of sodium. Fibbi et al.[122] has examined mesenchymal stromal cells, the precursor to bone-forming osteoblasts, and found an inclination towards adipocyte differentiation instead of osteoblasts in an environment of hyponatremia. This has the potential to hamper bone formation in humans.

Separate cellular studies of vasopressin knock-out in relation to bone resorption and bone anabolism has implicated a causal relationship between increased vasopressin and changes in cellular metabolism, favoring resorption by activating osteoclasts[123,124]. Other studies, among them an in vitro study by Sun et al.[124], showed no relation between vasopressin and bone resorption, but stressed that the relationship is intricate to other pituitary hormones, i.e. oxytocin. Increased proliferation of osteoblast-like cell has been shown by Lagumdzija et al.[125]. Conversely, the condition of absent vasopressin production, diabetes insipidus, has also been associated with detrimental bone loss[126].

1.5 – HYPONATREMIA, FALLS AND FRACTURE RISK

Several retrospective population studies have shown evidence that hyponatremia affects fracture risk negatively, i.e. non-vertebral fracture risk in The Netherlands[119], overall fracture risk in Ireland[127] and Belgium[128] and both hip and overall fracture risk in North American studies[129,130]. Specifically for falls, regardless of fracture occurrence, hyponatremia was significantly associated with falls in hospitalized Japanese patients[131].

A plausible explanation for the increased fracture risk was published by Renneboog et al., who showed gait stability and postural balance to be negatively affected by hyponatremia, but reversible with normalization of serum sodium[8]. Further neurological and cognitive deterioration[94,132] symptoms and hypotension-prone reactions to hyponatremia have also been speculated to affect fall and fracture risk. In an older dose-response study by Arieff et al.[133], a correlation was found between lower serum sodium models in humans and rabbits with greater severity of certain neurological symptoms, i.e. somnolence and seizure incidence. A further symptomatology study has been performed by Chow et al.[134]. Through chart reviews, hyponatremia was shown to be commonly related to dizzy spells and to vasovagal symptoms, e.g. vomiting, which could temporary lower systolic blood pressure and cause fainting episodes.

1.6 – THIAZIDES

Thiazide diuretics (ATC codes *C03AB* and *C03AA*) form a group of pharmaceuticals used to treat hypertension and disturbances of calcium excretion. The first commercially available thiazide, chlorothiazide, was introduced in 1958 and has since been widely used to increase renal excretion of sodium not only in hypertension, but also cirrhosis, congestive heart failure and chronic renal failure with edema[135–137]. Thiazides act on the distal tubule of the nephron by inhibiting sodium reabsorption in the NCC[138–140]. This results in several antihypertensive mechanisms whose relative importance is not fully understood; decreased renal reabsorption of sodium causes increases diuresis[141–143], lower extracellular volume decreases cardiac output[144–146] and, speculatively, lowers peripheral vascular resistance over time.

In Denmark, thiazide diuretics are a widely used antihypertensive agent and is recommended by the Danish Hypertension Society as on the four primary choices for older patients with no competing comorbidity that warrant newer agents, e.g. ACE-inhibitors and ARBs[147]. In registry data of 3.1 million Danes used for the papers of this thesis, thiazide diuretics are among the top ten most widely used agents of all the 7-digit ATC agents[2]. While this widespread use indicates that thiazides are a well-tolerated agent in the general population, side-effects have been documented both as subjective symptoms and as asymptomatic alterations of electrolyte metabolism. Hyponatremia, hypercalcemia and hypomagnesemia are known to occur in 0.1-1% of users, while hypokalemia and hyperglycemia are known to occur for more than 10% of users (Appendix 8).

1.6.1 – THIAZIDES AND HYPONATREMIA

For a subset of patients, this intended renal excretion of sodium caused by thiazide diuretics can reach inappropriate proportions and stimulate other physiological mechanisms that lower plasma sodium levels disproportionally. The phenomenon of thiazide-induced hyponatremia has been known since the introduction of the pharmaceutical[148] and was feared for its possible neurological complications[134,149–151]. Thiazide-induced hyponatremia is thought to occur due to inappropriate responses to the pharmaceutical and its effect on water and osmolality physiology. Increased renal excretion of sodium lowers intravascular volume and sodium content[152–155], which inappropriately increases vasopressin production and augments the effect of ADH on AQP-receptors in the distal portions of the nephron. This results in increased subjective sensations of thirst and increased oral hypotonic water intake[148,156,157] in thiazide users, diluting plasma sodium. More than 10% of Danish thiazide users are estimated to develop hyponatremia after commencing thiazide therapy (Appendix 8). The absolute decrease in plasma sodium is however unpredictable and relates to concurrent medication use[158,159], i.e. SSRIs and other natriuretic agents. Comorbidities affecting water metabolism, i.e. SIADH-prone malignancy, chronic inflammation and edema-related conditions also figure with unknown weights, as do anthropometrics, i.e. low body weight and higher age[160–162].

1.6.2 - THIAZIDES AND OTHER ELECTROLYTE DISTURBANCES

Thiazide diuretics similarly alter renal excretion of other electrolytes, i.e. calcium, potassium and magnesium.

Increased renal reabsorption of calcium is known to occur with thiazide use relative to the increases in renal excretion of sodium. This can lead to clinical hypercalcemia[163–165]. Thiazide-induced inhibition of the NCC decreases luminal availability of sodium which facilitates increased activity in the NCX1[166,167]. This increases reabsorption of calcium from the urine side via the calcium-selective TRPV5 protein[168,169]. This effect has been documented to occur within 14 days of commencing thiazide diuretics[170]. The effect was originally considered to increase parathyroid activity in canine models[171], but has in later randomized controlled trials been shown to maintain PTH levels[172], as hypomagnesaemia can also occur with thiazide use[173]. In patients with PHPT, serum calcium levels can further increase with thiazide use, although this is related to hypochloremia-related alkalosis pseudo-increases in albumin-adjusted serum calcium[174].

Hypokalemia is known to occur in more than 10% of thiazide users, particularly if the pharmaceutical is administered without potassium chloride supplementation[175]. The proposed mechanism relates to NCC inhibition of sodium reabsorption in the distal tubule, which increases availability of sodium in the collecting ducts.

Increased availability of sodium facilitates reabsorption through the Na^+/K^+-ATPase and increases renal excretion of potassium[176–179]. Thiazide-induced hypokalemia is related to metabolic alkalosis and arrhythmias ranging from premature ventricular contractions to ventricular fibrillation[180].

Hypomagnesemia is known to occur in 0.1-1% of Danish thiazide users (Appendix 8). Similar to hypokalemia, the condition is caused by altered availability of sodium at the cellular level of the distal convoluted tubule which increases renal reabsorption of calcium. This downregulates activity of the TRPM6 transporter and facilitates magnesium reabsorption to the luminal side[181]. The phenomenon is similar to inherited diseases of hypomagnesaemia, i.e. Gittelman's disease[182–184].

1.6.3 – THIAZIDES AND BMD

The effect of thiazides on osteoporosis and disorders of calcium metabolism has been studied for several decades, both retrospectively and through prospective clinical trials.

A randomized, clinical trial spanning four years was conducted by the Reid and Bolland group[185,186] between 2000 and 2007 and documented small, positive effects on total body, mid- and distal forearm BMD. A randomized controlled trial of alendronate versus alendronate and thiazides by Arrabol-Polo et al.[187] in 2013 showed improved total hip, femoral neck and lumbar spine BMD with added thiazide use. In 2000, LaCroix et al. documented a protective effect on hip and lumbar spine BMD with thiazide use compared to placebo[23].

A large retrospective, cross-sectional study by Wasnich et al. in 1983 discovered an association between thiazide use and increased BMD in distal portions of the upper and lower extremities[188]. A neutral effect of thiazide on hip and radial BMD was documented by Ooms et al. in a retrospective cross-sectional study in 1993[189]. Cauley et al. documented a positive association between thiazide use and distal radius BMD in 1993[190]. A detrimental 5-year effect on radial bone by thiazide use was documented by Sowers et al. in a cohort study in 1993[191]. Sigurdsson reported a positive effect of thiazide use on lumbar spine BMD in women in a cross-sectional study in 2001[192].

On the cellular level of bone, thiazides have been shown to increase osteopontin and runx2 levels as an indicator of stimulated osteoblast differentiation. A possible explanation is NCC-receptors similar to the distal tubule located directly on osteoblasts[193].

1.6.4 – THIAZIDES, FALLS AND FRACTURE RISK

Several large, retrospective epidemiological studies have examined the role of thiazides and thiazide-induced hyponatremia in fracture and fall risk. Prospective research has also been performed on the effect of thiazides on gait instability and postural balance.

In cross-sectional population-based studies, thiazides have been associated with greater risks of falls in older people[194–197], while some studies have shown the association to be neutral[190,198]. A probable explanation for the increased incidence of falls is poor postural balance and gait instability associated with hyponatremia, a condition often caused by thiazide use[8]. By correcting hyponatremia, it has been shown that gait stability can be improved and thereby limit the risk of falls[8].

Conversely, when examining thiazide use and fracture risk regardless of falls, thiazides have been shown to be protective against fractures in European and North American studies. This has been documented in prospective cohort studies[199,200], retrospective cohort studies[201] and case-control studies[202,203]. A duration-response effect has been shown in some studies[204,205] and rejected in others[11]. Berry et al. documented an increased fracture risk shortly after the onset of thiazide therapy which then subsided with continued use[206]. Specific studies have shown mixed effect sizes, i.e. protection from hip and wrist fractures, but neutral risk towards other osteoporotic fractures[190]. Neutral effects have also been documented[14,207]. One specific study by Chow et al.[208] of patients with thiazide-induced hyponatremia did not show a significant association with fracture risk. A harmful effect has also been published in one case-control study[209].

1.7 – SUMMARY

Several large, population-based studies across various territories have shown a protective effect of thiazide use on fracture risk, with some studies also showing further improvement with longer duration and higher treatment doses. This can be explained pharmacologically by thiazide-induced increases in renal calcium reabsorption which provides components for mineralization and improves bone formation directly on the cellular level of osteoblasts. Several randomized controlled trials of thiazides and BMD have been performed and shown causal improvements at specific regions of interest, but no universal effect that has warranted recommendation in clinical practice.

Hyponatremia is a condition of diluted serum sodium due to several solitary or combined pharmaceuticals and diseases. Primarily in large retrospective studies, but also prospectively, this condition has in itself been associated with increased falls and fracture risk, likely due to poor gait stability, physical performance and postural

balance. On a cellular and epidemiological level, hyponatremia has been associated with greater bone resorption, lower BMD and a higher risk of osteoporosis. With and without osteoporosis, hyponatremia has been shown to increase the risk of falls and fractures due to the aforementioned symptomatology. Thiazides have been shown to causally induce hyponatremia in a subgroup of patients.

Absent from this intertwined positive and negative relationship between thiazides, hyponatremia and osteoporosis is a clear and stratified treatment strategy. No summarized conclusion has been made as to which patient groups should be prescribed the agent to improve BMD and protect from fractures, and who should refrain from commencing therapy due to risks of hyponatremia and the increased fracture risk associated herewith.

2. AIM AND HYPOTHESES

2.1 - PRIMARY HYPOTHESES

1. Hyponatremia is associated with lower BMC and BMD, and a higher risk of osteoporosis compared to normonatremia.
2. Thiazide use is protective against fracture occurrence in certain female age groups.

2.2 - SECONDARY HYPOTHESES

1. Chronic hyponatremia is associated with worse progression of osteoporosis compared to chronic normonatremia.
2. Thiazide use improves BMD compared to none-use.
3. Long-term thiazide use is necessary to obtain a protective effect against fracture occurrence.
4. Thiazide use alters urinary excretion of electrolytes in a matter that is beneficial to osteoporosis.
5. Hyponatremia is associated with greater comorbidity and economic burdens among hospitalized patients.
6. Hyponatremia is predictive of mortality.

2.3 - AIMS OF THIS THESIS

1. To examine an association between hyponatremia, bone mineral density and osteoporosis in a population-based sample.
2. To examine the effect of chronic hyponatremia on osteoporosis progression.
3. To examine the relative risk of fractures in thiazide users versus non-users based on age of commencing therapy.
4. To investigate the effect of long-term thiazide use on fracture risk.
5. To examine the combined evidence of a beneficial role of thiazides on bone mineral density from existing clinical trials.
6. To rank the relative importance of hyponatremia in mortality and incident immobility among older men.
7. To investigate the effects of hyponatremia on hospitalization length of stay and cost of stay.

3. PAPERS

This thesis is based on seven scientific papers.

Paper 1
Kruse C, Eiken P, Vestergaard P. Hyponatremia and osteoporosis: insights from the Danish National Patient Registry. Osteoporos Int. 2015;26(3):1005-1016. doi:10.1007/s00198-014-2973-1.
A retrospective cross-sectional study of serum sodium and DXA-based BMD values

Paper 2
Kruse C, Eiken P, Verbalis J, Vestergaard P. The effect of chronic mild hyponatremia on bone mineral loss evaluated by retrospective national Danish patient data. Bone. 2016;84:9-14. doi:10.1016/j.bone.2015.12.002.
A retrospective cohort study of chronic hyponatremia and progression of DXA-based BMD values

Paper 3
Kruse C, Eiken P, Vestergaard P. Continuous and long-term treatment is more important than dosage for the protective effect of thiazide use on bone metabolism and fracture risk. J Intern Med. 2016;279(1):110-122. doi:10.1111/joim.12397.
A retrospective matched cohort study of weekly fracture risk with continuous thiazide use relative to no use.

Paper 4
Kruse C, Eiken P, Vestergaard P. Optimal age of commencing and discontinuing thiazide therapy to protect against fractures. Osteoporos Int. 2016;27(5):1875-1885. doi:10.1007/s00198-015-3451-0.
A retrospective matched cohort study of 5-year fracture risk when commencing thiazide diuretics at a certain age relative to non-users.

Paper 5
Kruse C, Goemaere S, de Buyser S, Lapauw B, Eiken P, Vestergaard P. Predicting Mortality and Incident Immobility in Older Belgian Men by Characteristics Related to Sarcopenia and Frailty. Submitted 2017 July to Osteoporosis International, In Review
A prospective cohort study predicting 5-year mortality and incident immobility in older Belgian men and ranking predictor importance using machine learning algorithms.

Paper 6
Kruse C, Eiken P, Vestergaard P. Hyponatremia is Associated with Greater Hospital Length of Stay and Cost of Stay in Danish patients. Submitted 2017 July to Journal of General Internal Medicine

A retrospective cohort study of length of stay and costs of stay for hospitalized patients, relative to nadir serum sodium values.

Paper 7

Kruse C, Eiken P, Vestergaard P. Thiazide diuretics, bone mineral density and electrolytes: a systematic review and meta-analysis. Submitted 2017 July to Journal of Hypertension

A meta-analysis of randomized controlled trials examining thiazide use on BMD and electrolyte concentrations in serum and urine samples.

3.1 - PAPER 1

Osteoporos Int
DOI 10.1007/s00198-014-2973-1

ORIGINAL ARTICLE

Hyponatremia and osteoporosis: insights from the Danish National Patient Registry

C. Kruse · P. Eiken · P. Vestergaard

3.1.1 MATERIALS AND METHODS

3.1.1.1 - DESIGN

This work was a retrospective cross-sectional study examining the relationship between serum sodium and DXA-based BMD values in Danish outpatients. Data available from 2004 to 2011 were linked to national Danish patient registries to investigate a potential association and potential causes of hyponatremia to further investigate.

3.1.1.2 - REGISTRIES AND EXPOSURE VARIABLES

3.1.1.2.1 - REGIONAL DXA SCAN DATABASE

The DXA scan database consisted of data from one regional center conducting DXA scans on referred outpatients; the Department of Endocrinology at Aalborg University Hospital, Denmark. DXA scan data were available from 2004 to 2011. The database included two separate tables of hip and lumbar spine DXA scans and the underlying anthropometry data of scanned patients (i.e. height in centimeters and weight in kilograms). Scanned regions of interest at the hip were femoral neck, trochanteric, intertrochanteric, Ward's triangle and total hip. Scanned regions of interest at the lumbar spine were L1-L4 and the total lumbar spine. Collected variables were BMC, bone area and BMD for these regions. For the total hip, femoral neck and total lumbar spine, BMD was used to calculate T-score based on sex, age and ethnicity. All the DXA scan data had been collected using Hologic™ machines (Hologic 1000, Hologic 2000, Hologic Discovery). All machines underwent a QC program of phantom scans and cross-calibration to limit drift over time. LSC was below 3% for lumbar spine and total hip measurements and below 5% for femoral neck measurements.

3.1.1.2.2 - REGIONAL BIOCHEMICAL SAMPLES DATABASES

From the same center, data on all biochemical samples drawn from the aforementioned scanned patients were added to this work. The data collection

period spanned 1996 to 2012 and was collected from the Department of Biochemistry at Aalborg University Hospital. Both were certified by national agencies (i.e. DANAK, Appendix 9) with publicly available information of precision error. The data consisted of date and time of sampling, analysis label (i.e. NPU code), numerical value and reference intervals. The data were structured in a long format for relational purposes.

3.1.1.2.3 - NATIONAL DANISH PATIENT REGISTRIES

Data on prior prescription reimbursement and existing patient diagnoses were collected from the National Danish Patient Registry.
Prescription information included date of reimbursement, ATC code up to a 7-digit level, numerical and DDD doses, price of reimbursement, package size and number of packages. Patient diagnoses information consisted of date of diagnosis, ICD-10 code up to decimal sub-description and whether the diagnosis was made in an in- or outpatient setting.

3.1.1.3 - THE STUDIED POPULATION

The DXA scan tables of hip and lumbar spine data were examined separately. The scans were linked by CPR identification number to biochemical data via this identifier. Serum sodium values drawn closest to each scan date, if drawn within 14 days before or after the scan date, were selected. From the resulting serum sodium values, values at or below 145 mmol/L were included for further studied. A further categorization of "hyponatremia" as $[Na^+] < 135$ mmol/L and "normonatremia" as $[Na^+] = [135\text{-}145]$ mmol/L was done. Non-numeric and incomplete DXA scans were excluded. Subjects younger than 25 years of age, a weight below 30 kg or a height below 130 cm were excluded. BMI was calculated from these weights and heights. Cases without complete data on weight and height were excluded. Based on the scan dates, total numerical consumptions in DDD of selected pharmaceuticals within the prior 5 years were calculated. BMD values were used to establish T-scores and WHO-defined bone status, i.e. normal BMD, osteopenia or osteoporosis. Further categorizations of "Osteoporosis without hyponatremia" and "Osteoporosis with hyponatremia" were performed.

3.1.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. Between-group comparisons were performed by independent samples T-tests. Comparisons were made between "Hyponatremia" and "Normonatremia", and between "Osteoporosis with hyponatremia" versus "Osteoporosis without hyponatremia". For categorical data, odds-ratios were calculated and statistical comparisons performed between groups performed through Chi-square ($\chi 2$) analysis. Logistic regression was performed to establish dose-response risk of numerical serum sodium and risk of osteoporosis. Crude odds ratios were

established, followed by odds ratio calculations adjusted for age, gender and BMI. Multiple linear regressions were performed between serum sodium levels below 135 mmol/L and BMD, BMD and T-scores in all regions of interest, both crude slopes and slopes adjusted for age, gender and BMI.

3.1.3 - SUMMARY OF RESULTS

1,575 patients met the inclusion criteria. Of these, 104 (6.6%) had hyponatremia while 1,462 (93.4%) were normonatremic (Table 3). Patients with hyponatremia were significantly older (mean age 68.77 ± 10.07 vs 62.71±13.78 years, $p < .05$), presented with lower weight (67.15 ±16.61 vs 72.31 ±16.29 kg, $p < .05$), height (1.62 ± .09 vs 1.65 ± .09 meters, $p < .05$) and BMI (25.48 ± 5.12 vs 26.52 ± 5.21, $p < .05$), and ran higher risks of existing lumbar fractures (3.8% vs. 1.2%, $p < .05$) (Table 3). Hyponatremic patients had significantly higher use of benzodiazepines, beta-receptor antagonists and opioids and were more likely to have existing diagnoses of gynecological and urological malignancy (OR 3.40 and 4.80 respectively, $p < .05$), epilepsy (OR 4.79, $p < .05$), sero-positive rheumatoid arthritis (OR 3.25, $p < .05$) and ischemic heart disease (1.76, $p < .05$). Numerical increases in serum sodium below 145 mmol/L were associated with a lower risk of categorical osteoporosis at either the lumbar spine or hip. This association persisted after adjustment at either the hip or lumbar spine (OR .951 [.916-.986], $p < .05$) and the hip regardless of lumbar spine BMD (OR .948 [.910-.989], $p < .05$), but not in the lumbar spine alone regardless of hip BMD (OR 0.979 [.943-1.017], $p = .277$) (Table 4). A linear relation was established between serum sodium below 135 mmol/L and both BMC, BMD and T-scores in all regions of the hip, but not of the lumbar spine. This relationship persisted after adjustment (Table 5).

Table 3: Baseline characteristics and DXA scan results between hypo- and normonatremic patients

	Normonatremia; mean, SD, range	Hyponatremia; mean, SD, range	Normonatremia vs. hyponatremia p value
Number of cases	1.462	104	
Age at scan	62.71 (13.78) (69.00)	68.77 (10.07) (51.00)	.000 *
BMI	26.52 (5.21) (39.30)	25.48 (5.12) (32.15)	.049 *
Yearly salary	210,987.43 (139,692.17) (1,147,210.00)	185,785.29 (106,135.47) (527,634.00)	.157 *
Height	1.65 (.09) (.62)	1.62 (.09) (.48)	.001 *
Weight	72.31 (16.29) (114.40)	67.15 (16.61) (103.10)	.002 *
Sex, female (percentage)	1129 (77.2 %)	81 (77.9 %)	.876
Prior lumbar spine fracture	18 (1.2 %)	4 (3.8 %)	.038 *
Prior hip fracture	51 (3.5 %)	6 (5.8 %)	.235
Spine, total BMC	56.27 (14.92) (106.23) (1462)	51.72 (15.61) (97.16) (104)	.003 *
Spine, total BMD	0.93 (.18) (1.15) (1024)	0.88 (.19) (.99) (81)	.036 *
Spine, T-score	-1.24 (1.58) (10.44) (1462)	-1.59 (1.63) (8.62) (104)	.029 *
Hip, neck BMC	3.69 (.84) (5.27) (1462)	3.41 (.88) (5.16) (104)	.001 *
Hip, neck BMD	0.69 (.14) (1.08) (1462)	0.64 (.14) (.65) (104)	.000 *
Hip, total BMC	31.15 (8.45) (54.50)	28.69 (8.53) (45.14)	.004 *
Hip, total BMD	0.82 (.16) (1.16) (1462)	0.76 (.16) (.82) (104)	.000 *
Hip, T-score (total hip)	-1.39 (1.28) (9.66) (1462)	-1.91 (1.25) (6.85) (104)	.000 *

*: $p < .05$

Table 4: Total OR for osteoporosis

Condition	OR	95% CI	Significant p-value
Total OR for osteoporosis, either total hip or lumbar spine - Increasing plasma concentration Na+ (for [Na+] <145 mmol/L) adjusted odds ratio (age, gender, BMI)	.951	[.916-.986]	.007*
Total OR for osteoporosis, total hip region regardless of lumbar spine - Increasing plasma concentration Na+ (for [Na+] <145 mmol/L) adjusted odds ratio (age, gender, BMI)	.948	[.910-.989]	.013 *
Total OR for osteoporosis, lumbar spine regardless of total hip - Increasing plasma concentration Na+ (for [Na+] <145 mmol/L) adjusted odds ratio (age, gender, BMI)	.979	[.943-1.017]	.277

*: $p < .05$

Table 5: Multiple regression, the relationship between [Na+], BMC, BMD, and T-scores for [Na+] <135 mmol/L

Characteristics	Slope	Standard error	Unadjusted significant p value	Adjusted (age, gender, BMI) p value
Spine, L1 BMC	.038	.028	.168	.187
Spine, L1 BMD	.002	.001	.215	.522
Spine, L2 BMC	.053	.031	.085	.108
Spine, L2 BMD	.002	.002	.156	.448
Spine, L3 BMC	.068	.034	.044 *	.061
Spine, L3 BMD	.002	.002	.354	.744
Spine, L4 BMC	.068	.038	.075	.090
Spine, L4 BMD	.002	.002	.242	.470
Spine, total BMC	.228	.123	.063	.076
Spine, total BMD	.001	.002	.472	.717
Spine, T-score	.009	.013	.464	.998
Hip, trochanter BMC	.050	.018	.005 *	.001 *
Hip, trochanter BMD	.004	.001	.001 *	.005 *
Hip, intertrochanteric BMC	.100	.049	.041 *	.019 *
Hip, intertrochanteric BMD	.004	.002	.005 *	.037 *
Hip, neck BMC	.020	.007	.003 *	.005 *
Hip, neck BMD	.003	.001	.005 *	.041 *
Hip, Ward's BMC	.005	.002	.002 *	.020 *
Hip, Ward's BMD	.004	.001	.001 *	.015 *
Hip, total BMC	.174	.069	.012 *	.003 *
Hip, total BMD	.004	.001	.002 *	.012 *
Hip, T-score	.034	.010	.001 *	.012 *

*: $p < .05$

3.2 - PAPER 2

Bone 84 (2016) 9–14

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

The effect of chronic mild hyponatremia on bone mineral loss evaluated by retrospective national Danish patient data☆

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3.2.1 - MATERIALS AND METHODS

3.2.1.1 - DESIGN

This study was a retrospective cohort work examining the relationship between persistent hyponatremia and its temporal effects on DXA-based BMD values in Danish outpatients. Data available from 2004 to 2011 were linked to national Danish patient registries to perform the study.

3.2.1.2 - REGISTRIES AND EXPOSURE VARIABLES

3.2.1.2.1 - REGIONAL DXA SCAN AND BIOCHEMICAL SAMPLES

DATABASE

The DXA scan databases consisted of all data from two regional centers conducting DXA scans on outpatients; the Departments of Endocrinology at Aalborg and Aarhus University Hospital, both in Denmark between 2004 and 2011. The biochemical samples database was extracted from the Departments of Biochemistry at Aalborg University Hospital and Departments of Biochemistry and Microbiology at Aarhus University Hospitals, both DANAK certified institution (Appendices 9 and 11), based on the list of scanned patients.

3.2.1.2.2 - NATIONAL DANISH PATIENT REGISTRIES

Data on prior prescription reimbursement and existing patient diagnoses were collected from the National Danish Patient Registries.

3.2.1.3 - THE STUDIED POPULATION

Patients were included among all DXA scanned patients of both sexes who were older than 25 years of age, above 120 cm tall, weighted more than 30 kg and had undergone more than one lumbar spine or hip scan. The procedures were then done separately for hip and lumbar spine scan tables. The first and last hip and lumbar spine DXA scans for each patient were selected. Patients were excluded if less than 24 months had gone between the first and last scans. Patients with known diabetes mellitus were excluded based on existing diagnoses, HbA1c measurements above 48 mmol/mol or antidiabetics use. Patients without at least one sodium measurement in 40% of observed quarters between the dates were excluded. For all DXA scanned regions of interest, absolute and annualized changes in BMC and BMD were calculated. Absolute and annualized changes in height, weight and BMI were also calculated. Between the dates of the first and last scan, all blood samples of sodium were pooled, and from them, mean and 95% CI of the mean calculated. From the CIs, patients were categorized as having either "normonatremia", defined as a sodium level of [137.00;147.00] mmol/L or "mild hyponatremia" as a sodium level of [130.00;137.00] mmol/L. For all subjects, categorical presence of co-existing diagnoses between January 1st, 1996 and the scan date was established. CCI scores were calculated for each individual at the scan date. Prescription reimbursements for specific pharmaceuticals were calculated from January 1st, 1996 to the date of the first scan, and from between the first to the last scan. Changes in BMC, BMD in all regions of interest were calculated for each group both as crude changes and change adjusted for age, sex, length of observation, prior hip and lumbar spine fractures and exposure to glucocorticoid (ATC code *H02AB*), bisphosphonate (ATC code *M05BA*) and drugs used in alcohol dependence (ATC code *N07BB*) from ATC codes.

3.2.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. For categorical data, odds ratios were calculated and statistical comparisons performed between groups through Chi-square ($\chi 2$) analysis. 95% CI was calculated from a t-distribution of +/-1.96. Independent samples t-tests were performed between "normonatremia" and "mild hyponatremia". Multiple regression was used to adjust for age, sex, length of observation, medication use and existing diagnoses.

3.2.3 - SUMMARY OF RESULTS

945 patients with hip DXA scans and 1,130 patients with lumbar spine DXA scans were included in the final analysis (Tables 6 and 7). The population sample was predominantly female (hip scans; normonatremia 85.7%, mild hyponatremia 84.5%). Mean length of exposure ranged between 12.97 quarters (Table 7, mild hyponatremia, spine scans) and 14.33 quarters (Table 6, normonatemia, hip scans) on average for the two scan categories, with sodium measurements available in 77%

to 80% of quarters. In the group of hip scans, hyponatremia was present in 58 patients (6.2%), while the condition was present in 58 of the lumbar spine scans (5.1%). Patients with “mild hyponatremia” were significantly older (Table 7, hip scans; 67.36 ± 10.12 vs 62.48 ± 11.97 years), weighted less (Table 7, hip scans; 64.54 ±15.57 vs 69.01 ±15.11 kg) and had higher use of opioids, sedatives, beta-receptor antagonists, selective serotonin reuptake inhibitors and glucocorticoids. Patients with “mild hyponatremia”, with the exception of intertrochanteric BMD, presented with significantly lower BMC and BMD values at all regions of the hip compared to normonatremic patients (Table 8, total hip BMD 0.722 ± .120 vs. 0.785 ± 0.137 g/cm^2,$p < .05$). In the lumbar spine, “mild hyponatremia” was associated with lower BMD in L1 and total lumbar spine (Table 9, 0.817 ± 0.171 vs 0.846 ± 0.155 g/cm^2,$p < .05$) at baseline. At follow-up, patients with “mild hyponatremia” progressed with greater absolute loss of trochanteric BMC (-0.234 ± 0.784 vs 0.2247 ± 2.999, $p < .05$) and BMD, femoral neck BMD (-0.014 ± 0.042 vs 0.0143 ± 0.164*, $p < .05$), annualized femoral neck BMC (-2.3% ± 8.8%* vs. 2.4% ± 33.5%*, $p < .05$) and absolute total hip BMD (-0.012 ± 0.045 vs. 0.0122 ± 0.173, $p < .05$) (Table 10). In the lumbar spine, adjusted absolute losses of L1 BMC (-0.407 ± 0.337 vs. 0.389 ± 0.33, $p < .05$), L3 BMC (-0.384 ± 0.329 vs. 0.335 ± 0.323, $p < .05$) and L4 BMC (-0.488 ± 0.426 vs. 0.490 ± 0.418, $p < .05$) were greater for patients with “mild hyponatremia” (Table 11).

Table 6: Descriptive data for hip scan periods, epidemiology, medication use and CCI scores.

	Normonatremia (n=884) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
Gender (% female)	85.7%	84.5%
Age at baseline (years)	62.48 ± 11.97*	67.36 ± 10.12*
Weight at baseline (kg)	69.01 ± 15.11*	64.54 ± 15.57*
Height at baseline (cm)	164.13 ± 8.40	162.12 ± 7.27
Calculated BMI at baseline (kg/m2)	25.53 ± 4.74	24.47 ± 5.28
Observation period (months)	43.00 ± 16.25	39.32 ± 14.05
Section 2: biochemical data		
Number of sodium measurements	9.77 ± 17.68*	23.6 ± 30.50*
Quarters of observations	14.33 ± 5.42	13.11 ± 4.68
Quarters with at least one sodium measurement	9.86 ± 2.84	9.88 ± 2.84
Fraction measurements/observations quarters	.73 ± .21	.78 ± .21
Mean HBA1C IFCC (mmol/mol)	38.65 ± 3.39 (n = 343)	39.16 ± 3.58 (n = 37)
Lower 95% CI of mean sodium during observation	139.32 ± 1.35*	133.10 ± 1.54*
Upper 95% CI of mean sodium during observation	141.53 ± 1.55*	135.54 ± 1.20*

*: $p < .05$

Table 7: Descriptive data for spine scan periods, epidemiology, medication use and CCI scores.

	Normonatremia (n=884) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
Gender (% female)	84.9%	86.4%
Age at baseline (years)	62.18 ± 11.80*	67.65 ± 10.09*
Weight at baseline (kg)	68.64 ± 14.32*	64.18 ± 15.04*
Height at baseline (cm)	164.69 ± 8.31	162.63 ± 7.64
Calculated BMI at baseline (kg/m2)	25.23 ± 4.50	24.18 ± 5.04
Observation period (months)	41.42 ± 15.81	38.90 ± 13.52
Section 2: biochemical data		
Number of sodium measurements	10.74 ± 19.00*	24.06 ± 29.10*
Quarters of observations	13.81 ± 5.27	12.97 ± 4.51
Quarters with at least one sodium measurement	10.04 ± 2.66	10.12 ± 2.81
Fraction measurements/observations quarters	.77 ± .21	.80 ± .20
Mean HBA1C IFCC (mmol/mol)	38.51 ± 3.59* (n = 412)	39.08 ± 3.53 (n = 40)
Lower 95% CI of mean sodium during observation	139.45 ± 1.43*	132.97 ± 1.62*
Upper 95% CI of mean sodium during observation	141.71 ± 1.64*	135.38 ± 1.28*

*: $p < .05$

Table 8: Baseline and follow-up DXA scan measurements for hip regions

	Normonatremia (n=884) [137.00–147.00] mmol/L Mean ± SD	Normonatremia (n=884) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
	Baseline	***Follow-up***	***Baseline***	***Follow-up***
Trochanteric BMC	6.926 ± 1.853*	6.972 ± 1.886*	6.268 ± 1.326*	6.043 ± 1.270*
Trochanteric BMD	0.601 ± 0.114*	0.603 ± 0.113*	0.557 ± 0.104*	0.542 ± 0.111*
Intertrochanteric BMC	18.803 ± 4.996	18.570 ± 4.911*	17.525 ± 4.494	17.091 ± 4.318*
Intertrochanteric BMD	0.924 ± 0.168*	0.928 ± 0.171*	0.842 ± 0.144*	0.829 ± 0.154*
Femoral neck BMC	3.555 ± 0.759*	3.482 ± 0.746*	3.266 ± 0.606*	3.108 ± 0.587*
Femoral neck BMD	0.666 ± 0.121*	0.655 ± 0.119*	0.612 ± 0.107*	0.587 ± 0.109*
Ward's triangle BMC	0.559 ± 0.187*	0.552 ± 0.176*	0.487 ± 0.160*	0.478 ± 0.166*
Ward's triangle BMD	0.478 ± 0.147*	0.474 ± 0.142*	0.415 ± 0.117*	0.407 ± 0.129*
Total hip BMC	29.285 ± 7.144*	29.024 ± 7.038*	27.059 ± 6.094*	26.244 ± 5.770*
Total hip BMD	0.785 ± 0.137*	0.785 ± 0.137*	0.722 ± 0.120*	0.707 ± 0.129*
Hip region T-score	-1.643 ± 1.114*	-1.643 ± 1.111*	-2.165 ± 1.002*	-2.287 ± 1.077*
Hip region Z-score	-0.449 ± 1.069*	-0.260 ± 1.066*	-0.754 ± 1.090*	-0.692 ± 1.220*

*: $p < .05$

Table 9: Baseline and follow-up DXA scan measurements for lumbar spine regions

	Normonatremia (n=1069) [137.00–147.00] mmol/L Mean ± SD	Normonatremia (n=1069) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
	Baseline	***Follow-up***	***Baseline***	***Follow-up***
L1 BMC	9.779 ± 2.972	10.314 ± 3.184	9.113 ± 2.422	9.360 ± 2.513*
L1 BMD	0.753 ± 0.159*	0.778 ± 0.167*	0.710 ± 0.155*	0.726 ± 0.149*
L2 BMC	11.757 ± 3.294	12.220 ± 3.338	11.232 ± 3.347	11.557 ± 3.230
L2 BMD	0.830 ± 0.167	0.849 ± 0.167	0.803 ± 0.181	0.818 ± 0.174
L3 BMC	13.575 ± 3.451	14.034 ± 3.590*	12.891 ± 3.768	13.086 ± 3.941*
L3 BMD	0.872 ± 0.168	0.888 ± 0.171	0.846 ± 0.196	0.854 ± 0.201
L4 BMC	15.314 ± 3.793	15.819 ± 3.942	14.905 ± 3.930	15.068 ± 3.949
L4 BMD	0.906 ± 0.171	0.924 ± 0.173	0.892 ± 0.202	0.900 ± 0.196
Total lumbar spine BMC	50.281 ± 12.81*	52.047 ± 13.23*	47.480 ± 13.180*	48.380 ± 13.000*
Total lumbar spine BMD	0.846 ± 0.155*	0.865 ± 0.154*	0.817 ± 0.171*	0.829 ± 0.163*
Total lumbar spine T-score	-1.882 ± 1.399	-1.711 ± 1.384	-2.136 ± 1.547	-2.034 ± 1.476
Total lumbar spine Z-score	-0.422 ± 1.465	-0.054 ± 1.497*	-0.377 ± 1.557	-0.092 ± 1.560*

*: $p < .05$

Table 10: Change in hip region DXA data during follow-up period, adjusted.

	Normonatremia (n=884) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
Change in trochanteric BMC, absolute units	0.2247 ± 2.999*	-0.234 ± 0.784*
Change in trochanteric BMC, % annualized	3.0% ± 44.8%*	-3.2% ± 11.7%*
Change in trochanteric BMD, absolute units	0.0143 ± 0.164*	-0.014 ± 0.042*
Change in trochanteric BMD, % annualized	2.5% ± 29.0%*	-2.6% ± 7.6%*
Change in intertrochanteric BMC, absolute units	0.1630 ± 6.608	-0.134 ± 1.729
Change in intertrochanteric BMC, % annualized	0.6% ± 32.0%	-0.4% ± 8.6%
Change in intertrochanteric BMD, absolute units	0.0122 ± 0.219	-0.012 ± 0.057
Change in intertrochanteric BMD, % Annualized	1.2% ± 25.5%	-1.2% ± 6.7%
Change in femoral neck BMC, absolute units	0.0800 ± 1.176	-0.078 ± 0.307
Change in femoral neck BMC, % annualized	2.4% ± 33.5%*	-2.3% ± 8.8%*
Change in femoral neck BMD, absolute units	0.0131 ± 0.177*	-0.012 ± 0.046*
Change in femoral neck BMD, % annualized	2.2% ± 27.7%*	-2.1% ± 7.2%*
Change in Ward's triangle BMC, absolute units	0.0066 ± 0.330	-0.004 ± 0.086
Change in Ward's triangle BMC, % annualized	2.7% ± 101.2%	-2.3% ± 26.4%
Change in Ward's triangle BMD, absolute units	0.0075 ± 0.240	-0.005 ± 0.062
Change in Ward's triangle BMD, % annualized	3.0% ± 98.8%	-2.7% ± 25.9%
Change in total hip BMC, absolute units	0.4678 ± 8.869	-0.446 ± 2.320
Change in total hip BMC, % annualized	1.4% ± 29.4%	-1.3% ± 7.7%

Change in total hip BMD, absolute units	0.0122 ± 0.173*	-0.012 ± 0.045*
Change in total hip BMD, % annualized	1.6% ± 23.5%	-1.6% ± 6.2%

*: $p < .05$

Table 11: Change in lumbar spine region DXA data during follow-up period, adjusted.

	Normonatremia (n=1069) [137.00–147.00] mmol/L Mean ± SD	Mild hyponatremia (n= 58) [130.00–137.00] mmol/L Mean ± SD
Change in L1 BMC, absolute units	0.389 ± 0.33*	-0.407 ± 0.337*
Change in L1 BMC, % annualized	4.1% ± 3.7%	-4.2% ± 3.8%
Change in L1 BMD, absolute units	0.016 ± 0.020	-0.015 ± 0.020
Change in L1 BMD, % annualized	2.1% ± 3.0%	-2.0% ± 3.0%
Change in L2 BMC, absolute units	0.240 ± 0.293	-0.221 ± 0.299
Change in L2 BMC, % annualized	1.6% ± 2.8%	-1.5% ± 2.8%
Change in L2 BMD, absolute units	0.011 ± 0.015	-0.010 ± 0.016
Change in L2 BMD, % annualized	1.2% ± 2.0%	-1.1% ± 2.0%
Change in L3 BMC, absolute units	0.335 ± 0.323*	-0.384 ± 0.329*
Change in L3 BMC, % annualized	2.5% ± 2.5%	-2.8% ± 2.5%
Change in L3 BMD, absolute units	0.014 ± 0.016	-0.014 ± 0.016
Change in L3 BMD, % annualized	1.5% ± 2.0%	-1.6% ± 2.0%
Change in L4 BMC, absolute units	0.490 ± 0.418*	-0.488 ± 0.426*
Change in L4 BMC, % annualized	3.1% ± 2.8%	-3.0% ± 2.8%
Change in L4 BMD, absolute units	0.017 ± 0.018	-0.017 ± 0.018
Change in L4 BMD, % annualized	1.7% ± 2.1%	-1.7% ± 2.1%
Change in total lumbar spine BMC, absolute units	1.186 ± 1.114	-1.226 ± 1.136

Change in total lumbar spine BMC, % annualized	2.4% ± 2.8%	-2.4% ± 2.9%
Change in total lumbar spine BMD, absolute units	0.013 ± 0.013	-0.013 ± 0.013
Change in total lumbar spine BMD, % annualized	1.5% ± 1.6%	-1.4% ± 1.6%

*: $p < .05$

3.3 - PAPER 3

Original Article | Journal of INTERNAL MEDICINE

doi: 10.1111/joim.12397

Continuous and long-term treatment is more important than dosage for the protective effect of thiazide use on bone metabolism and fracture risk

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3.3.1 - MATERIALS AND METHODS

3.3.1.1 - DESIGN

This paper was a retrospective matched cohort study examining the weekly risk of fracture occurrence on thiazide-use versus non-use in Danish patients. The work also sought to investigate patterns of thiazide prescription in Danish patients.

3.3.1.2 - REGISTRIES AND EXPOSURE VARIABLES

3.3.1.2.1 - NATIONAL DANISH PATIENT REGISTRIES

Multiple tables from the National Danish Patient Registry were used to conduct this study. The original data source comprised approximately 3.1 million Danish citizens; 1.6 million men, women and children who had at one point sustained a fracture, and 1.5 million propensity matched individuals who had never sustained a fracture at the date the sampling was performed. Data were available from between January 1st 1996 and December 31st 2011. From the originating 3.1 million individuals, data between January 1st 1977 and December 31st 2011 were extracted regarding prescription use, hospital admissions and hospital outpatient visits, yearly socioeconomic status, migration and mortality. Prescription data consisted of all reimbursed prescriptions included date of reimbursement, ATC code, pack size and both numerical and WHO-defined daily dose equivalent. Patient diagnoses were gathered from the Danish National Hospital Discharge Register and consisted of date of diagnosis, ICD-10 code and contact category (i.e. admission or outpatient visit). Migration data consisted of date of immigration/emigration and country of origin/destination. Mortality data consisted of date of death, cause of death and causal diagnosis when applicable.

3.3.1.2.2 - THE STUDIED POPULATION

Initially, all prescriptions of thiazide diuretics were selected based on the ATC code C03AB. Grouping individuals by social security number ("CPR"), prescription reimbursements were sorted and ranked by date of collection (earliest to latest). Based on pack size, number of packs and DDD, and assuming consumption of one DDD per day, each prescription was reconstructed to an approximate stop date. Each prescription was prolonged by 20% to account for possible non-compliance and time lag to collection of a new prescription. By the ranked dates, data were transposed and overlaps between prescriptions used to prolong the earliest prescription. From the resulting periods, a washout period of 14 days was deemed necessary for a new thiazide exposure period to commence, otherwise the two periods were merged to one. The resulting periods were ranked by number for each individual, and based on the start date of each exposure period, each thiazide-exposed individual was matched with *control* individuals by exact birthdate and sex. The *control* individuals were then excluded if they were exposed to thiazides during the shared observation time. For each individual and each date, a "run-in" period of 52 weeks before the start of observations was constructed. Fracture occurrence for each individual was established using ICD-10 diagnoses; spinal fractures (DM484, DM485, DM485A, DM495, DM809C, DS32, DS320, DS320A, DS320B, DS320C, DS320D, DS320E, DS327, DS327A, DS328, DS328A, DS220, DS221 and DT08), hip and femoral region (DM809B, DS72, DS720, DS721, DS721A, DS721B, DS722, DS723, DS724B, DS724C, DS727, DS728A and DS729), wrist and hand (DM809A, DS52, DS521A, DS521B, DS522, DS523, DS524, DS525, DS525A, DS525B, DS525C, DS526, DS527, DS528, DS528A, DS528B, DS528C, DS529, DS620 and DS621) and proximal upper arm and shoulder fractures (DS422, DS422A, DS422B, DS422C, DS423, DS423A, DS424 and DS427). Time in weeks from observation start to fracture occurrences were calculated, and from this, it was established categorically if a fracture occurred during each week or not. Individuals were followed for up to 15 years with weeks excluded from the analysis in case of loss-to-follow up, migration or mortality. For each week, odds ratio of fracture occurrence was calculated using logistic regression between exposed and non-exposed individuals; crude and adjusted for thiazide exposure length, prior thiazide exposure, pre-existing diagnoses and medication use. During the periods of the run-in period, weeks 0-42 and from 43 to the end of the study, incidence rates and incidence rate ratios were calculated, both crude and age-adjusted.

3.3.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. Independent samples t-tests were performed between "thiazide exposure" and "thiazide non-exposure" for numerical values with the exception of CCI scores where Mood's median test was performed. Incidence rates and incidence rate ratios were calculated from number of events divided by observation time.

3.3.3 – SUMMARY OF RESULTS

A total of 1,602,141 thiazide exposure periods and 1,530,233 matched non-exposure periods were eligible for inclusion (Table 12). In total, 468,271 individuals with thiazide exposure and 655,399 users of non-exposure were included. At the start of exposure, thiazide exposed individuals were significantly older (69.47 ±13.97 vs. 69.17 ±14.07 years, $p < .05$), more likely to be female (OR 1.04 [1.03;1.04] vs 0.97 [.96;.97], $p < .05$) and presented with lower yearly salaries (205,274.20 ±182,021.28 vs. 226,118.41 ±242,711.14, DKK, $p < .05$) compared to non-users (Table 12). Thiazide users had higher concurrent use of other antihypertensive agents, glucocorticoids, insulin, thyroid hormone supplements and antiresorptive agents. Thiazide-exposed individuals were more likely to have existing cases of cerebral stroke (OR 1.13 [1.12-1.14]), hypertension (OR 2.82 [2.80; 2.84]), ischemic heart disease (OR 1.07 [1.06-1.07]), osteoporosis (OR 1.05 [1.04; 1.06]) and diabetes mellitus type 2 (OR 1.02 [1.01; 1.03]). During the majority of the 52-week run-in period, thiazide exposure was associated with higher OR during each week (Figure 1). From weeks 0 to 42 after commencing thiazide exposure, OR was increased with thiazide exposure and a simple moving average showed an increasing trend (Figure 1). After week 43, fracture risk progressively declined until weeks 116 where sporadic weeks of lower risk were observed (Figure 1). Overall adjusted incidence rate ratios increased from the run-in period (age-adjusted IRR 1.44 [1.42; 1.47]) to the week-0-to-42 period (age-adjusted IRR 1.27 [1.24; 1.29]), then further decreased during the week-43-to-780 period (age-adjusted IRR 1.14 [1.11; 1.18]) (Table 13).

Table 12: Descriptive data, epidemiological data, and medication exposure during the observation period (in DDD per day) for C03AB alone

	Thiazide exposure case periods (n = 1,602,141) 468,271 individuals (3.42 period/individual)	Thiazide nonexposure control periods (n = 1,530,233) 655,399 individuals (2.33 periods/individual)	Statistical significance P [95% CI]
Age at beginning of exposure period, years	69.47 (13.97)	69.17 (14.07)	0.000* [0.27; 0.33]
Gender (female), odds ratio [95% CI]	1.04 [1.03; 1.04]	0.97 [.96; .97]	0.000*
Yearly income year prior to start of exposure period, Danish Kroner (Euros)	205274.20 (182021.28) 27535.26€ (24416.13€)	226118.41 (242711.14) 30331.28€ (32557.00€)	0.000 [20178.01; 2151.41] 0.000 [2706.66€; 288.59€]
Number of previous periods of thiazide exposure	5.67 (7.57)	0.23 (0.97)	0.000* [5.43; 5.45]
Number of previous periods of thiazide nonexposure	5.39 (7.08)	1.07 (0.69)	0.000* [4.31; 4.33]
Number of days of thiazide exposure in previous thiazide exposure periods	77.05 (117.68)	34.33 (153.53)	0.000* [42.42; 43.02]
Number of days of thiazide nonexposure in previous thiazide nonexposure	204.69 (520.18)	2808.80 (1785.98)	0.000* [2601.23; 2606.99]
Total number of days of thiazide exposure in	462.35 (569.56)	60.52 (234.95)	0.000* [400.87; 402.79]

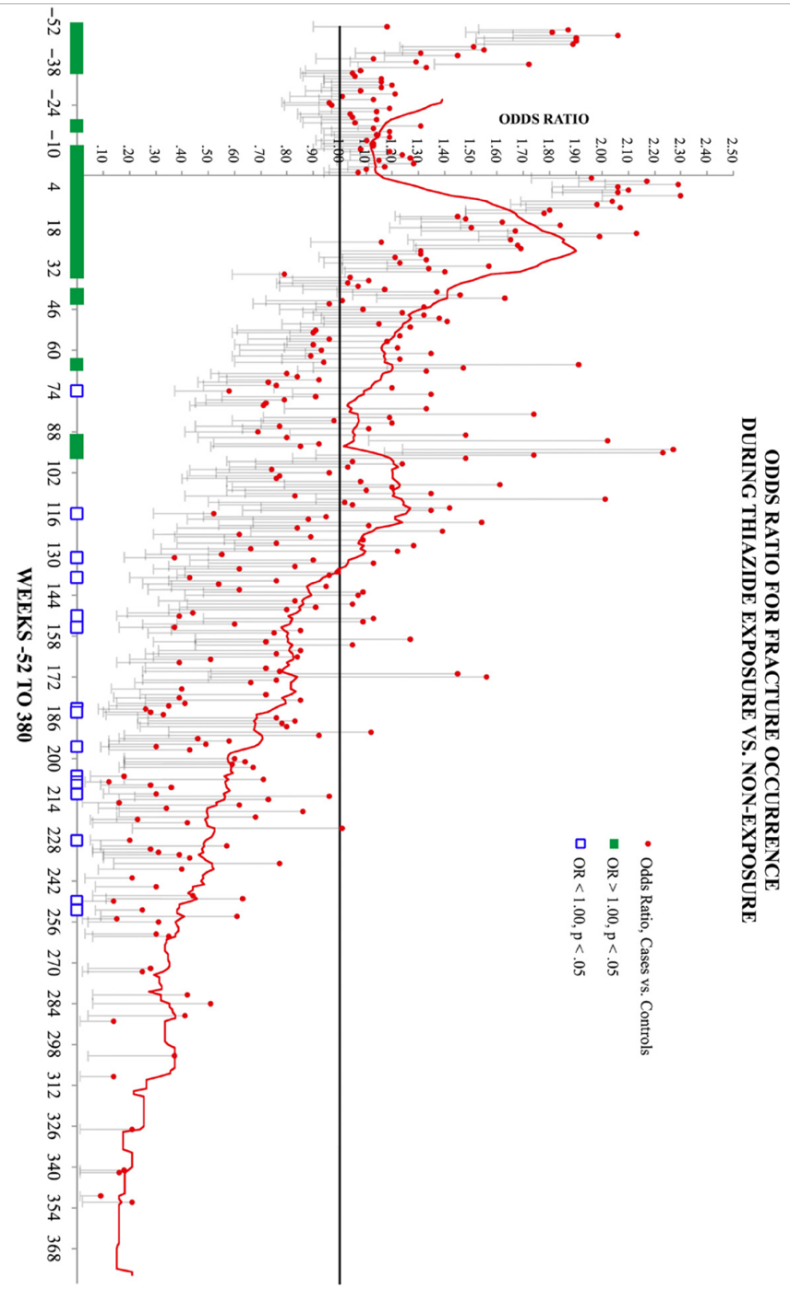
previous thiazide exposure periods			
Total number of days of thiazide absence in previous thiazide nonexposure periods	562.00 (778.48)	2833.62 (1770.48)	0.000* [2268.62; 2274.61]
Number of days since end of last thiazide exposure period	231.14 (559.81)	1495.70 (1159.77)	0.000* [1261.26; 1267.84]
CCI			
0	344.946 (21.5%)	304.650 (19.3%)	
1–2	461.992 (28.8%)	481.500 (30.6%)	
3–4	605.512 (37.8%)	585.547 (37.2%)	
5–6	157.177 (9.8%)	148.726 (9.4%)	
>6	32.514 (2.0%)	54.630 (3.5%)	
Median Charlson Index score (IQR)	2 (3)	3 (3)	0.000*

*: $p < .05$

Table 13: Incidence rates and incidence rate ratios for fracture occurrence: thiazide exposure periods versus nonexposure periods

	IR Fracture rate/ 1000 person-years	Unadjusted IRR	Age-adjusted (decades) IRR
Run-in period			
• Thiazide exposure	26.34	1.48 [1.46; 1.51]	1.44 [1.42; 1.47]
• Thiazide nonexposure	17.76		
Weeks 1–42			
• Thiazide exposure	264.64	1.65 [1.62; 1.69]	1.27 [1.24; 1.29]
• Thiazide nonexposure	159.90		
Weeks 43–780			
• Thiazide exposure	32.98	1.12 [1.09; 1.15]	1.14 [1.11; 1.18]
• Thiazide nonexposure	29.48		

Figure 1: Adjusted weekly odds ratios (ORs) for risk of fracture occurrence for thiazide exposure versus nonexposure during the period of thiazide exposure/nonexposure



3.4 - PAPER 4

Osteoporos Int
DOI 10.1007/s00198-015-3451-0

ORIGINAL ARTICLE

Optimal age of commencing and discontinuing thiazide therapy to protect against fractures

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Received: 14 August 2015 / Accepted: 2 December 2015

3.4.1 - MATERIALS AND METHODS

This paper was a retrospective matched cohort study examining the 10-year risk of fracture occurrence when commencing thiazide diuretics at certain ages versus non-users, in order to investigate if certain age groups show different risks of thiazide-related fractures or fracture protection.

3.4.1.1 - REGISTRIES AND EXPOSURE VARIABLES

3.4.1.1.1 - NATIONAL DANISH PATIENT REGISTRIES

Data originated from the National Danish Patient Registry and comprised of approximately 2.93 million Danish citizens between January 1st 1996 and December 31st 2011. We reused prescription data, patient diagnoses data, socioeconomic data, mortality data and migration data.

3.4.1.1.2 - THE STUDIED POPULATION

Patients were selected initially as "thiazide users" if they at one point had collected a prescription for thiazide diuretics. Two separate analyses were performed for ATC categories C03AB ("thiazides with potassium supplementation") and C03AA ("thiazides, plain") and C03AB ("thiazides with potassium supplementation") combined. The start date was defined as the date the first thiazide prescription was collected. Similar to the technique in "paper 3", thiazide exposure periods were then reconstructed based on collection dates, pack size, DDD and prolongation of 20% to account for non-compliance and time lag to prescription collection. Age and sex were established at the individual start dates and females above 50 years of age included for further analysis. Exposure periods between January 1st, 1999 and December 31st 2001 were included. Thiazide-exposed individuals were matched by

sex and exact birthdates to individuals who had never been exposed to thiazides. Fracture occurrence was defined as one patient diagnosis of spinal fractures (M484, M485, M485A, M495, M809C, S32, S320, S320A, S320B, S320C, S320D, S320E, S327, S327A, S328, S328A, S220, S221, and T08), hip and femoral region fractures (M809B, S72, S720, S721, S721A, S721B, S722, S723, S724B, S724C, S727, S728A, and S729), wrist and hand fractures (M809A, S52, S521A, S521B, S522, S523, S524, S525, S525A, S525B, S525C, S526, S527, S528, S528A, S528B, S528C, S529, S620, and S621) or proximal upper arm and shoulder fractures (S422, S422A, S422B, S422C, S423, S423A, S424, and S427) occurring within 10 years of the observation start. Lost to follow-up was defined as dates of death, date of emigration, 10-year observation or December 31st 2011, whichever came first. Medication use in DDD from January 1st 1996 to the start of observation, and from the start to end of observation were calculated. Categorical presence of comorbidities associated with increased risk fractures at the start of observation was established. Grouped by absolute age in years, floored to the nearest integer, Cox proportional hazards models were constructed for 10-year fracture risk both crude and adjusted for fracture-prone medication use, treatment length, CCI and Boolean comorbidity presence. A secondary analysis of 10-year fracture occurrence after discontinuation of thiazide diuretics compared to non-users was performed using the same methodology and adjustments.

3.4.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. Median and interquartile ranges were calculated for ordinal variables. Independent samples t-tests were performed between “thiazide exposure” and “thiazide non-exposure” for numerical values with the exception of ordinal variables where Mood’s median test was used. Chi-square ($\chi 2$) analysis with calculation of odds ratio was used for categorical data. Cox proportional hazard models were used as time-to-event analyses with estimation of hazard ratios.

3.4.3 - SUMMARY OF RESULTS

58,790 females above 50 years of age were included versus 1,357,829 non-exposed matched individuals (Table 14). Thiazide-exposed individuals were significantly older as a whole (71.99 ± 11.22 vs. 69.80 ±11.44, $p < .05$), had higher median CCI scores (3 (IQR 4) vs. 2 (IQR 3), $p < .05$) and higher prior use of analgesics, sedatives, cardiovascular medication, antidepressants and antiresorptive agents than non-exposed individuals. Thiazide-exposed individuals were also more likely to have existing hypertension and stroke, but less likely to have chronic kidney disease, Parkinson’s disease and chronic obstructive pulmonary disease (Table 15). The age-grouped Cox regression models revealed increased 10-year HR of fracture occurrence for C03AB when exposure began at and after age 73 years, lasting until age 96, compared to non-users (Figure 2). From 76 years upwards, a trend towards increasing risk year by year was found (Figure 2). Between ages 50 and 73, risks

were comparable and sporadically increased with thiazide exposure. Discontinuing thiazide therapy between the ages of 63 and 97 showing a trend towards decreasing 10-year crude HR of fracture compared to non-users (Figure 3). Between ages 50 and 63 years of age, risks were comparable between thiazide exposed and non-exposed.

Table 14: Descriptive data, epidemiological data, and medication exposure during the observation period (in DDD per day) for C03AB alone

	Cases Mean (SD) (range) (N)	Controls Mean (SD) (range) (N)	p value
Age at beginning of exposure period (years)	71.99 (11.22) (57) (58,790)	69.80 (11.44) (53) (1,357,829)	p<0.05*
Fracture sustainment during observation period			
Hip and pelvic fractures	7.042 (12.0 %)	114.539 (8.4 %)	p<0.05*
No fracture	42.156 (71.7 %)	1.044.435 (76.9 %)	p<0.05*
Spinal fracture	1.076 (1.8 %)	16.088 (1.2 %)	p<0.05*
Upper extremity fracture	3.852 (6.6 %)	70.043 (5.2 %)	p<0.05*
Wrist Fracture	4.664 (7.9 %)	112.724 (8.3 %)	p<0.05*
CCI			
0	16.381 (27.9 %)	296.475 (21.8 %)	
1–2	11.964 (20.4 %)	384.313 (28.3 %)	
3–4	26.843 (45.7 %)	573.826 (42.3 %)	
5–6	3.218 (5.5 %	83.247 (6.1 %)	
>6	384 (0.6 %)	19.968 (1.5 %)	
Median CCI (IQR)	3 (4)	2 (3)	p<0.05*
Previous exposure and absent exposure of thiazide diuretics			p<0.05*
Avg. no of days of "thiazide non-exposure" all previous "thiazide non-exposure periods"	253.52 (579.01) (4592) (58,790)	3088.94 (1448.83) (4747) (1,357,829)	p<0.05*
Avg. no of days of "thiazide treatment" in all"thiazide treatment periods"	815.70 (1,206.79) (4743) (58,790)	N/A	N/A
Length of first period of "thiazide treatment"	699.89 (1,228.25) (4743) (58,790)	N/A	N/A
Total length of "thiazide non-exposure"	593.50 (934.69) (4592) (58,790)	3088.94 (1448.83) (4747) (1,357,829)	p<0.05*

Total length of "thiazide treatment"	1421.63 (1408.62) (4743) (58,790)	0 (0) (0) (1,357,829)	p<0.05*
Total no of "thiazide non-exposure" periods	3.69 (9.30) (198) (58,790)	1 (0) (0) (1,357,829)	p<0.05*
Total no of "thiazide treatment" periods	4.69 (9.30) (198) (58,790)	0 (0) (0) (1,357,829)	p<0.05*

*: $p < .05$

Table 15: Co-morbidity for underlying individuals exposed to C03AB at the beginning of the observation period. OR for preexisting diagnoses

	Cases OR, 95 % CI, p value
Conditions of higher OR among exposed individuals	
• Angina pectoris (I20)	1.23 [1.17; 1.29], p<0.05*
• Cerebral infarction (I63)	1.08 [1.00; 1.18], p<0.05*
• Essential (primary) hypertension (I10)	1.89 [1.80; 1.98], p<0.05*
• Fracture at wrist and hand level (S62)	1.14 [1.07; 1.21], p<0.05*
• Fracture of forearm (S52)	1.09 [1.05; 1.13], p<0.05*
Conditions of lower OR among exposed individuals	
• Anemia, unspecified (D649)	0.83 [0.76; 0.91], p<0.05*
• Chronic ischemic heart disease (I25)	0.87 [0.82; 0.91], p<0.05*
• Chronic kidney disease, unspecified (N189)	0.25 [0.17; 0.37], p<0.05*
• Chronic obstructive pulmonary disease with (acute) exacerbation (J441)	0.82 [0.75; 0.89], p<0.05*
• Fracture of femur (S72)	0.82 [0.79; 0.86], p<0.05*
• Heart failure (I50)	0.50 [0.47; 0.54], p<0.05*
• Other chronic obstructive pulmonary disease (J44)	0.86 [0.82; 0.91], p<0.05*
• Parkinsons sygdom (G209)	0.61 [0.52; 0.72], p<0.05*
• Type 1 diabetes mellitus (E10)	0.92 [0.84; 0.99], p<0.05*
• Type 2 diabetes mellitus (E11)	0.91 [0.86; 0.97], p<0.05*
• Unspecified dementia with behavioral disturbance (F039)	0.73 [0.66; 0.80], p<0.05*

*: $p < .05$

Figure 2: Age-stratified 10-year crude HR of fracture risk, thiazide exposure vs. non-exposure

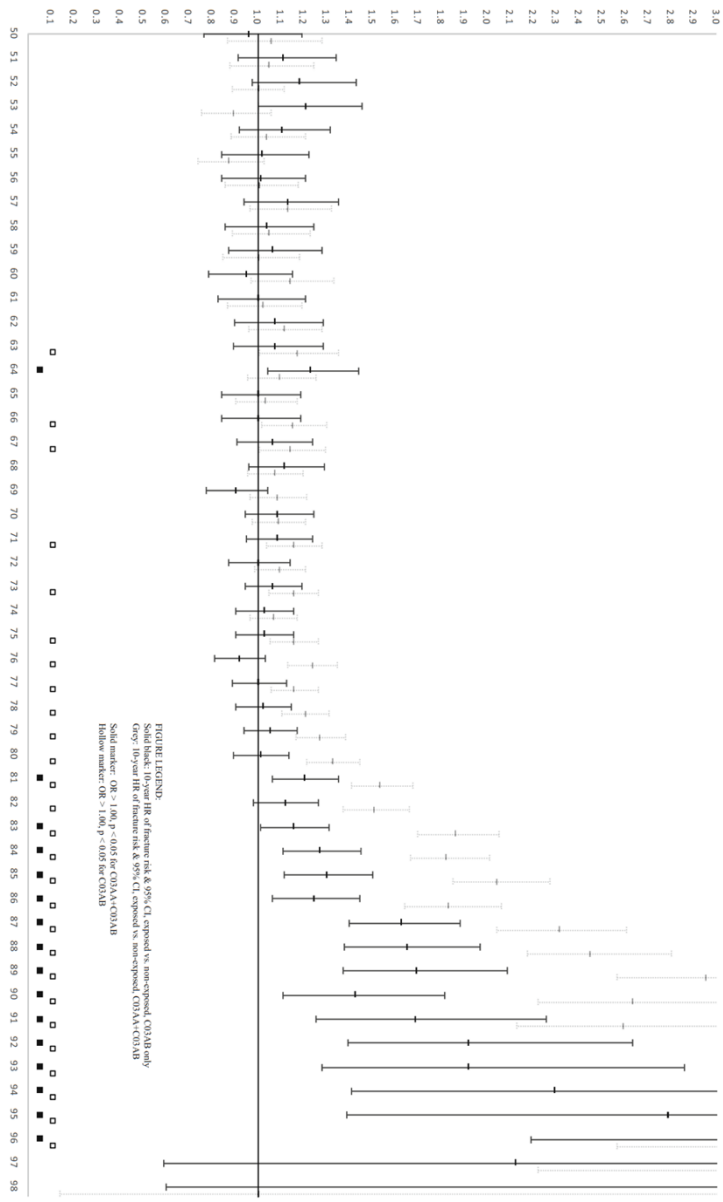
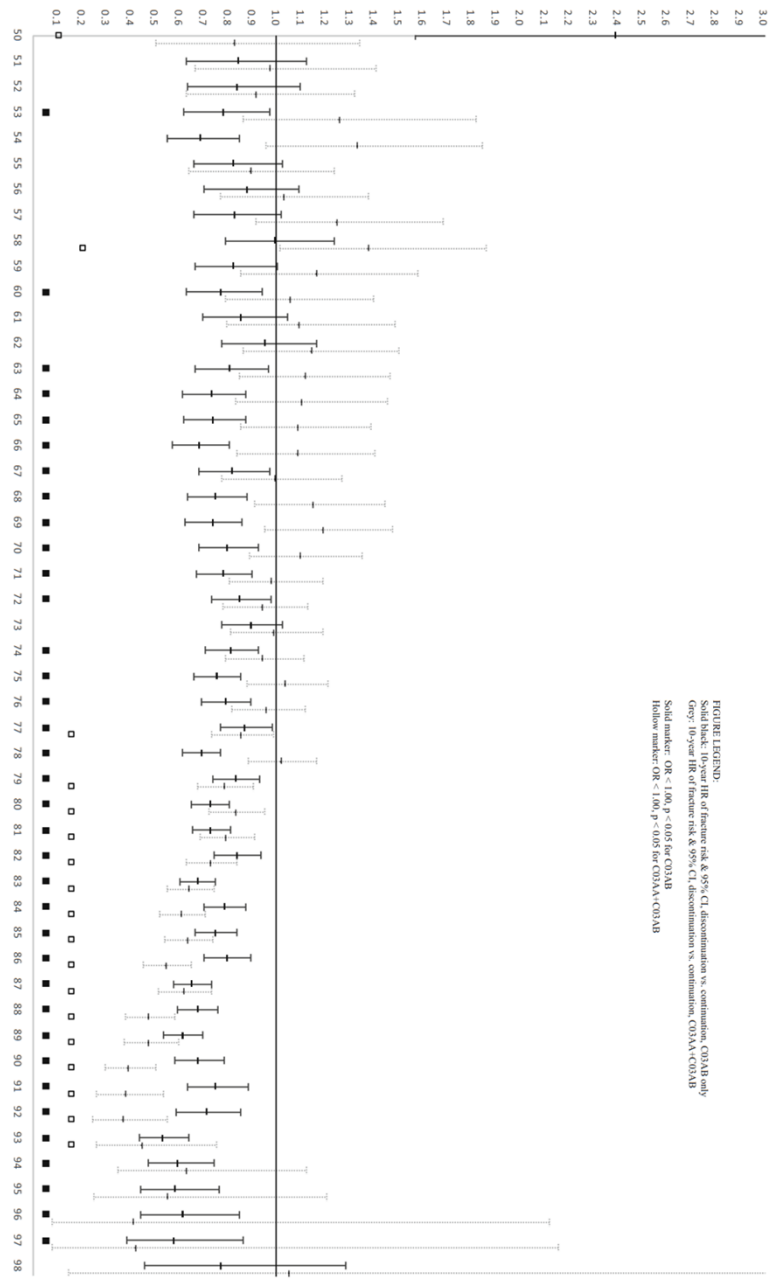


Figure 3: Age-stratified 10-year adjusted HR of fracture risk, thiazide exposed versus non-exposed



3.5 - PAPER 5

Kruse C, Goemaere S, de Buyser S, Lapauw B, Eiken P, Vestergaard P.
Predicting Mortality and Incident Immobility in Older Belgian Men by Characteristics Related to Sarcopenia and Frailty.
Submitted 2017 July to Osteoporosis International, In Review

3.5.1 - MATERIALS AND METHODS

3.5.1.1 - DESIGN

This study was a retrospective cohort study using data on older Belgian men. The purpose of the study was to investigate the predictability of 5-year mortality and 3-year incident immobility in this patient group based on DXA scans, physical performance, blood samples and gerontology battery tests. An aim was to rank predictor importance of the same outcomes to assess the relative importance of hyponatremia and thiazides.

3.5.1.2 - DATA SOURCES AND EXPOSURE VARIABLES

3.5.1.2.1 - THE MERELBEKE STUDY

This study used data from the Merelbeke Study which was anchored at Gent University Hospital in Gent, Belgium[210]. From 1996 onwards, the study collected data on a cohort of older Belgian men from a semi-rural province of Merelbeke (population 20,000) for several purposes, primarily to investigate the effect of andropause and androgens on mortality and bone health. All men between ages 70 to 85 years were invited by mail. Between 1996 and 2000, annual visits took place for all participants with another follow-up in 2003. Annual statuses of mortality have since been collected via telephone calls to patients and relatives. The scope of the yearly visits varied, but could consist of blood and urine samples, whole-body and bone DXA scans (forearm, lumbar spine and hip), gerontology battery tests (e.g. SF-36[211], RDRS-2[212], GDS[213]) and physical tests (TGUG[214], handgrip strength), alongside collection of comorbidity and medication use information.

3.5.1.2.2 - THE STUDIED POPULATION

This work centered around the second annual visit in 1997 where the majority of tests were undertaken for all individuals. Further exposure variables that were only collected in 1996 and considered stable, i.e. height and weight, were included. The date of the second visit was used as start of observation and for each individual, all measurements of blood samples, all separate answers to the gerontology battery tests, all recorded performances of the physical tests and all BMC and BMD measurements from the performed DXA scans were selected for each individual. Two outcomes were defined; 1) 5-year mortality, and 2) 3-year incident severe

immobility based on subjective reporting from the SF-36 questionnaire (from "no" to "yes" when answering one of two questions, 3E; "Are you limited in coping a flight of stairs?", and 3H; "Are you limited in talking several blocks?"). A systematic machine learning procedure was used to assess predictability of different models on unseen data samples, followed by extraction of ranked predictor importance for the final model. Descriptive analysis of mortality versus non-mortality, and of included versus non-included patients, were performed.

3.5.1.2.3 - THE MACHINE LEARNING PROCEDURE.

The separate datasets for the two outcomes were split into "*training*" (75% of data points) and "*test*" (25% of data points) datasets, by balanced outcome proportion in the four groups. Across all model types, *k*-5, 10-repeat cross-validation was used across the scope of hypercomplexity parameters for each model to assess the complexity with which the highest AUC was attained. A total of 29 model types were trained: parametric models, non-parametric models, tree-based with boosted and bagged versions of the same. For models requiring complete cases, random forest imputation was used through 1,500 trees and 5 iterations. Ranked predictor importance based on error reduction capability and hierarchical presence were extracted from the best performing model. To assess optimal cut-off values for the resulting predictors, classification tree models were trained across all data points with these sole predictors.

3.5.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. Median and 25^{th}-75^{th} percentiles were calculated for non-parametric data. Welch tests were performed between "mortality" and "non-mortality"/"survival" for numerical values with the exception of ordinal variables where a Wilcoxon rank sum test was used. Data distributions were assessed by QQ-plots, histograms and kernel smoothing. Chi-square ($\chi 2$) analyses with calculation of odds ratios were used for categorical data.

3.5.3 - SUMMARY OF RESULTS

352 men formed the original Merelbeke cohort in 1996 (participation rate 47.1%). In this study, 264 subjects were included from the visit in 1997. For the mortality outcome, 56 mortalities occurred within five years (21.2%) (Table 16). In the mortality group, subjects were significantly older (77.98 ± 4.54 years versus 75.61 ± 3.55), had lower intertrochanteric BMD and T-score, lower trochanteric BMD, higher TGUG duration in seconds and higher RDRS-2 scores (Table 16). The best predictive model type of this outcome was a Bayesian generalized linear model with a test AUC of .85 [.73 ; .97], a sensitivity of 78% and a specificity of 89% at a Youden probability cut-off of 22.3%. Ranked predictor importance revealed that plasma 25-hydroxycholecalciferol, plasma albumin, trochanteric BMD and T-scores

and total hip region BMD to be the best predictors of mortality (Table 17). Serum sodium and thiazide use did not figure in the most important predictors. For the outcome “incident immobility”, 39 subjects became severally immobile over a 3-year period, while 102 remained mobile (outcome rate 27.7%). The best performing model was the multivariate adaptive regression splines model with a test AUC of .74 [.57;.91], a sensitivity of 67% and a specificity of 78% at a Youden probability cut-off of 14.2%. The most important predictors for this outcome were SF-36 Physical Domain Numerical Score, BMI, SF-36 Physical Domain Standardised Score, IPSS-35 Total Score, plasma SHBG levels, plasma testosterone and GDS Question 1 Numerical Score (Table 17). Nor serum sodium nor thiazide use figured in the ranked important predictors.

Table 16: Descriptive data. grouped by 5-year mortality vs. survival

	Mortality n = 56 (21.2%)	Survival n = 208 (78.8%)	Statistical Significance Level
	Mean/Median (SD/25th-75th perc.)	Mean/Median (SD/25th-75th perc.)	
Age (years)	77.98 ±4.54	75.61 ±3.55	***
Height (cm)	168.1 ±7.5	167.9 ±6.0	
Weight (kg)	72.3 ±13.7	75.0 ±11.3	
BMI (kg/m^2)	25.47 ±3.97	26.55 ±3.32	*
Biochemistry			
Creatinine, Serum (µmol/L)	1.26 ±.34	1.22 ±.2	
Natrium, Serum (mmol/L)	141.02 ±3.29	141.61 ±2.35	
Creatinine, Urine (mg/dL)	.95 ±.5	1.11 ±.46	*
Natrium, Urine (mmol/L)	85.54 ±52.4	94.79 ±48.06	
DXA, Hip			
Lowest T-score. hip/femoral neck (SD)	-1.81 ±.9	-1.42 ±.86	**
Total, BMC (g)	41.25 ±8.69	44.75 ±8.15	**
Total, BMD (g/cm^2)	.86 ±.15	.94 ±.14	***
Total, Z-Score (SD)	-.14 ±1.02	.26 ±.93	**
Femoral Neck, BMC (g)	4.4 ±.86	4.71 ±.81	**
Femoral Neck, BMD (g/cm^2)	.69 ±.12	.74 ±.12	**
Trochanteric Region, BMC (g)	8.99 ±2.07	10.13 ±2.05	***
Trochanteric Region, BMD (g/cm^2)	.65 ±.12	.72 ±.12	***
Intertrochanteric Region, BMC (g)	27.86 ±6.32	29.91 ±5.84	*
Intertrochanteric Region, BMD (g/cm^2)	1.0 ±.19	1.1 ±.17	***
Ward's Triangle, BMC (g)	-2.59 ±.77	-2.23 ±.77	**
Ward's Triangle, BMD (g/cm^2)	.47 ±.13	.52 ±.12	**

Muscle Function Tests			
Timed Up-And-Go (seconds)	14.4 ±7.21	11.15 ±3.16	***
Grip Strength, Smedley, Maximum (kg)	28.36 ±7.49	30.91 ±7.55	*
Balance Test			
- Completed 6/6 (n, %)	10 (17.8%)	74 (35.9 %)	**
- Completed <6 (n, %)	46 (82.2%)	132 (64.1 %)	
Cognitive Function			
RDRS-2, Total Score	21 (20-23)	20 (19-21)	***
GDS, Total Score	6 (3-9.75)	4 (2-7)	**
SF-36, Total Score	74 (62.75-78.25)	77 (68.75-81.25)	*
Sarcopenia Consensus Variables			
Janssen BIA Predicted Absolute Muscle Mass (kg)	17.85 ±3	18.87 ±2.01	**
SMI, Predicted from Janssen BIA (kg/m^2)	10.25 ±1.61	10.74 ±1.17	*

*: $p < .05$. **: $p < .01$. *** $p < .001$

Table 17: Ranked Variable Importance for the 5-Year Mortality Outcome Predicted Using a Bayesian Generalized Linear Model

Rank	Variable Name	Variable Importance (Normalized 0-100) *	Optimal Cut-off	Survival/Mortality Below Cut-off	Survival/Mortality Above Cut-off
1.	Plasma 25-hydroxycholecalciferol (nmol/L)	100	24.5 nmol/L	85/32	125/13
2.	Plasma albumin (g/L)	91	N/A	N/A	N/A
3.	DXA: Trochanteric BMD (g/cm^2)	88	.58 g/cm^2	35/17	172/28
4.	DXA: Hip Trochanteric T-Score (SD)	88	-1.56	35/17	172/28
5.	DXA: Hip Total BMD (g/cm^2)	87	.86 g/cm^2	74/27	133/18
6.	DXA: Hip Total T-Score (SD)	87	-1.12	74/27	133/18
7.	DXA: Hip Intertrochanteric BMD (g/cm^2)	86	.93 g/cm^2	39/17	168/28
8.	DXA: Hip Intertrochanteric T-Score (SD)	86	-1.49	39/17	168/28
9.	Lean Body Mass (%)	84	68%	35/15	165/28
10.	DXA: Trochanteric BMC (g)	83	6.48 g	7/1	200/39
11.	Balance Score	82	N/A	N/A	N/A
12.	SF-36: 11A Score[a]	80	N/A	N/A	N/A
13.	DXA: Trochanteric Z-Score	79	-.91	34/16	170/28
14.	DXA: Ward's Triangle BMD (g/cm^2)	79	.39 g/cm^2	53/20	154/25

15.	DXA: Ward's Triangle T-Score (SD)	79	-2.80	53/20	154/25
16.	DXA: Total forearm (g/cm^2)	78	.49 g/cm^2	31/15	178/30
17.	DXA: Lower Arm T-Score (SD)	78	-3.49	31/15	178/30
18.	DXA: Radius BMD (g/cm^2)	78	.62 g/cm^2	29/14	180/31
19.	DXA: Radius T-Score (SD)	78	-3.75	29/14	180/31
20.	Plasma Testosterone (nmol/L)	78	3.8 nmol/l	11/5	174/30

*: Variable importance by predictor presences across ensembled tree-models weighted by reduction in error, then normalized to 0-100.
[a]: "I become ill more frequently than my peers"

Table 18: Ranked Variable Importance for the Incident Immobility Outcome Predicted Using a Multivariate Adaptive Regression Spline Model

Rank	Variable Name	Variable Importance (Normalized 0-100)	Optimal Cut-off	Immobility/No Immobility Below Cut-off	Immobility/No Immobility Above Cut-off
1.	SF-36: Physical Domain Numerical Score	100	18.5	70/28	44/4
2.	BMI (kg/m^2)	79	30.39 kg/m^2	100/31	14/1
3.	SF-36: Physical Domain Standardised Score (1-100)	68	37.5	10/2	104/24
4.	IPSS-35 Total Score	55	7.5	45/19	68/13
5.	Plasma SHBG (nmol/l)	47	42.2 nmol/L	47/18	54/8
6.	Plasma Testosterone (nmol/L)	31	N/A	N/A	N/A
7.	GDS: Question 1 Numerical Score[a]	22	N/A	N/A	N/A

[a]: "Are you basically satisfied with your life?"

3.6 - PAPER 6

Kruse C, Eiken P, Vestergaard P.
Hyponatremia is Associated with Greater Hospital Length of Stay and Cost of Stay in Danish patients.
Submitted 2017 July to Journal of General Internal Medicine

3.6.1 - MATERIALS AND METHODS

3.6.1.1 - DESIGN

This paper was a retrospective cohort study examining LoS, CoS and readmission risk for hospitalized patients relative to nadir serum sodium values during the hospitalizations.

3.6.1.2 - REGISTRIES AND EXPOSURE VARIABLES

For this study, a combination of regional biochemical sample databases and national Danish patient registries were used, identical to "paper 1", with no updates to data since publication.

3.6.1.2.1 - REGIONAL BIOCHEMICAL SAMPLES DATABASE

The biochemical samples database was the same as the one used in "paper 1" with no updates since time of publication, extracted from the list of scanned patients. The data comprised biochemical samples for all patients who had at one point been in contact with the Department of Endocrinology at Aalborg University Hospitals in Denmark. Data spanned 1996 to 2012 and was extracted from the Department of Biochemistry at Aalborg University Hospital, a DANAK certified institution (Appendix 9).

3.6.1.2.2 - NATIONAL DANISH PATIENT REGISTRIES

Data on prior prescription reimbursement, patient diagnoses, mortality and migrations were collected from the National Danish Patient Registry. Further database tables of primary healthcare sector use on the same patients were also utilized. Further data on hospital admissions costs between January 1st 2007 and December 31st 2010 were included.

3.6.1.3 - THE STUDIED POPULATION

Primary inclusion in this study were all individuals with at least one hospitalization, regardless of ICD-10 diagnosis code, who had had at least one blood sample drawn within the administrative Region of North Denmark. Based on a selected list of admission codes from the ICD-10 registry, dates of admission and discharge were selected with overlaps resulting in prolongation of the observation periods.

Admissions were only included if they had taken place in the administrative Region of North Denmark. After the date of discharge, a further follow-up date ("post period") was selected at the first occurring readmission, emigration, mortality or one year after the date of discharge. Dichotomous readmission within 30 days was established. From the table of hospital admissions costs related to specific admissions, the total monetary costs in DKK were calculated for both the hospitalization itself and the following convalescence. The nadir sodium value, the sodium value closest to admission and closest to discharge were selected of either arterial, venous or capillary origin. From the date of admission and one year prior, the total reimbursed dosage of certain pharmaceuticals and presence of specific comorbidities were computed. During the follow-up period, monetary primary sector expenses were computed for GP visits, emergency GP services, dental services, podiatrists and physical therapy. Admissions were grouped as "hyponatremia" (serum sodium below 137 mmol/L), "normonatremia" ([Na+] 137-147 mmol/L) and "not measured" if no sodium samples were drawn. LoS and readmission within 30 days were established.

3.6.2 - STATISTICAL ANALYSIS

Descriptive statistics included mean, SD and range for numerical variables. Median and 25^{th}-75^{th} percentiles were calculated for non-parametric data. Welch T-tests with Bonferroni correction were performed between "hyonatremia", "normonatremia" and "not measured" for numerical values with the exception of ordinal variables where Wilcoxon rank sum tests were used. Pairwise G-tests with Holm correction were performed for categorical data between "hyonatremia", "normonatremia" and "not measured".

Linear regression models of 1) nadir sodium to length of stay, 2) nadir sodium to cost of stay were fitted for crude relationships and with adjustments for sex, age (years) at admission, CCI total score and reimbursed total DDD doses of pharmacologic agents, i.e. DPP-4 inhibitors (A10BH), thiazides and potassium in combination (C03AB), ARBs (C09CA), glucocorticoids (H02AB), hydantoin derivatives (N03AE) and SSRIs (N06AB). Linear models were fitted to both the entire data and group-wise for each primary ICD-10 diagnosis. Logistic regression models were fit both for crude and adjusted models (also for factors above) between nadir sodium and 30-day risk of readmission. Multiple comparisons were adjusted with Bonferroni correction.

3.6.3 - SUMMARY OF RESULTS

The original data sources comprised 32,392 patients. Following selection by inclusion criteria, 9,912 individuals with at least one inpatient hospitalization and one blood sample were identified between January 1^{st} 2007 and December 31^{st} 2010 (Table 19). In the final analysis, 1,612 individuals with a total of 5,809 admissions and readmissions were included for the examined diagnoses. Hyponatremia by nadir sodium occurred in 919 (15.8%) of admissions, normonatremia was present in 1,593

(27.4%) while no sodium measurements were drawn in 3,297 (56.7%). The mean age of hyponatremic patients was 69.01±12.43 years (vs. 69.04±12.61 year for normonatremia, not significant), 67.5% were female (vs. 68.2%, not significant) and the average LoS was 12.10 ±12.23 days (vs 8.27±8.97, $p < .001$) (Table 19). The mean nadir sodium value was 132.8 ±3.2 mmol/L. Hyponatremic subjects had high, but lower CCI scores than normonatremic subjects (median 11 (25^{th} pct. 8; 75^{th} pct. 14) vs. 10 (25^{th} pct. 7; 75^{th} pct. 14)), higher oral antidiabetics use, higher antihypertensive use. Compared to patients with normonatremia, hyponatremic patients had longer LoS (12.10 vs. 8.27 days, $p < .001$), greater CoS (55.18 vs. 43.69 thousand DKK, $p < .001$) and convalescence costs (930 vs. 700 DKK, $p <. 001$). Fewer GP visits and lower total GP expenses were observed in the ”post” period for hyponatremic patients compared to normonatremic (Table 19). A significant relationship was discovered between increasing nadir sodium during admissions and both LoS and CoS, both crude and after adjustments. In adjusted models, total LoS decreased .49 day per 1 mmol/L increase in nadir sodium, while total CoS decreased 1,857 DKKs per 1 mmol/L increase in nadir sodium (Table 20). 30-day readmission occurred in 22.2% of hyponatremic cases. For the patient group as a whole, no significant association was found between nadir sodium value and readmission risk (Table 21). For specific diagnoses, lower readmission risk was found for ”Unspecified atrial fibrillation and atrial flutter, I48.9”, ”Heart failure, unspecified, I50.9”, ”Acute respiratory failure, J96.0”, while the risk was higher for ”Pneumonia, unspecified organism, J18.9” (Table 4) (Table 21).

Table 19: Descriptive Statistics, Hyponatremia During Admission and Readmission

	Hyponatremia Mean ± SD (n) or Median (25^{th}-75^{th} percentile) (n=919)	**Normonatremia** Mean ± SD (n) or Median (25^{th}-75^{th} percentile) (n=1,593)	**No Sodium Measurements** Mean ± SD (n) or Median (25^{th}-75^{th} percentile) (n=3,297)	**Statistical Significance**		
				1v2	1v3	2v3
Age at Admission (Years)	69.01 ±12.43	69.04 ±12.61	64.30 ±13.31		***	***
Sex (Nº Females)	620 (67.5%)	1,086 (68.2%)	2,408 (73%)		**	**
Admission Number (Nº)	1 (1;2)	1 (1;2)	1 (1;2)			
Length of Stay (Days)	12.10 ±12.23	8.27 ±8.97	3.80 ±6.21	***	***	***
Readmission	204 (22.2%)	308 (19.3%)	553 (16.8%)		***	
Readmission (Days after Discharge)	11 (5;18)	9 (5;16)	9 (4;18)			
Cost of Admission (thousand DKK)	55.18 ±68.64	43.69 ±55.43	33.67 ±40.28	***	***	***
Cost of Convalescence (thousand DKK)	.93 ±1.65	.70 ±1.41	.37 ±1.09	***	***	***
P-Sodium, Nadir (mmol/L)	132.8 ±3.2	140.3 ±2.4	N/A	***		
P-Sodium, Closest to Admission (mmol/L)	135.8 ±3.1	141.4 ±2.5	N/A	***		

P-Sodium, Closest to Discharge (mmol/L)	136.2 ±3.1	141.6 ±2.4	N/A	***		
Primary Healthcare Sector Use One Year After Discharge						
GP Expenses, 1 yr after Discharge (total DKK)	10,138.88 ±23,572.32	14,934.67 ±40,960.70	9,318.24 ±25,145.53	***		***
GP Visits, 1 yr after Discharge (N°)	37.0 (22.0;58.0)	38.0 (23.0;69.0)	31.0 (17.0;53.0)	*	***	***
Emergency GP Expenses, 1 yr after Discharge (total DKK)	3,261.82 ±29,883.04	12,418.72 ±83,725.63	3,863.39 ±19,915.89	***		***
Dentist Expenses, 1 yr after Discharge (total DKK)	348.22 ±1,594.98	294.03 ±591.98	398.94 ±1,001.48			**
PT Expenses, 1 yr after Discharge (total DKK)	1,376.60 ±5,427.90	1,123.27 ±4,631.02	1,784.64 ±6,673.42			***
Podiatrist Expenses, 1 yr after Discharge (total DKK)	7.85 ±58.45	29.57 ±180.46	14.22 ±115.56	***		***
Charlson Comorbidity Age-Adjusted Index at the Time of Admission						
Charlson Total Sum	10 (7;14)	11 (8;14)	10 (7;14)	***		***

***: p < .001, **: p < .01, * < .05

Table 20: The Association Between Increasing Nadir P-Sodium (mmol/L), Length of Stay (LOS) and Cost of Stay (COS)

	Length of Stay (Days) per 1 mmol/L increase in P-Sodium Adjusted Slope	Cost of Stay (DKKs) per 1 mmol/L increase in P-Sodium Adjusted Slope
All Admissions		
Nadir P-Sodium (n=2,512)	-.49***	-1,857***
Individual Diagnosis Codes		
Sepsis, unspecified organism (A41.9,n=26)	N/A	N/A
Erysipelas (A46.9,n=84)	-1.03***	-92
Malignant neoplasm of breast of unspecified site (C509,n=14)	N/A	N/A
Volume depletion, unspecified (E86.9,n=23)	N/A	N/A
Anemia, unspecified (D64.9,n=34)	-.46	-614
Thyrotoxicosis, unspecified (E05.9,n=67)	-2.24***	-152***
Primary hyperparathyroidism (E21.0,n=251)	.23	-958
Alcohol dependence (F10.2,n=170)	-.42	-6,240
Multiple sclerosis (G35.9,n=232)	N/A	N/A
Epilepsy and recurrent seizures (G40.9,n=243)	-1.33	-1,595
Angina pectoris, unspecified (I20.9,n=828)	-.66	-2,531
Non-ST elevation (NSTEMI) myocardial infarction (I21.4,n=161)	-.16	-17
ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction (I21.9,n=139)	N/A	N/A
Chronic ischemic heart disease, unspecified (I25.9,n=464)	-.39	-6,076
Pulmonary embolism without acute cor pulmonale (I26.9,n=102)	-1.71	828
Nonrheumatic aortic valve disorders (I35.0,n=285)	-.89	-14,024
Unspecified atrial fibrillation and atrial flutter (I48.9,n=2,380)	-.56**	-3,969**
Left ventricular failure (I50.1,n=70)		

Heart failure, unspecified (I50.9,n=549)	-.65	-3,087
Cerebral infarction, unspecified (I63.9,n=700)	.30	-3,854
Stroke (I64.9,n=202)	-.40	3,268
Atherosclerosis of native arteries of the extremities (I70.2,n=103)	N/A	N/A
Phlebitis and thrombophlebitis of lower extremities, unspecified (I80.3,n=110)	.69	1,505
Unspecified bacterial pneumonia (J15.9,n=152)	-.69	1,360*
Pneumonia, unspecified organism (J18.9,n=1,355)	-.80***	-1,349
Chronic obstructive pulmonary disease with acute lower respiratory infection (J44.0,n=202)	-.39	179
Chronic obstructive pulmonary disease with (acute) exacerbation (J44.1,n=1,836)	-.12	-1,421
Chronic obstructive pulmonary disease, unspecified (J44.9,n=3,598)	-.48***	-2,268*
Other and unspecified asthma (J45.9,n=184)	-.67	-902
Acute respiratory failure (J96.0,n=94)	-2.73*	-8,817
Respiratory failure, unspecified (J96.9,n=630)	-.60	-575
Crohn's disease, unspecified (K50.9,n=124)	-.25	-3,395
Alcoholic cirrhosis of liver (K70.3,n=122)	-.95	875
Calculus of gallbladder without cholecystitis (K80.2,n=166)	-.42	268
Acute pancreatitis, unspecified (K85.9,n=93)	.83	-19
Rheumatoid arthritis with rheumatoid factor, unspecified (M05.9,n=712)	-1.04*	-1,546
Rheumatoid arthritis, unspecified (M06.9,n=306)	-.21	640

Unilateral primary osteoarthritis of hip (M16.1,n=217)	.05	-326
Bilateral primary osteoarthritis of knee (M17.0,n=178)	.25	-463
Unilateral primary osteoarthritis of knee (M17.1,n=276)	-.50	-1,917
Chronic kidney disease, unspecified (N18.9,n=34)	-.46	-5,537
Calculus of kidney (N20.0,n=15)	-.30***	2,594
Acute cystitis (N30.0,n=51)	-.55	-36
Cystitis, unspecified (N30.9,n=10)	-8.77	-15,910***
Urinary tract infection, site not specified (N39.0,n=57)	-.38	-309
Fracture of lumbar vertebra (S32.0,n=21)	-.52	3,646
Fracture of upper end of humerus (S422,n=32)	-.29	583
Fracture of lower end of radius (S525,n=19)	.46	2,347
Fracture of head and neck of femur (S72.0,n=172)	-.27**	-735
Pertrochanteric fracture (S72.1,n=86)	-.13	-532
Subtrochanteric fracture of femur (S72.2,n=9)	-.12	-1,415

***: $p < .001$, **: $p < .01$, * < .05

Table 21: Risk of Readmission According to P-Natrium

	Odds Ratio, Readmission Within 30 Days Adjusted
P-Na, Nadir (mmol/L)	.986 [.964;1.007]
P-Na, Closest to Admission (mmol/L)	1.014 [.988;1.040]
Sodium Corrected Before Discharge (True/False)	1.002 [.738;1.344]
Diagnosis (Nadir Sodium)	
Sepsis, unspecified organism (A41.9,n=26)	N/A
Erysipelas (A46.9,n=84)	.86 [.63;1.09]
Malignant neoplasm of breast of unspecified site (C50.9,n=14)	N/A
Anemia, unspecified (D64.9,n=27)	.95 [.40;1.88]
Volume depletion, unspecified (E86.9,n=23)	N/A
Unspecified dementia (F03.9,n=5)	N/A
Alcohol dependence (F10.2,n=22)	N/A
Multiple sclerosis (G35.9,n=7)	N/A
Epilepsy, unspecified (G40.9,n=9)	N/A
Angina pectoris, unspecified (I20.9,n=42)	.76 [.52;.98]
Non-ST elevation (NSTEMI) myocardial infarction (I21.4,n=22)	1.71 [1.05;6.96]
ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction (I21.9,n=9)	N/A
Chronic ischemic heart disease, unspecified (I25.9,n=49)	.99 [.85;1.16]
Pulmonary embolism without acute cor pulmonale (I26.9,n=14)	.96 [.53;1.79]
Nonrheumatic aortic (valve) stenosis (I35.0,n=15)	N/A
Unspecified atrial fibrillation and atrial flutter (I48.9,n=226)	.91 [.83;1.00] *
Heart failure, unspecified (I50.9,n=41)	.81 [.63;.97] *
Stroke (I64.9,n=20)	.94 [.40;1.91]
Atherosclerosis of native arteries of the extremities (I70.2,n=8)	N/A
Phlebitis and thrombophlebitis of lower extremities, unspecified (I80.3,n=13)	N/A
Unspecified bacterial pneumonia (J15.9,n=13)	N/A
Pneumonia, unspecified organism (J18.9,n=213)	1.09 [1.00;1.18] *
Chronic obstructive pulmonary disease with acute lower respiratory infection (J44.0,n=44)	1.17 [.90;1.60]
Chronic obstructive pulmonary disease with (acute) exacerbation (J44.1,n=308)	1.06 [.99;1.15]

Chronic obstructive pulmonary disease, unspecified (J44.9,n=302)	1.00 [.94;1.07]
Other and unspecified asthma (J45.9,n=18)	N/A
Acute respiratory failure (J96.0,n=28)	.68 [.43;.92] *
Respiratory failure, unspecified (J96.9,n=36)	N/A
Crohn's disease, unspecified (K50.9,n=13)	N/A
Alcoholic cirrhosis of liver (K70.3,n=16)	1.49 [.95;3.85]
Calculus of gallbladder without cholecystitis (K80.2,n=15)	N/A
Acute pancreatitis, unspecified (K85.9,n=20)	N/A
Rheumatoid arthritis with rheumatoid factor, unspecified (M05.9,n=74)	1.01 [.76;1.39]
Rheumatoid arthritis, unspecified (M06.9,n=62)	.91 [.76;1.08]
Unilateral primary osteoarthritis of hip (M16.1,n=26)	N/A
Unilateral primary osteoarthritis of knee (M17.1,n=32)	N/A
Osteoarthritis of first carpometacarpal joint, unspecified (N18.9,n=34)	.81 [.51;1.14]
Calculus of kidney (N20.0,n=15)	N/A
Acute cystitis (N30.0,n=51)	1.00 [.84;1.22]
Urinary tract infection, site not specified (N39.0,n=57)	1.28 [.97;1.89]
Benign neoplasm of cerebral meninges (S32.0,n=21)	N/A
Fracture of upper end of humerus (S42.2,n=32)	.79 [.47;1.16]
Fracture of lower end of radius (S52.5,n=19)	N/A
Fracture of head and neck of femur (S72.0,n=172)	1.08 [.96;1.25]
Pertrochanteric fracture (S72.1,n=86)	.86 [.66;1.10]

***: $p < .001$, **: $p < .01$, * < .05

3.7 - PAPER 7

Kruse C, Eiken P, Vestergaard P.
Thiazide diuretics, bone mineral density and electrolytes: a systematic review and meta-analysis. Submitted 2017 July to Journal of Hypertension

3.7.1 - MATERIALS AND METHODS

The study was a systematic review and meta-analysis of randomized controlled trials examining thiazide use on BMD and electrolyte concentrations in serum and urine samples.

3.7.1.1 - SYSTEMATIC REVIEW

3.7.1.1.1 - LITERATURE STUDY

A systematic review was performed in February 2017 regarding thiazide use, bone mineral density progression (DXA-based) and changes in serum and urine samples of specific bone-related analyses. PRISMA guidelines were followed. The scope of the literature search comprised the Cochrane Library, EmBASE, PubMed, SCOPUS, Web of Science and SveMed+. For exposure keywords, general compound terms for thiazides and specific chemical forms were used. For outcome terms, bone mineral-related keywords, DXA-related keywords, osteoporosis-related keywords and the specific electrolytes were included.

3.7.1.1.2 - STUDY INCLUSION AND EXCLUSION

Studies were included for further analysis based on the following inclusion criteria; 1) randomized controlled trial design, 2) adult study participants, 3) female or mixed study population, 4) thiazide diuretics as solitary agent or combined with antiresorptives, 5) thiazide diuretics not compared to other diuretic agent, 6) study duration of minimum 6 months. Studies were similarly excluded if the following criteria were met: 1) not a randomized controlled trial and/or no control group, 2) publications that were conference abstracts, review articles or letters to the editor, 3) if included patients suffered from osteoporosis, this could not of a secondary nature, e.g. rheumatoid arthritis, malignancy-induced, gastroenterology disease.

3.7.1.1.3 - STUDY ASSESSMENT

Meta-data from all abstracts of all database sources were extracted and combined to one common format with the following information; 1) sequential ID number, 2) database source, 3) year, 4), journal, 5) authors, 6) title and 7) full abstract text. An interactive system was constructed in the R Statistical Software Package to present one abstract text and title at a time with blinding of journal, authors and year. Two of the authors, CK and PV, each assessed all abstracts as either “Accepted” or “Rejected”, the latter with associated reasons for decline. Decisions were stored to

allow for Kappa agreement calculations. Missing abstract texts were manually sought after by internet searches. Disagreements between reviewers were discussed internally and final decisions were made. From the accepted abstracts, full-texts were gathered either from online or Danish national library sources. All full-texts were assessed by CK and accepted or rejected according to outlined criteria for inclusion and exclusion.

3.7.1.1.4 - DATA EXTRACTION

From the final full-texts, the following data were extracted: 1) first author name, 2) title, 3) publication year, 4) journal name and abbreviation, 5) patient age (mean or median where applicable), 6) number of participants in either intervention or control group, 7) intervention and control group agent, name of pharmacologic agent and role as solitary or additive agent. Baseline and follow-up mean and standard deviations of the aforementioned end-points were calculated.

3.7.1.1.5 - BIAS ASSESSMENT

The included studies were assessed using the Cochrane Risk of Bias[215] tool to evaluate bias in reporting, detection, bias and selection. We used three groups of risk; low risk, moderate risk, high risk, and a separate category if "not applicable".

3.7.2 - STATISTICAL ANALYSIS

For each outcome, at least 2 studies were required for completion. Both fixed and random effects models were applied for each outcome, with study heterogeneity then deciding if the fixed or random effects model effect sizes were presented. Continuous outcomes were presented with standardized mean differenced and 95% CI. A p-value of $< .05$ was considered statistically significant. I2 above .50 (50%) and a Q statistical significance level below 5% were used to assess and conclude study heterogeneity.
Publication bias was assessed by funnel plots and linear regression tests of plot asymmetry.

3.7.3 - SUMMARY OF RESULTS

2,780 abstracts including duplicates were found using the search strategy. Reviewer #1 (CK) accepted 41 abstracts while reviewer #2 (PV) accepted 35 abstracts. 29 abstracts were assessed as not complete and gathered from external sources. In the final comparison, 25 abstracts were agreed upon, while 10 abstracts were disagreed upon (Unweighted Kappa agreement 83% [73% ; 93%]). The individual disagreements were discussed and 34 abstracts were selected for full-text inclusion. Seven (n=7) studies were included in the quantitative synthesis.

3.7.3.1 - BONE MINERAL DENSITY

The fixed effects model showed a non-significant, albeit borderline positive effect on BMD among thiazide users compared to control groups (Figure 4, SMD 0.0854 [-0.005 ; 0.175], p = .063). Heterogeneity was not present (I2 = 0.0%, Q = .95 (p = 1.00)). Test for between-subgroup differences was insignificant (Q = .50, p = .99) in the fixed effects model (Q = .50, p = .99). The contour funnel plot did not indicate publication bias (Figure 5).

Figure 4: Forest plot of BMD meta-analysis

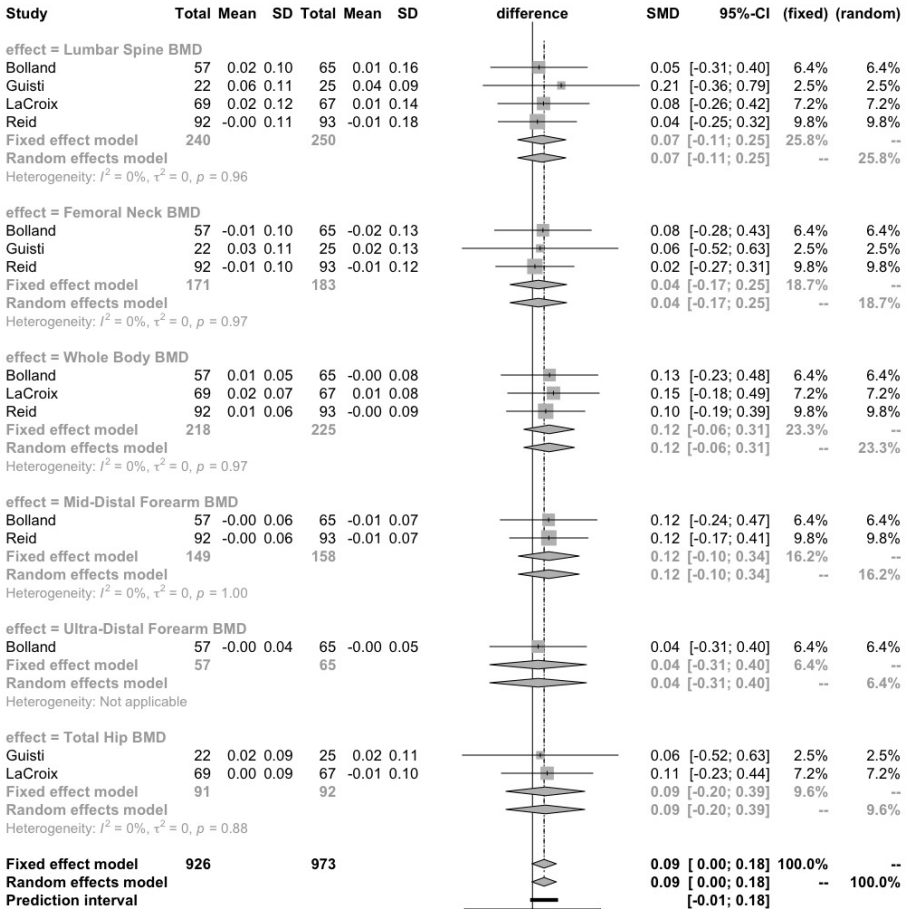
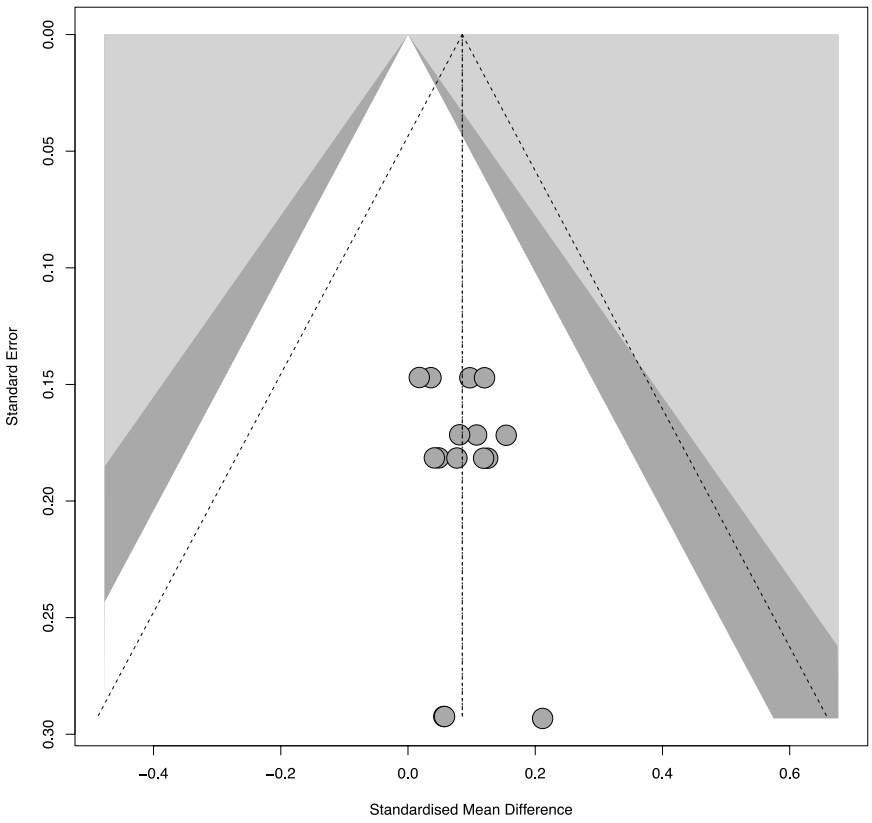


Figure 5: Contour funnel plot, BMD outcome



3.7.3.2 - SERUM CALCIUM

The random effects model showed a significantly increased Se-Ca among thiazide users compared to control groups (Figure 8, SMD .44 [.08 ; .81], p < .05). Significant study heterogeneity was discovered (I2 = 66.0%, Q = 11.85 (p < .05). A contour funnel plot (Figure 9) was symmetrical and agreed on a positive, significant effect. A linear regression model of funnel plot asymmetry did not indicate significant asymmetry (t = 1.98, p = .14).

Figure 8: Forest plot; serum calcium outcome

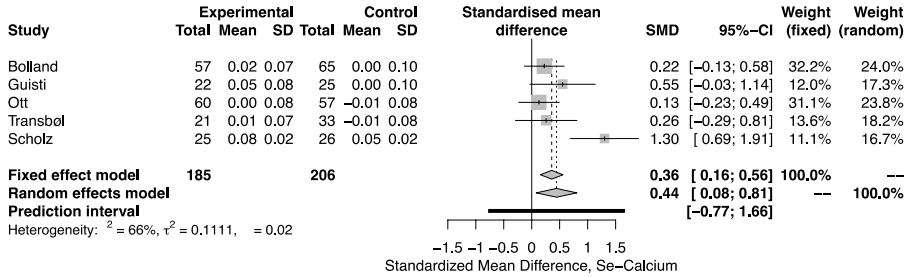
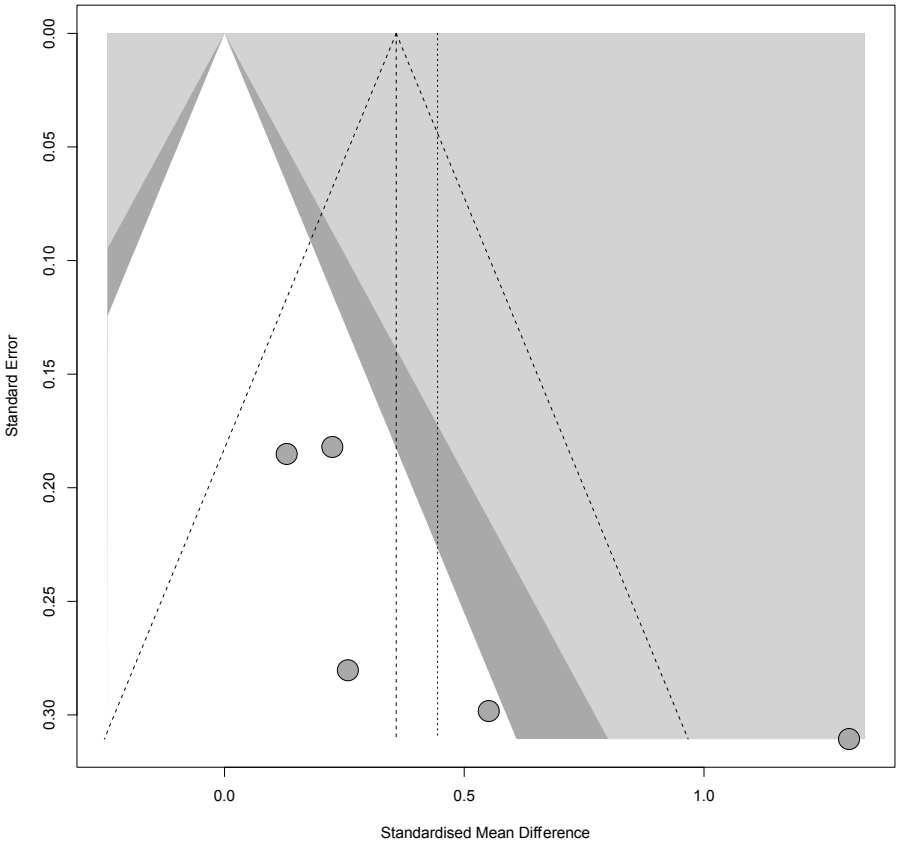


Figure 9: Contour funnel plot; serum calcium outcome



3.7.3.3 - SERUM PHOSPHATE

The random effects model did not show significant changes in serum phosphate among thiazide users compared to control groups (Figure 10, SMD -.41 [-1.05 ; .23], p = .21). Significant study heterogeneity was discovered (I2 = 84.0%, Q = 18.22 (p < .05).). A contour funnel plot did not indicate small-study bias (Figure 11). A linear regression model of funnel plot asymmetry did not indicate significant asymmetry (t = -.74, p = .53).

Figure 10: Forest plot, serum phosphate outcome

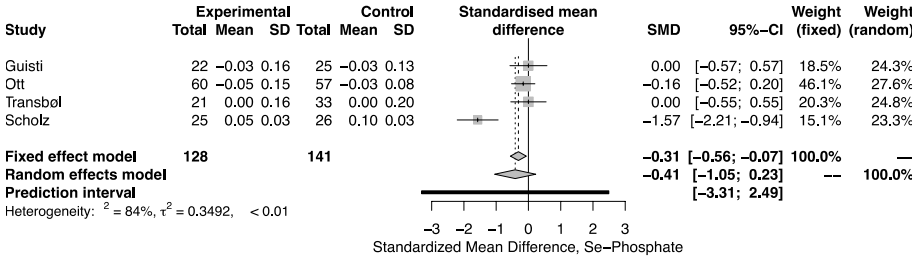
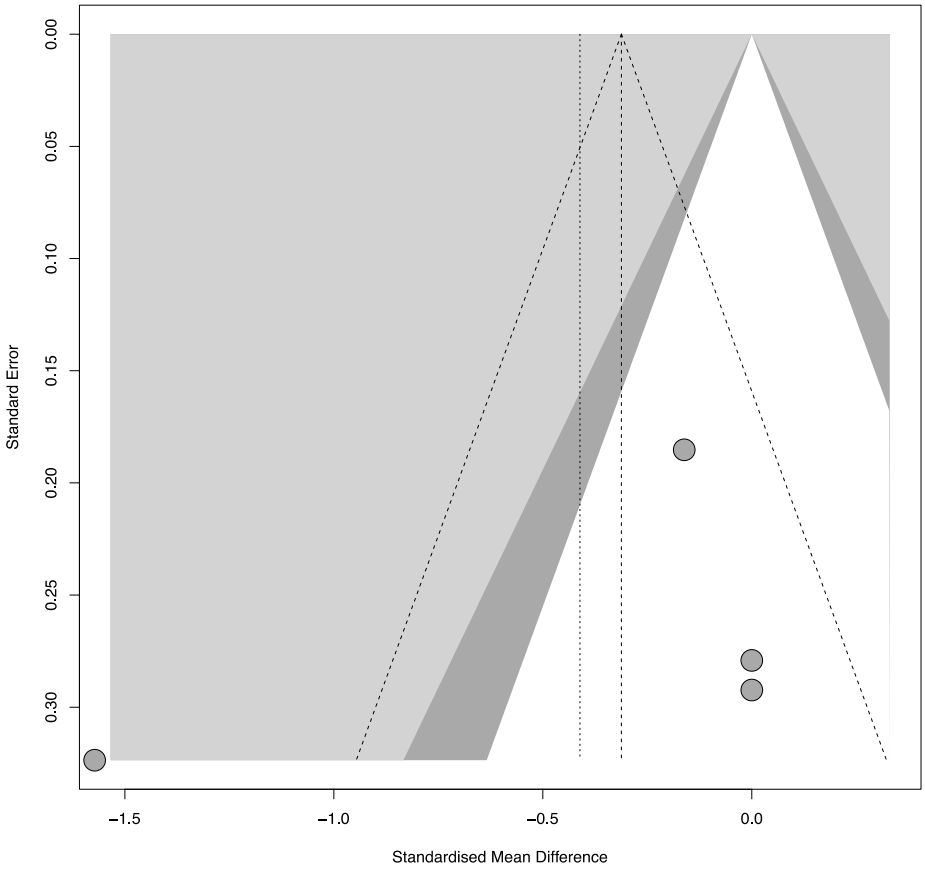


Figure 11: Contour funnel plot, serum phosphate outcome



3.7.3.4 - PARATHYROID HORMONE

The random effects model showed a borderline significant lower PTH among thiazide users compared to control groups (Figure 12, SMD -.42 [-.95 ; .11], p = .12). Significant study heterogeneity was discovered (I2 = 70.0%, Q = 6.68 (p < .05). A contour funnel plot did not indicate small-study bias (Figure 13). A linear regression model of funnel plot asymmetry did not indicate significant asymmetry (t = -.83, p = .56).

Figure 12: Forest plot, parathyroid hormone outcome

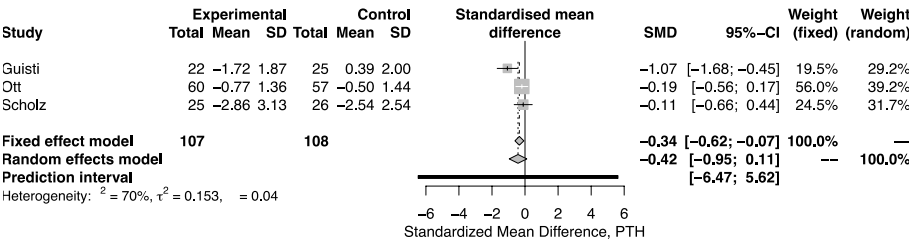
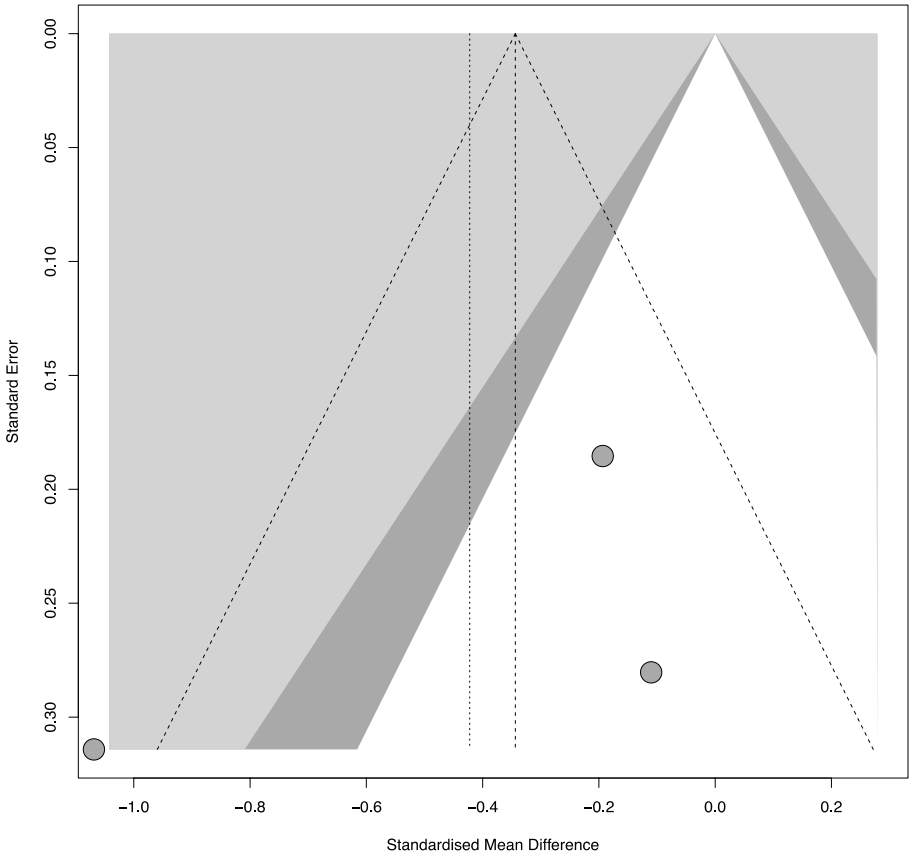


Figure 13: Contour funnel plot, parathyroid hormone outcome



3.7.3.5 - ALKALINE PHOSPHATASE

The fixed effects model did not show significant changes in ALP among thiazide users compared to control groups (Figure 14, SMD .08 [-.31 ; -.48], p = .68). No study heterogeneity was discovered (I2 = 0.0%, Q = 0.06 (p = 0.80). A contour funnel plot did not indicate small-study bias (Figure 15). A linear regression model of funnel plot asymmetry could not be completed.

Figure 14: Forest plot, alkaline phosphatase outcome

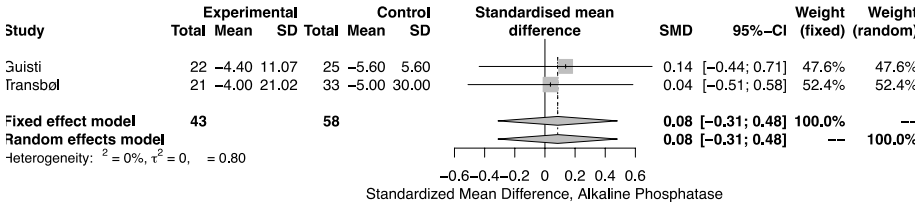
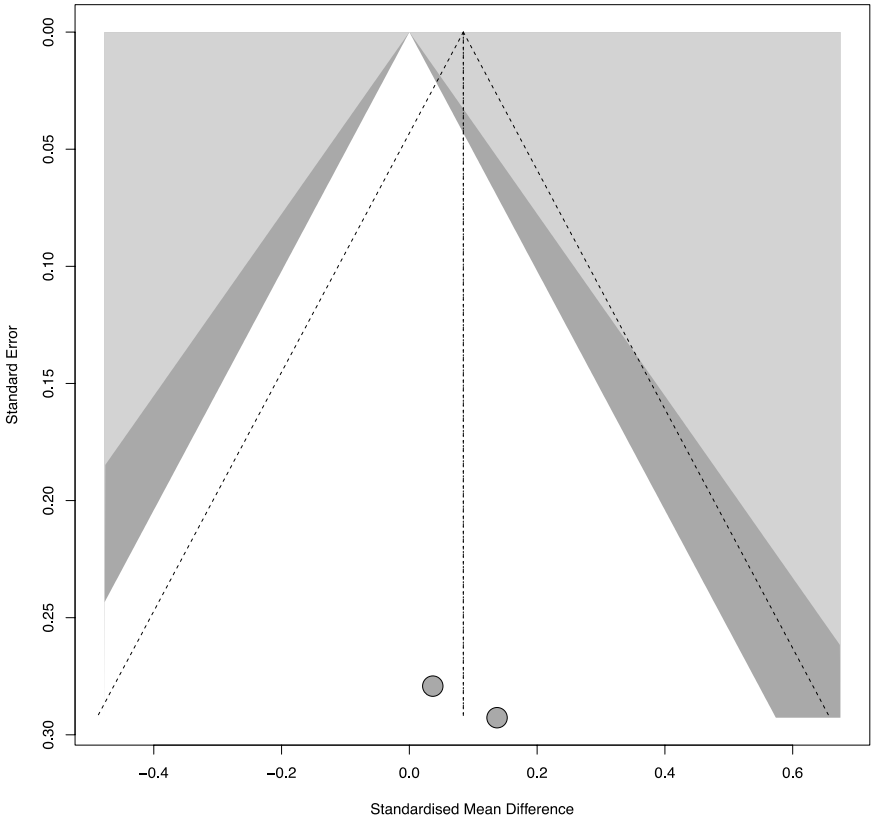


Figure 15: Contour funnel plot, alkaline phosphatase outcome



3.7.3.6 - URINARY CALCIUM

The random effects model showed a significantly lower U-Ca among thiazide users compared to controls (Figure 6, SMD -.96 [-1.72 ; -.20], p < .05). Significant study heterogeneity was discovered (I2 = 90.0%, Q = 30.1 (p < .05). The contour funnel plot indicated publication bias in the sense that positive effects were more likely to be published (Figure 7). A linear regression model of funnel plot asymmetry could not be completed.

Figure 6: Forest plot; urinary calcium outcome

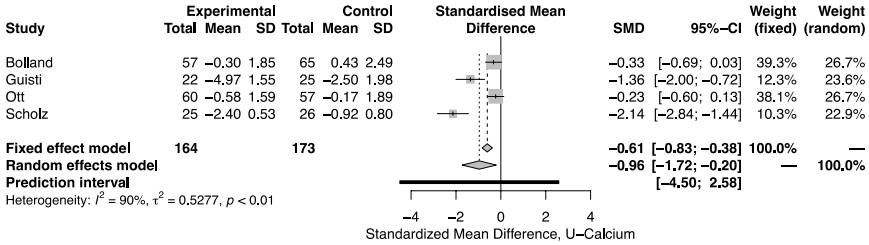
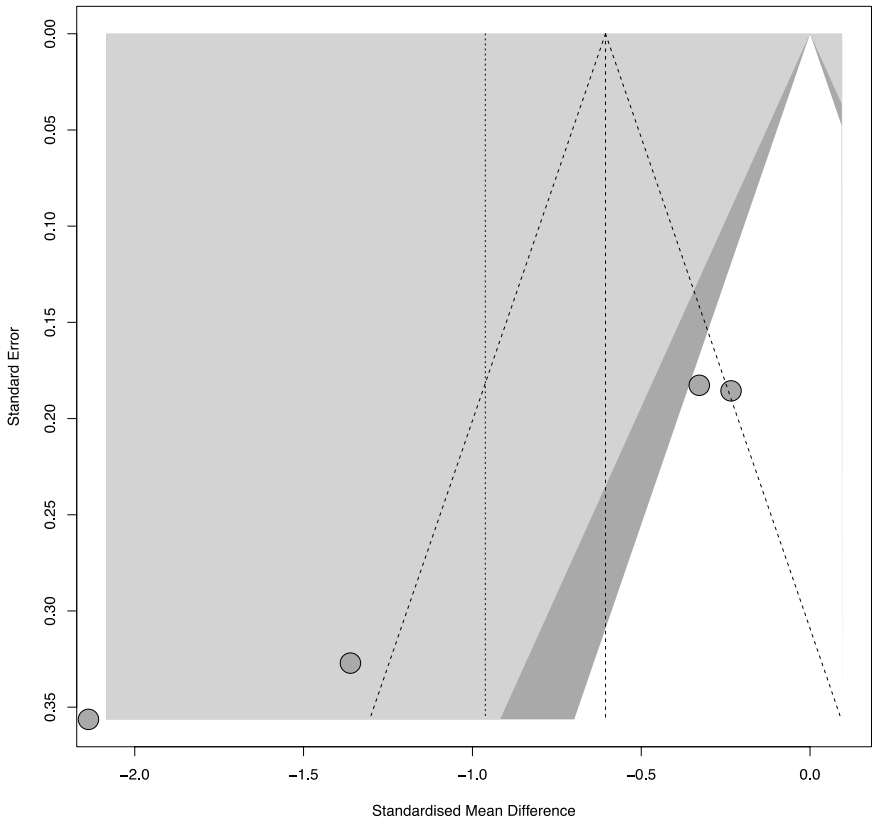


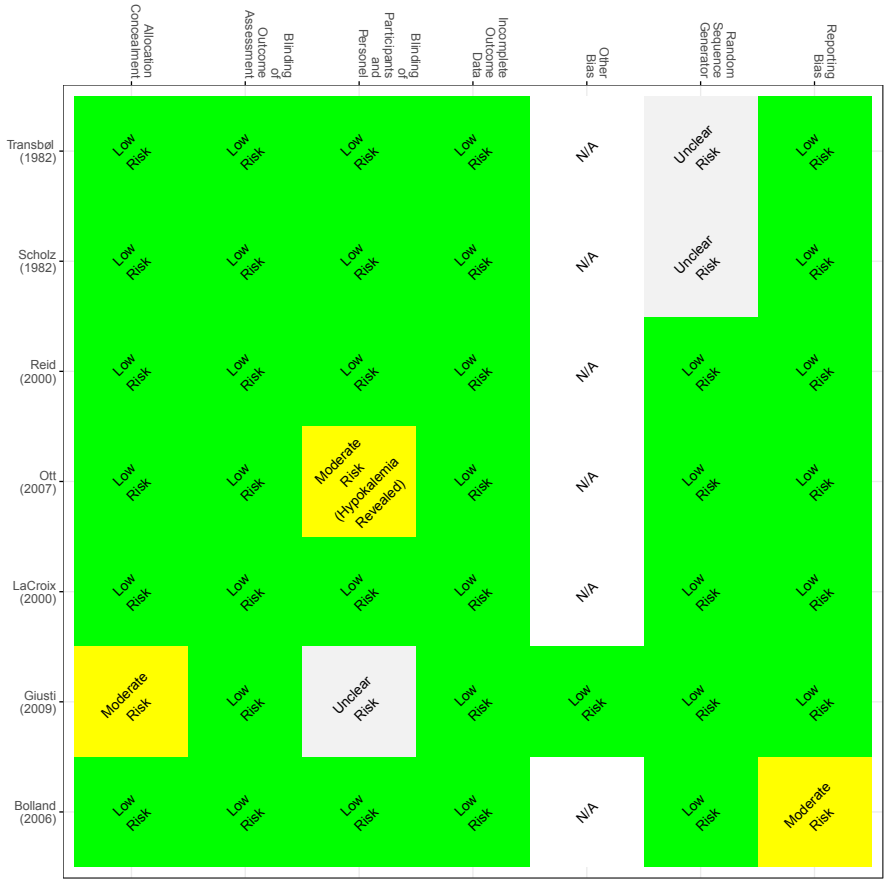
Figure 7: Contour funnel plot, urinary calcium outcome



3.7.3.7 - BIAS ASSESSMENT

Generally, a low risk of bias was found in all aspects of selection, blinding and outcome data collection. In certain studies, with no apparent pattern of year of publication, not all visit measurements were made available and there was a greater focus on visualizing progression of measurements compared to table presentations. No other obvious sources of bias were discovered (Figure 16).

Figure 16: Cochrane Bias Assessment of included studies



3.7.3.8 - SENSITIVITY ANALYSIS

For the outcome “serum calcium”, all study combinations without the study of Scholz et al[216] resulted in lower I^2 while maintaining a significantly positive effect size for the random effects model (Figure 17). For the outcome “serum phosphate”, exclusion of the Scholz et al[216] study decreased both the study I^2 and effect size of the random effects model to non-significance (Figure 18). For the outcome “urinary calcium”, study heterogeneity remained for all study combinations (Figure 19).

Figure 17: Sensitivity Analysis for the Serum Calcium outcome

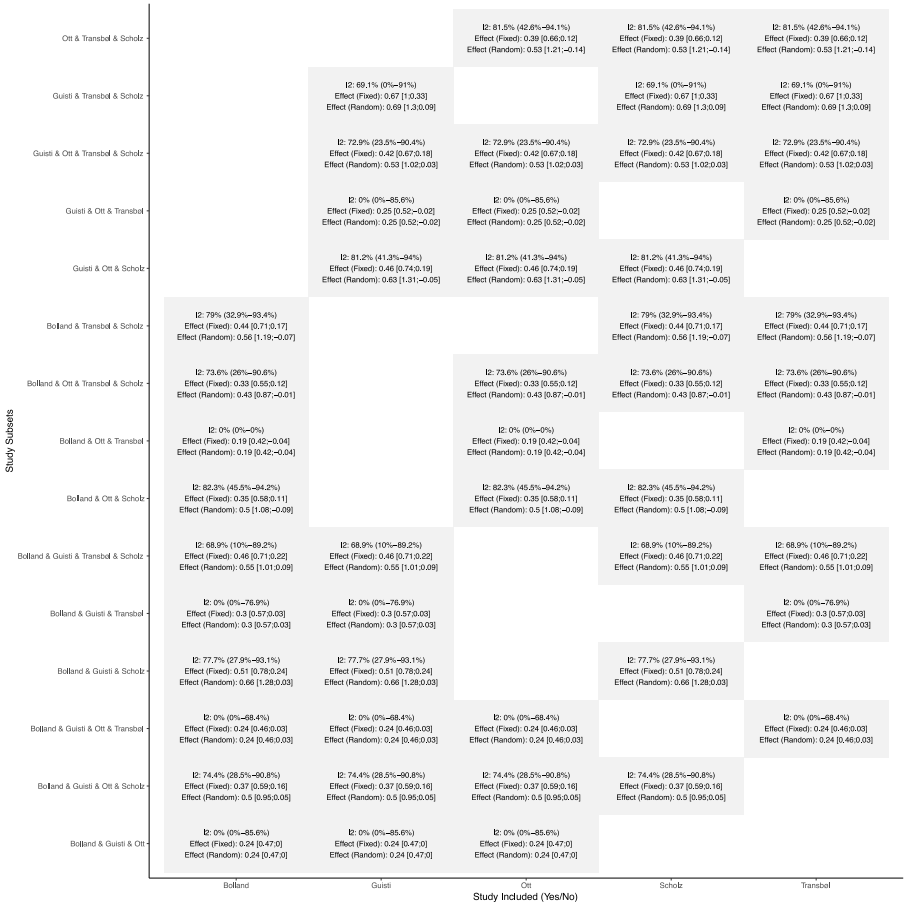


Figure 18: Sensitivity Analysis for the Serum Phosphate outcome

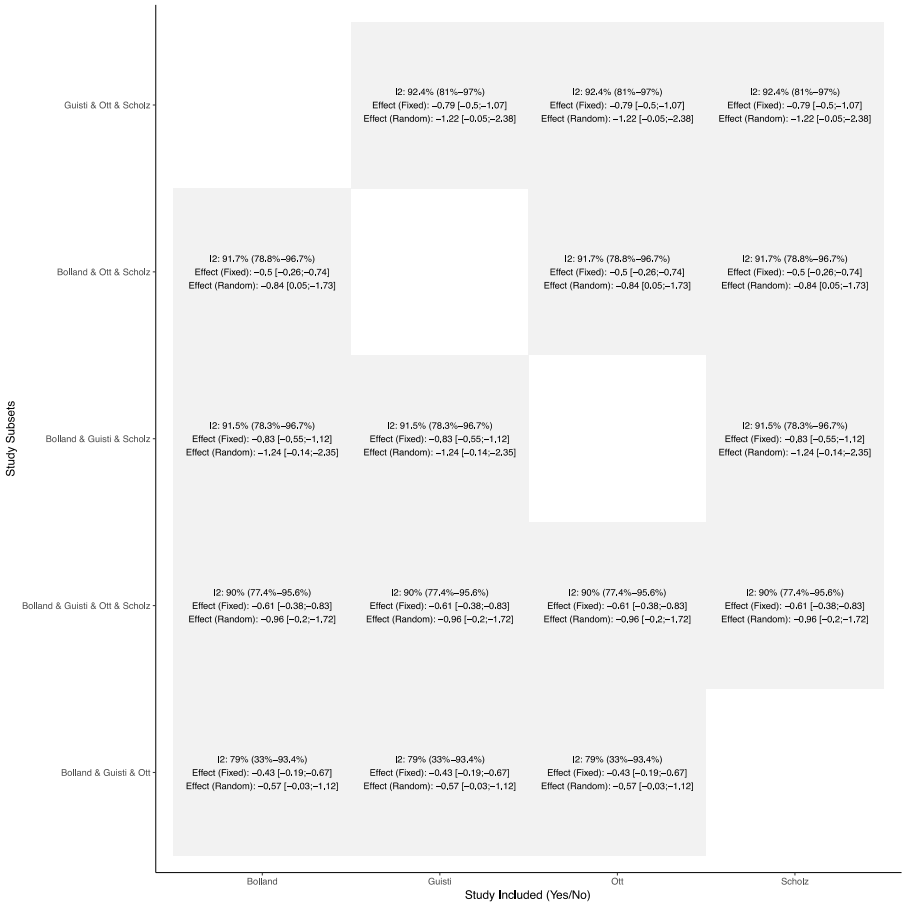
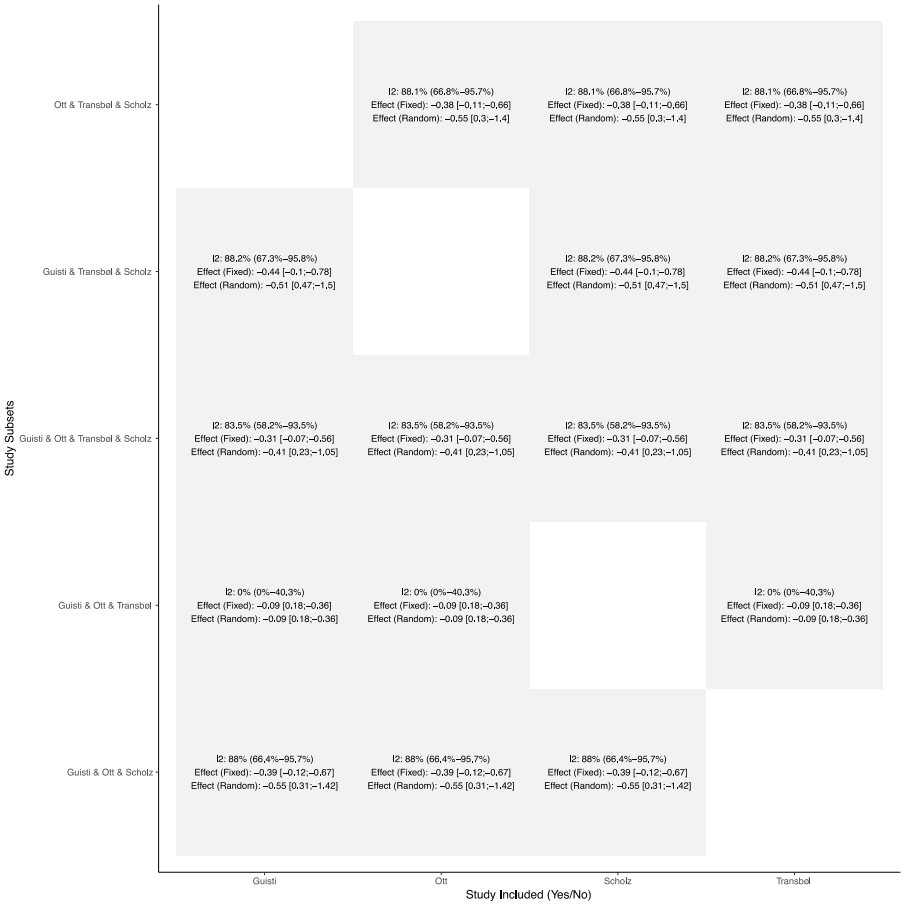


Figure 19: Sensitivity Analysis for the Urinary outcome



4. METHODOLOGICAL CONSIDERATIONS

4.1 - PAPERS 1-4, 6 (REGIONAL AND NATIONAL DANISH DATA):

Five of the included papers in this thesis are based on patient registry data collected on a regional level at Aalborg and Aarhus University Hospitals, combined with national data from the National Danish Patient Registry spanning several million individuals. There are both strengths and weaknesses to utilizing data resources of such a magnitude. The data reflects a prospective, real-life clinical setting and therefore reveals behavioral patterns that would be observed on a population-level outside a controlled research setting. Treatment adherence and effects related to age and temporal changes can illustrate use of certain pharmaceuticals and risk factors outside a controlled clinical setting. The large sample size also allows for sub-group analyses without limiting statistical power by selection.

However, data will be analyzed retrospectively and considerations about the scope and control of data collected are needed. For the DXA and biochemical data used in these papers, the population sample consisted of patients who had been referred to a DXA scan by their GP, not as a general research sample from the entirety of the originating population. This results in an inherent risk of selection bias, as patients who are more health-oriented, are within certain age groups and/or have risk factors for osteoporosis will more likely be referred than the general population. Severe cases of non-manifest osteoporosis due to poor health awareness, poor socioeconomic conditions or GP adherence could decrease the likelihood of being referred to a DXA scan. The same arguments can be placed on the blood samples, as data were collected based on blood samples in a normal clinical routine setting, not a research setting.

Specifically, for paper 1, when investigating the relationship between serum sodium, BMC and BMD, it is likely that patients with a serum sodium measurement available had this procedure performed due to greater health awareness or specific comorbidity compared to patients without serum sodium measurements. As hyponatremia was associated with parameters of greater frailty, i.e. older age and lower weight, it can be speculated that a population group with more severe hyponatremia and/or osteoporosis is not represented fully in the available data. Further in paper 2, the exclusion of patients with infrequent samples (assessed by calendar quarters with sodium samples) also contains a risk of selection bias, as patients with readily available sodium samples are likely more health oriented and well-adherent compared to those with less frequent samples. For both studies, the study period of data collection was long, which would normally cause concern about temporal changes in sample collection and analyses. However, low drift over time was observed for the DXA scan data and a daily QC program was in place for both sites, limiting the risk of compromised data quality.

Papers 3 and 4 do not utilize DXA or biochemical data and reflect how data in the national Danish patient registry are collected automatically and prospectively. The risk of information bias from the patients is therefore limited, but due to the retrospective nature of analyzing the data, certain sources of bias can be introduced. The reconstruction algorithm was not ascertained for the entirety or parts of the sample, and an estimate of adherence, e.g. percentage consumed of reimbursed doe, cannot be provided. Prolongation by 20% could be insufficient in some cases where large doses are reimbursed, e.g. as C03AB exists in 250 tablet sizes. Overlaps did not account for expiration dates of the medicine or repurchases, so the assumption that patients were fully adherent for several years cannot be ascertained easily.

4.2 - PAPER 5 (REGIONAL BELGIAN DATA):

This paper uses data collected prospectively over several years and investigates them at a cross-sectional level to predict future events. Several precautions were made to ensure high data quality. Patients had blood samples drawn in a fasting condition by trained laboratory technicians with certified Belgian credentials. DXA scanners were subjected to a daily QC program to ensure low drift over time. CVs were not available. Questionnaires were validated and internationally used tools. Blood pressure devices, stopwatches and dynamometers were calibrated. Grip strength, balance test and TGUG were performed several times to allow for mean and maximum value calculations.

The main methodological concern of this paper relates to the sample size and the prediction error intervals resulting from this approach. For the mortality outcome, the data quality of both the visit date and the ascertainment of mortality can be regarded as high, yet the exclusion of participants who participated in 1996 and not in 1997 resulted in only a moderate sample size being available when the data was split into training and test datasets. By further choosing an allocation of 75% to the *training* dataset and 25% to the *test* dataset, this infers a trade-off between higher predictive capability and subgroup pattern recognition with more training data, but greater test error when applying the model to new and unseen data.

Greater sample sizes than those available could have increased predictive capability. Thereby, a higher AUC would lend greater strength to the relative predictor importance. Probability calibration is also too difficult to perform credibly with a low sample size, as very small probability bins of events will occur if the entire spectrum of probabilities is not represented. A similar concern is the stability of the model predictions and predictor importance with increasing sample sizes. Models such as the “random forest” and “multivariate adaptive regression spline” are considered stable in terms of predictive power.

4.3 - PAPER 7 (SYSTEMATIC REVIEW AND META-ANALYSIS):

The main methodological concern of performing the meta-analysis in paper 7 relates to data extraction, as precautions were made to ensure a reproducible and agreed-upon study selection and sensitivity analysis procedure. Data were extracted from the published versions of the included papers which did not all present data transparently by means, SD or equivalent, transformable parametric data. For some studies, only graphical presentations of the results could be used to extract data on the specific end-points and time periods that were studied. The transformation from standard error or confidence interval to mean and SD is not as accurate as extracting parameters directly from datasets.

Further consideration relates to the age of certain papers. For certain end-points, i.e. serum phosphate, relatively few studies were included and a substantial proportion among them were older studies, possibly with different temporal effects and treatment standards. The strengths of the study were the novel, iterative sensitivity analysis to study the effects of heterogeneity when excluding specific study. This adds weight to the perception that the findings can be reproduced in new studies, as the excluded study consisted of a patient group with certain added characteristics, i.e. nephrolithiasis in the Scholz et al. study[216]. The literature study was performed with exhaustive combinations of search terms related to the outcomes, which led to a large number of abstracts needed screening, but also limited the risk that relevant studies were excluded. Using an interactive selection procedure limited risk of false exclusions and allowed for calculations of agreements between the two observers.

4.4 - DATA SOURCE QUALITY

4.4.1 - DXA SCAN DATA

The DXA scan data used for papers 1 and 2 were collected in a clinical setting by trained laboratory staff. Scans were performed using Hologic™ machines (Hologic 1000, Hologic 2000, Hologic Discovery) that all underwent a QC program of phantom scans and cross-calibration with low drift over time. The precision errors were very low; columna LSC 2.49%, total hip 2.78% and femoral neck 4.96%.

4.4.2 - BIOCHEMISTRY DATA

The biochemistry data originated from two departments of biochemistry at Aalborg and Aarhus University Hospitals in Denmark. Both institutions were certified by national agencies, i.e. DANAK for both the Department of Biochemistry at Aalborg University Hospitals (Appendix 9) and the Department of Biochemistry and Microbiology at Aarhus University Hospitals (Appendix 11). For the serum sodium samples used in papers 1 and 2, the sample was analyzed on a Roche-Cobas 6000/ 8000 with measureable interval between 80 and 180 mmol/L. At the low and high ends of the reference interval, the precision error was 2 SD (Appendix 10).

4.4.3 - DANISH PATIENT REGISTRY DATA VALIDITY

Data originating from the National Danish Patient Registry are collected prospectively and automatically, and is governed by national institutions to ascertain collection procedures are followed. Logical rules are applied to data to limit inconsistencies. The validity of diagnoses codes used in the National Danish Patient Registry has been studied with different scrutiny depending on specialties and disease categories.

For fractures specifically, few studies exist that all present favorable metrics on hip fracture validity. In a validity study of female Danish nurses[217], the positive predictive value was estimated at 89% for hip fractures, 75% for wrist fractures and 54% for upper arm fractures. In an older study, the precision of fracture diagnosis has further been estimated at 82%[218], while for certain patient groups, the validity is estimated to exceed 97%[219].

For other diagnoses, particularly the outcome diagnoses examined in paper 6, there is a generally high degree of validity in the National Danish Patient Registry[220]. Diagnoses within cardiology have a high degree of validity for general diagnoses of arrhythmia[221] and for myocardial infarction[222]; rheumatoid arthritis has a lower degree of validity[223].

5 - DISCUSSION

5.1 - SUMMARY OF RESULTS

- Hyponatremia was associated with lower BMC and BMD and a higher risk of osteoporosis in all regions of interest in DXA-scanned hips compared to normonatremic patients.
- No association was found between hyponatremia, decreasing serum sodium and BMC or BMD in the lumbar spine DXA-scanned regions of interest, nor with a higher risk of WHO-defined osteoporosis in the lumbar spine alone.
- Chronic mild hyponatremia ([Na+] = 130-137 mmol/L) was associated with lower BMC and BMD at baseline and with worse progression of BMC and BMD in several regions of interest in the hip. In the lumbar spine, only sporadically was chronic mild hyponatremia associated with worse progression of BMC and BMD.
- Thiazide users have an increased weekly risk of fracture prior to commencing therapy, which shows trends towards further increases during weeks 0-42 after commencing therapy. From weeks 43, continued use was associated with a linear trend towards decreasing per-week risk of fracture.
- From the age of 73, commencing therapy with thiazide diuretics is associated with an increased 10-year risk of fracture compared to non-users. Between 50 and 73 years of age, the risk is comparable to non-users.
- Previous randomized controlled trials show a borderline positive effect of thiazide use on BMD.
- Thiazide use causes lower urinary calcium, higher serum calcium, lower serum phosphate and borderline significantly lower plasma parathyroid hormone, but no change in serum alkaline phosphatase.
- Neither serum sodium nor thiazide use are relatively good predictors of mortality for older men compared to markers of physical performance.
- Hyponatremia during hospital admissions is associated with longer lengths of stay and greater costs of stay compared to normonatremic patients.

5.2 - BONE MINERAL CONTENT IN HYPONATREMIA

The primary hypothesis of this thesis was that hyponatremia is associated with lower BMD and a higher risk of osteoporosis compared to normonatremia. This hypothesis is based on previous epidemiologic studies of European and North American patients[18,116,120,224] as well as basal *in vitro* studies examining the effect of hyponatremia on osteoblast and osteoclast stem cells[20,122].

"Paper 1" reaffirmed this association using a large Danish patient sample and adds substantial knowledge about medication use and comorbidity presence.

Hyponatremia was found to be associated with lower BMC and BMD in all DXA-scanned regions of interest in the hip, with no association to osteoporosis in the lumbar spine. A novel finding was the difference between the hip and the lumbar spine in terms of severity, as lower sodium was linearly associated with lower BMC and BMD in all regions of the hip, while the same linear association was not shown between decreasing serum sodium and BMC or BMD in the lumbar spine's regions of interest. A possible explanation for this discrepancy is the difference in bone composition in the hip versus the lumbar spine. While the lumbar spine is composed mainly of trabecular bone to absorb gravitational energy and provide an elastic response hereto, the hip is composed mainly of cortical bone[225–229]. These differences are thought to arise from evolutionary needs of hip mobility versus shock absorption in the lumbar spine. The discovery in "paper 1" that regions of cortical bone seem to be more affected by hyponatremia is an original discovery and gives rise to a new hypothesis that aligns with older studies of sodium content in different areas of bone[230–232]. In canine studies as well as older isotope studies, it has been documented that cortical bone contains a relative abundance of sodium. A possible cause of hyponatremia-induced loss of bone mineral content is that the organism senses lower serum concentrations of sodium and mobilizes sodium from these bodily stores by osteoclast activity[231,232].

This hypothesis is supported by the previous *in vitro* findings of Verbalis et al. and Barsony et al.[18,20] where dilutional hyponatremia increased osteoclast activity and resorptive capability, even when the group adjusted for the resulting hypoosmolality of hyponatremia by insoluble mannitol. This technique strengthens the argument that the increased bone resorption observed in hyponatremia could be caused specifically by activation of sodium receptors and not by osmosensing receptors. One caution, however, is the conflicting role of vasopressin in bone and water metabolism. As noted previously, some studies have rejected that vasopressin activates and recruits osteoclasts[126], while others have shown an association between vasopressin and osteoporosis[123,124]. A very recent study by Greiller et al. showed further evidence that SIADH and the related abundance of vasopressin induces hypercalciuria which could aggravate bone resorption[233]. Further investigations of vasopressin are warranted, e.g. by stimulating cell culture responses of direct

vasopressin exposure when adjusting for hyponatremia and hypoosmolality through added sodium and mannitol.

An additional problematic aspect in the causal pattern of hyponatremia and lower bone mineral content are the phenotypes associated with hyponatremia and osteoporosis, and the resulting confounding that affects the overall understanding of the connection between the two. As it was documented in “paper 1”, patients with hyponatremia were older, weighted less and therefore had lower BMI. In hyponatremic patients, greater use of specific medication types known to cause hyponatremia or increased bone resorption, i.e. benzodiazepines, opioids and antihypertensives, were also found. In “paper 1”, adjustments were made for aggravating factors; age, gender and BMI, but inclusion of more factors would necessitate a larger sample size than 1,600 to allow for multiple comparison adjustments and to limit statistical type 1 and type 2 errors.

5.3 - PRO-RESORPTIVE MECHANISMS OF HYPONATREMIA

A secondary hypothesis of this study was that chronic hyponatremia exacerbates osteoporosis progression compared to normonatremia. This hypothesis was based on previous cellular works that investigated the responses of hyponatremia on mesenchymal and macrophage stem cells, and how this affected resorptive capabilities when exposed to bone tissue[18,20,122].

In “paper 2”, evidence was found that chronic hyponatremia is associated with worse progression of osteoporosis compared to normonatremic subjects.

An association was found between chronic mild hyponatremia ([Na^+] 130-137 mmol/L) and greater loss of trochanteric, femoral neck and absolute total hip BMD, and with greater loss of L1, L3 and L4 BMC. These findings were not as universal for one region as the association between lower serum sodium and lower BMD in all regions of the hip in “paper 1”, but patients with chronic mild hyponatremia did also start their periods of observation at a worse offset of lower hip BMC and BMD compared to normonatremic subjects.

Hyponatremic subjects began with lower BMD and suffered greater losses despite this fact. This gives rise to two hypotheses about this temporal effect. One is that hyponatremic patients are referred inappropriately late to their first DXA scan, at which it becomes relatively more difficult to sustain BMD and prevent fracture occurrence and progressive BMD loss. Evidence of this is the high mean age of 67.36 years (Table 6) at which hyponatremic patients underwent their first DXA scan, as well as the lower GP use observed in “paper 6” when hyponatremic patients are discharged. The age of 67 is above the ICSD recommendations that post-menopausal women should be referred to BMD estimation between ages 50-65 if one or more risk factors for low BMD are present. A further hypothesis is that hyponatremia is a risk factor that warrants a lower age cut-off. The second

hypothesis that "paper 2" gives rise to is the worrying aspect that adjustments for oral antiresorptives did not limit the increased bone loss caused by mild hyponatremia. If this finding is affirmed in prospective study, it would mean that the pro-resorptive mechanisms of hyponatremia-induced osteoporosis overwhelms the inhibition of mature osteoclasts by bisphosphonates, and possibly necessitates more aggressive therapy at the level of the RANK ligand immunoglobulin, denosumab[234]. This hypothesis is supported by the *in vitro* studies of Verbalis et al.[18] and Barsony et al.[20] where a dose-response relationship was found between lower sodium concentrations and both larger and more active (assessed via TARP-positive multinucleated cells[235,236]) osteoclasts, as well as more resorptive power assessed by resorption of dentin[20].

In future studies, an attempt to confirm this association should made by a cross-sectional study of human biomarker levels of bone resorption, i.e. CTX[237], with decreasing levels of serum sodium. From the perspective of the second major type of bone-related cell, the osteoblast, the finding from Fibbi et al.[122] of increased adipocyte differentiation with hyponatremia warrants further study and affirmation to understanding in this causal mechanism. While this hinders the presence of osteoblast and therefore a bone-forming response in regular physiology, this could also cause deteriorating bone quality as measurements of BMC or BMD in a DXA scan become unreliable with increased presence of fatty tissue. Secondly, mild hyponatremia ($[Na^+]$ 130-137 mmol) is generally considered a benign finding not associated with increased bodily distress necessitating diagnostic workup or therapeutic intervention[119,238,239]. If hyponatremia-induced osteoporosis commences at this mild level of hyponatremia, it warrants a new and more aggressive approach to hyponatremia management among this patient group.

5.4 - FRACTURE PROTECTION WITH THIAZIDE USE

The second primary hypothesis and a major aim of this thesis was to investigate potential age groups that benefit more from thiazide use than others. As substantial work has already been done to investigate a protective effect of thiazides on fracture risk, both through prospective and retrospective studies, the studies in "paper 3" and "paper 4" were designed not to reaffirm these associations, but to use the substantial sample size and automatic nature of data collection available from Danish registries to investigate temporal and age-related effects of this association. In "paper 4" of 10-year fracture risk of thiazide use, systematic age-groups of decreased fracture risk were not found, but strong signals of increased fracture risk, i.e. where not to commence and when to discontinue therapy, were found.

Commencing therapy between ages 50-63 was associated with a comparable 10-year risk; commencing therapy after age 73 was associated with increased 10-year fracture risk, and discontinuing therapy after age 63 was associated with lower 10-year fracture risk. These findings should be regarded in light of the physiological changes that occur in females after menopause and with further increases in age. It

has been shown in several studies that estrogen-depletion in menopause affects calcium homeostasis by increasing urinary excretion of calcium, and that supplementation of estrogen reduces this effect[240–243]. Lower calcium reabsorption causes a relatively lower abundance of mineralization components in serum and increases the need to mobilize calcium from bone and gastrointestinal absorption via increased PTH production[244,245]. The years between onset of menopause and age 65 are the ones of accelerating bone resorption until a progressively linear slope is observed[246].

The latest (2015) ISCD Official Positions[247] recommend that BMD be assessed for women above the age of 65 or post-menopausal women younger than 65 with one of more risk factors for low bone mass:

- Low body weight
- Prior fracture
- High risk medication use
- Disease or condition associated with bone loss.

Women during the menopausal transition are recommended to be referred if one or more clinical risk factors for fracture, i.e. low body weight, prior fracture or high-risk medication use, are present. If osteoporosis is diagnosed, treatment with oral antiresorptives, calcium and vitamin D is warranted[1].

From the findings of "paper 4", a new hypothesis can be made that thiazide-induced fracture protection occurs due to increased renal reabsorption of calcium and lower resorptive inclination by borderline lower PTH. This would explain the age-specific risk patterns between 50-63 where the risk was found to be comparable. A speculative explanation is that the calcium-retention effect provides the greatest degree of protection in this age group, while other mechanisms that increase fracture risk become relatively more predominant in older age groups, i.e. thiazide-induced hyponatremia. If this is true, it provides an explanation why the risk trends exponentially towards higher risk when commencing thiazide therapy after age 73. The finding that discontinuing therapy after age 63 is beneficial on 10-year fracture risk can be explained with lower hyponatremia risk when not receiving therapy.

If this rationale was to translate into clinical use, it would be one where thiazides are prescribed independently based on hypertension or an aim to protect from fractures and progression of osteoporosis. In the event that no comorbidities that necessitate thiazide use are present, e.g. hypertension, edema or calciuretic disease, thiazides would be prescribed at the onset of menopause and continued until other clinical recommendations of DXA scans come into effect at a higher age. In terms of duration of therapy, "paper 3" supports an approach where thiazides are used continuously and uninterrupted for several years, as the protective effects are likely to occur after 43 weeks of continuous use.

5.5 - THIAZIDE USE AND BONE MINERALIZATION

The patterns observed in "paper 3" and "paper 4" are substantiated by the causal effects observed in the meta-analysis of clinical trials in "paper 7". Evidence was found that thiazide use causes lower urinary excretion of calcium, increased serum calcium, borderline significantly lower PTH, lower serum phosphate, but no difference in serum alkaline phosphatase.

From this pattern, a hypothesis can be formed of how thiazides alter bone metabolism:

- Urinary calcium excretion decreases and causes an increase in serum calcium
- Increased serum calcium lowers serum PTH via inhibition of the CaSR
- Serum phosphate decreases due to decreased gastrointestinal absorption by lower PTH, lower 1,25-dihydroxycholecalciferol and increased urinary excretion due to thiazides
    - Lower serum phosphate decreases FGF-23 activity[57,248] and increases maximum availability of active 1,25-dihydroxycholecalciferol
    - Lower PTH and decreased 1,25-dihydroxycholecalciferol decreases bone mobilization of calcium[249]

The pattern of altered electrolyte metabolism simultaneously leads to unanswered questions:

- How does lower serum phosphate affect the mineralization quality of bone hydroxyapatite?[250,251]
- Should the unchanged concentration of alkaline phosphatase be seen as a marker of non-increased bone resorption?
- What is the relative weight between lower 1,25-dihydroxycholecalciferol by lower PTH and greater 1,25-dihydroxycholecalciferol by lower FGF-23 in a thiazide-exposed patient, particularly in relation to bone resorption?

The findings form a pharmacological argument for the use of thiazides in osteoporosis, as the patterns and possible causal chain are beneficial in an environment of increased bone resorption such as post-menopausal osteoporosis. However, the meta-analysis of the randomized controlled trial was only borderline significant in terms of a beneficial role of thiazides directly on bone mineral density progression. More statistical power via more clinical trials is needed to ascertain this protective pattern. As more than two studies were required for each end-point of the meta-analysis, insufficient studies were available to study the direct effects of thiazides on biochemical markers of bone resorption and anabolism. A lack of newer studies within the field also means that newer markers, e.g. CTX, P1NP[252], and osteocalcin[253–255], have not been studied previously. These are necessary to further examine the potential causal change in thiazides' effect on bone mineral density. This will aid our understanding of the mineralization quality and activity

caused by thiazides, as these are currently unanswered by the available clinical trials.

5.6 - BMD-INDEPENDENT FRACTURE RISK FACTORS

An added matter of complexity is represented by increased fracture risk independently of low BMD that seems to occur with hyponatremia. As exemplified by the work of Renneboog et al.[256], hyponatremia affects postural balance and gait stability in a negative way, which is reversible when attaining normonatremia through therapy.

In “paper 5”, the relative role of hyponatremia in predictive models of mortality and incident immobility was assessed in data of older European men. While not the same patient phenotype as the subjects of “paper 1” and “paper 2”, serum sodium and thiazide use did not figure among the most important predictors of either mortality or incident immobility. As a prediction model used at a cross-sectional level, no conclusions can be made about possible causalities, but the relatively low importance of serum sodium when predicting incident immobility indicates that other biochemical analyses such as 25-hydroxycholecalciferol and plasma testosterone infer greater weight on future physical performance.

Also in the descriptive comparison between 5-year mortality and 5-year survival, no difference in serum sodium was observed between the groups with very high statistical confidence, while markers of physical performance were worse in the mortality group (i.e. RDRS-2, TGUG, grip strength and SMI). This warrants further study of if and how hyponatremia causally affects physical performance, as the findings of *in vitro* studies[257] that hyponatremia can affect muscle tissue by collagen infiltration does not translate to lower physical performance *in vivo* in this paper. From an ethical standpoint, the effect on physical performance when correcting from chronic hyponatremia to normonatremia would be an apt first choice to study this inference in more detail.

5.7 - THRESHOLDS FOR HYPONATREMIA INTERVENTION

The findings of hyponatremia’s role in both osteoporosis, mortality and burdens of therapeutic care also warrant a discussion about thresholds for intervention and diagnostic workup. Currently, mild hyponatremia between 130-137 mmol/L is seldom seen as requiring full workup and intervention to correct to normonatremia, but a growing body of evidence within the scope of this thesis and other areas, particularly mortality[238,258,259], are increasingly suggesting a more aggressive approach to management of mild hyponatremia.

From the papers of this thesis, retrospective associations have been found between decreasing serum sodium and increased risk of osteoporosis, lower hip BMC and

BMD and increased lengths of stay and costs of stay during hospitalizations. Other recent epidemiologic studies[238,258], have shown strong associations between categorically lower sodium concentrations and increased mortality in patients at general practitioners. The papers of this thesis do not examine the effect of correcting hyponatremia on mortality and BMD progression, and as such, a new hypothesis warranting further investigation is whether this protects from mortality and fracture occurrence.

In a retrospective study by the Verbalis group[15], chronic hyponatremia with no correction further increased fracture risk compared to non-correction. A current clinical study in Cologne, the "Characterization of Neuropsychologic and Physical Performance in Geriatric Patients With Hyponatremia" study (ClinicalTrials ID: NCT02242604), is examining whether patients with serum sodium below 130 mmol/L are affected positively in terms of physical performance, cognitive ability and mortality by correction to normonatremia.

A further hypothesis that warrants investigation is whether correcting moderate and mild hyponatremia either by limiting the causal mechanism and/or altering water metabolism through vaptans cause improve bone mineralization through lower resorption and improved bone formation.

5.8 – VISUALIZING THE RELATIONSHIP BETWEEN HYPONATREMIA, THIAZIDES AND OSTEOPOROSIS

The intricate relationship between hyponatremia, thiazides and osteoporosis is visualized in figure 20 as a combination of state-of-the-art knowledge with the novel findings and hypotheses generated from this thesis. The points of contention needed to be further clarified are also illustrated.

5.8.1 – ELECTROLYTE DISTURBANCES

Evidence exists that thiazides increase the risk of several electrolyte disturbances, i.e. hyponatremia, hypomagnesemia, hypophosphatemia and hypercalcemia. Individually, these four have been shown to decrease PTH secretion and FGF-23 activity, but further study is warranted to document the relative importance of:

1) Decreased bone resorption by lower PTH secretion itself
2) Decreased calcium mobilization from bone by lower 1,25-dihydroxycholecalciferol by lower PTH
3) Greater or lower bone formation by greater 1,25-dihydroxycholecalciferol caused by lower FGF-23 activity

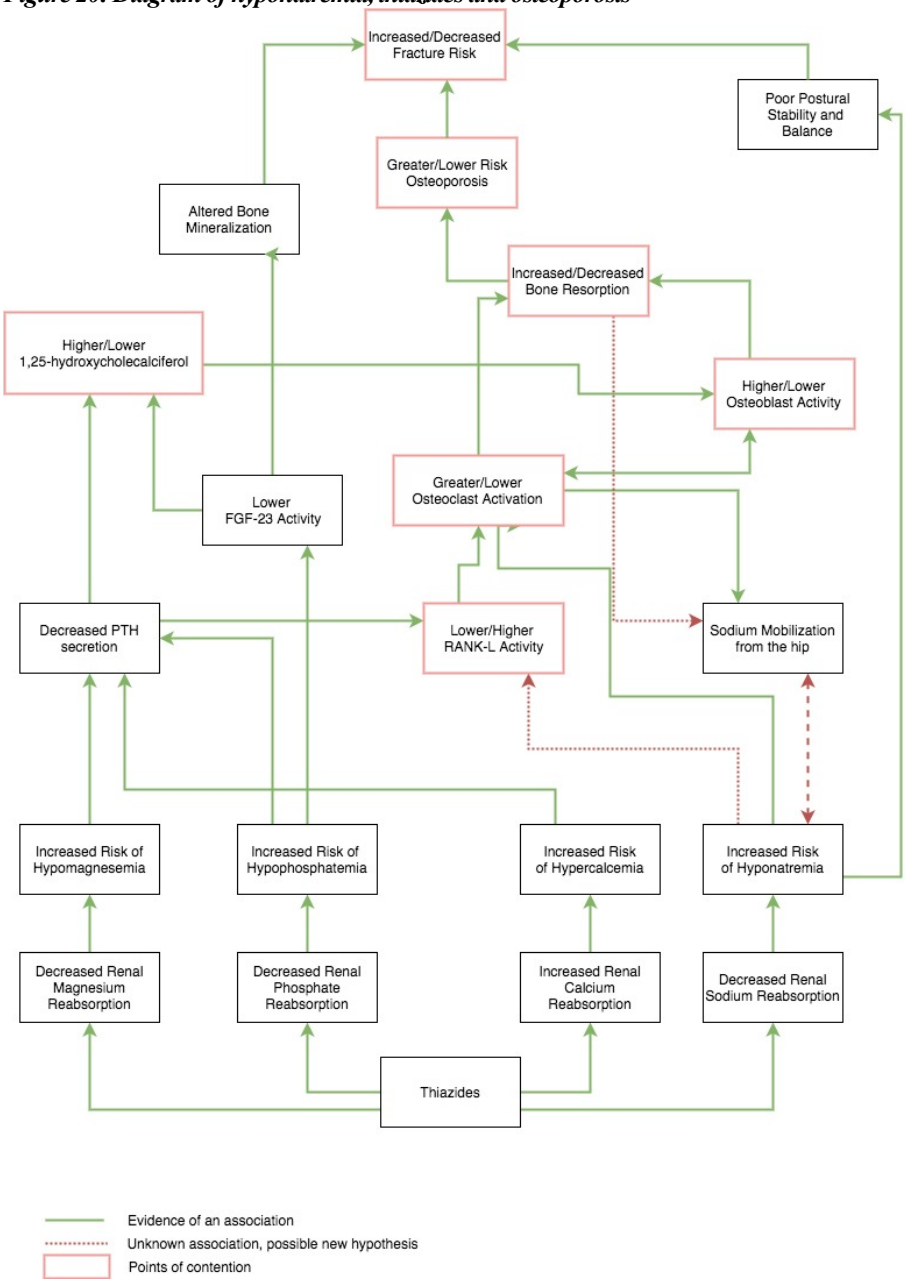
Novel hypotheses in this regard also warrant further research:

1) Does hyponatremia directly induce increased RANK-L activity?
2) What is the intricate relationship between bodily stores of sodium and hyponatremia?

5.8.2 – OSTEOCLAST AND OSTEOBLAST ACTIVITY

Hyponatremia and thiazides also appear to induce greater and/or lower osteoclast and osteoblast activity, where the relative weights between the two effects warrant further study. This also concerns the direct regulation between osteoblasts and osteoclasts in an environment of hyponatremia. With thiazide treatment, the works of this thesis indicate that short-term therapy increases the risk of fractures, which can be speculated to be due to electrolyte disturbances and their effect on postural balance. However, long-term therapy appears to decrease fracture risk, speculatively due to decreased bone resorption and increased BMD, which was found to be borderline positively affected by thiazide therapy. Substantial work both clinical and *in vitro* is needed to further this research topic.

Figure 20: Diagram of hyponatremia, thiazides and osteoporosis



6 - CONCLUSION

The initial hypotheses were:

6.1 - PRIMARY HYPOTHESES

1. Hyponatremia is associated with lower BMC and BMD, and a higher risk of osteoporosis compared to normonatremia.
2. Thiazide use is protective against fracture occurrence in certain female age groups.

6.2 - SECONDARY HYPOTHESES

1. Chronic hyponatremia is associated with worse progression of osteoporosis compared to chronic normonatremia.
2. Thiazide use improves BMD compared to none-use.
3. Long-term thiazide use is necessary to obtain a protective effect against fracture occurrence.
4. Thiazide use alters urinary excretion of electrolytes in a matter that is beneficial to osteoporosis.
5. Hyponatremia is associated with greater comorbidity and economic burdens among hospitalized patients.
6. Hyponatremia is predictive of mortality.

6.3 - CONCLUSIONS ON INITIAL HYPOTHESES

1. Hyponatremia and decreases in serum sodium are associated with lower BMC and BMD and a higher risk of osteoporosis compared to normonatremia in the hip region, not the lumbar spine.
2. Thiazide use was not found to be protective at specific age-groups compared to non-use, but age groups of substantially increased risk were found compared to non-users.
3. Chronic mild hyponatremia ([Na+] 130-137 mmol/L) is associated with lower BMC and BMD at baseline and with worse progression of BMC and BMD loss at certain areas of the hip.
4. The combined evidence of existing clinical trials on thiazides and BMD did not show a significantly positive effect of thiazide use on BMD progression, albeit the changes in BMD were borderline significantly positive.
5. Thiazides are associated with progressively lower weekly risk of fracture occurrence after long-term use beyond 43 weeks.
6. The combined evidence of existing clinical trials on thiazides and bone mineral density show that thiazides form a beneficial pattern of changes in

electrolyte concentrations by borderline lowering serum PTH, possibly by increased serum calcium.

7. Hyponatremia was not a relatively important predictor of mortality.
8. Hyponatremia is associated with a dose-response relationship between lower serum sodium and greater LoS and CoS in hospitalized patients, but not with readmission risk as a whole.

6.4 - OVERALL CONCLUSION

Hyponatremia was found to be associated with osteoporosis and worse progression of osteoporosis, particularly affecting the hip region. Further prospective study is warranted to ascertain this relationship and the possible effects of remedying the condition to normonatremia. Even mild hyponatremia ([Na+] 130-137 mmol/L) was associated with an increased risk of osteoporosis and osteoporosis progression in specific areas of the hip and lumbar spine despite a worse offset, warranting a new discussion about therapeutical thresholds and ages to intervene. Thiazides were associated with protection against fractures for users as a whole when used continuously and long-term beyond 43 weeks, but no systematic age groups with beneficial 10-year fracture risks after commencement were found. Thiazides provide a pattern of altered electrolyte metabolism that is likely beneficial against osteoporosis, but only shows a borderline significantly positive effect on BMD directly in current clinical trials. More randomized, controlled trials of thiazides and BMD are warranted to further support the argument for thiazides as a novel antiresorptive agent.

7 - PERSPECTIVE

As the first randomized controlled trials of thiazides as antiresorptive agents were conducted more than three decades ago, the hypothesis that increased renal reabsorption of calcium could prevent deteriorating bone strength is not a novel hypothesis of recent years. Despite many clinical trials, thiazides do not figure in European or American guidelines of osteoporosis management. This thesis has documented a positive pattern of altered electrolyte metabolism when thiazides are commenced in a controlled fashion, and the borderline significant, positive effect on BMD indicates that one more clinical trial showing beneficial effects could provide the final evidence that thiazides affect BMD positively.

The "BONATHIAD – Bone Association With Thiazide Diuretics" clinical trial investigating thiazides' effect on both BMD, bone architecture, balance and serum electrolyte changes over 48 weeks is currently taking place at the Department of Endocrinology, Aalborg University Hospital. The study is intended to conclude in late 2018 and could provide new information on fracture-predisposing risk factors with thiazides and hyponatremia, i.e. balance and physical performance, as well as a greater sample size for another systematic review and meta-analysis.

Provided that BMD improves with thiazide use in a randomized controlled setting, this gives an explanation of the many retrospective studies that have found a protective role of thiazides on fracture risk. This thesis adds to the understanding of this mechanism by showing that prolonged, continuous use for more than 43 weeks seems necessary to obtain the protective effect. However, as a substantial sample size is necessary in all randomized, clinical trials with fractures as the primary endpoint, it would require substantial, multi-center efforts with follow-ups for longer than a year to ascertain this evidence in a prospective and controlled matter.

Through the linear relationship between decreasing sodium, lower BMD and more burdensome hospitalization, this thesis adds to the understanding that hyponatremia even just below the reference interval should call for diagnostic workup and therapeutic management. As basal *in vitro* evidence is present that hyponatremia increases bone resorption in a dose-response relationship, an obvious next step is to investigate decreases in serum sodium and the effect on the near and long term on biochemical markers of bone resorption (e.g. CTX) and bone anabolism (e.g. P1NP). As there will be substantial ethical considerations of inducing hyponatremia in healthy individuals, a prospective trial should investigate if correcting hyponatremia to normonatremia alters bone metabolism, i.e. through the same biochemical bone markers.

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Hyponatremia, a condition of low serum concentrations of sodium, shares an intertwined and often paradoxical relationship with thiazide diuretics and osteoporosis. In retrospective studies, thiazides have been shown to protect against osteoporosis-related fractures, but also to cause hyponatremia which is associated with a higher risk of falling. In recent years, evidence has been found of an association between hyponatremia and osteoporosis in epidemiological and basal *in vitro* studies. Further research is needed to determine three aspects; who will benefit from thiazides in terms of fracture risk, why this is the case, and who are running an unnecessary risk of thiazide-induced hyponatremia when commencing therapy, predisposing to fractures.

The aim of this thesis was to investigate the role of hyponatremia in Danish osteoporosis patients, to investigate possible age groups that may benefit from thiazide therapy on fracture risk, and to examine the effect of hyponatremia on mortality, hospitalization burden and readmission risk.

This thesis is based on six retrospective epidemiological studies using Danish and Belgian data of regional and national origins, and one systematic review and meta-analysis reviewing clinical trials of thiazide use on bone mineral density and electrolyte metabolism.

SUMMARY

Hyponatremia, a condition of low serum concentrations of sodium, shares an intertwined and often paradoxical relationship with thiazide diuretics and osteoporosis. In retrospective studies, thiazides have been shown to protect against osteoporosis-related fractures, but also to cause hyponatremia which is associated with a higher risk of falling. In recent years, evidence has been found of an association between hyponatremia and osteoporosis in epidemiological and basal in vitro studies. Further research is needed to determine three aspects; who will benefit from thiazides in terms of fracture risk, why this is the case, and who are running an unnecessary risk of thiazide-induced hyponatremia when commencing therapy, predisposing to fractures. The aim of this thesis was to investigate the role of hyponatremia in Danish osteoporosis patients, to investigate possible age groups that may benefit from thiazide therapy on fracture risk, and to examine the effect of hyponatremia on mortality, hospitalization burden and readmission risk. This thesis is based on six retrospective epidemiological studies using Danish and Belgian data of regional and national origins, and one systematic review and metaanalysis reviewing clinical trials of thiazide use on bone mineral density and electrolyte metabolism.

ISSN (online): 2246-1302
ISBN (online): 978-87-7210-068-5

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