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A nationwide cohort study

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Title

The risk of osteoporosis is not increased after cholecystectomy. A nationwide cohort study

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Abstract

Cholecystectomy alters bile flow and quality which might compromise the absorption of Vitamin D and Vitamin K which are important for bone health. We aimed to assess the risk of osteoporosis after cholecystectomy. **Methods:** We performed a nationwide, register-based, cohort study using individual-level data from the Danish Health Registers. Individuals who had cholecystectomy performed between 1995 and 2017 were included as were five sex- and aged-matched controls for each case. We assessed fractures, ICD diagnosis of osteoporosis and osteoporosis treatments. We defined a composite endpoint for osteoporosis consisting of 1) hip or spine fractures, 2) ICD diagnosis of osteoporosis or 3) osteoporosis treatments. **Results:** We included 149.968 cases and 727.456 controls that were followed for a median of 9 years (inter-quartile range 4-15 years). In the cholecystectomy cohort, 6.4% reached the composite endpoint compared to 6.6% in the reference population (HR 0.98 (0.96-1.03)). In age-stratified analyses, minor risk increases were observed in the cholecystectomy cohort compared to the reference population in the composite endpoint for osteoporosis (age group ≤30 years, 0.8% vs. 0.6%, HR 1.42 (1.19-1.71)), fractures rates (≤30 years incidence rate ratio (IRR) 1.38 (1.32-1.43), age group 31-50 years IRR 1.10 (1.08-1.43)) and ICD osteoporosis diagnosis (≤30 years, 0.3% vs. 0.2% HR 1.40 (1.04-1.87)). **Conclusion:** The risk of osteoporosis was similar in patients with prior cholecystectomy and controls. In younger subgroups, the risk of osteoporosis and fractures was slightly increased but absolute risks were low. These findings do not support that cholecystectomy leads to any major calcium-metabolic disturbances.

1 Introduction

The primary function of the gallbladder is to store and secrete bile into the intestine after food intake. Gallstones are hardened deposits of bile and/or cholesterol in the gallbladder and may become symptomatic. The prevalence of symptomatic gallstones is 10-15% in the adult Caucasian population in developed countries (1). Cholecystectomy is a common abdominal surgical procedure that is most often performed for gallbladder stone associated diseases (2). After cholecystectomy, the liver keeps producing bile acids, but the bile is less concentrated (3). Bile is important for the absorption of fat-soluble vitamins such as vitamin D and vitamin K. Insufficiency of these vitamins is associated with an increased risk of fractures (4-6). Changes in bile flow or quality after cholecystectomy have generally not been considered to induce malabsorption of nutrients, minerals or vitamins, although it has been found that cholecystectomy is associated with lower levels of vitamin D (7-9).

Compromised absorption of fat-soluble vitamins after cholecystectomy could result in bone loss and an increased risk of osteoporosis. Two studies have evaluated bone mineral density (BMD) after

cholecystectomy. One study from Turkey showed that prior cholecystectomy was associated with lower 25-hydroxyvitamin D, higher parathyroid hormone levels and lower BMD in postmenopausal women compared to control subjects (7). Another study also from Turkey found that even though prior cholecystectomy was associated with lower vitamin D levels, these patients did not have a lower BMD (8). In addition, an epidemiological study from South Korea assessed the fracture risk following cholecystectomy and found a small increase in the incidence of fractures in individuals with prior cholecystectomy compared to controls (10).

This register-based nationwide cohort study aimed ~~aim~~ to assess fracture rates and risk of osteoporosis in individuals with prior cholecystectomy in comparison with age and sex-matched reference individuals from the general population. We hypothesized that cholecystectomy increases the risk of fractures and osteoporosis.

2. Subjects and Methods

2.1 Study design

This is a nationwide, population-based, cohort study using data from the Danish Health Registers (11). We included patients who had undergone cholecystectomy between January 1st 1996 and December 31st 2017. For every patient with prior cholecystectomy, we included five age- and sex-matched reference individuals. We defined the index date as the date of surgery for both cohorts. The participants were followed until the end of the study, migration or death.

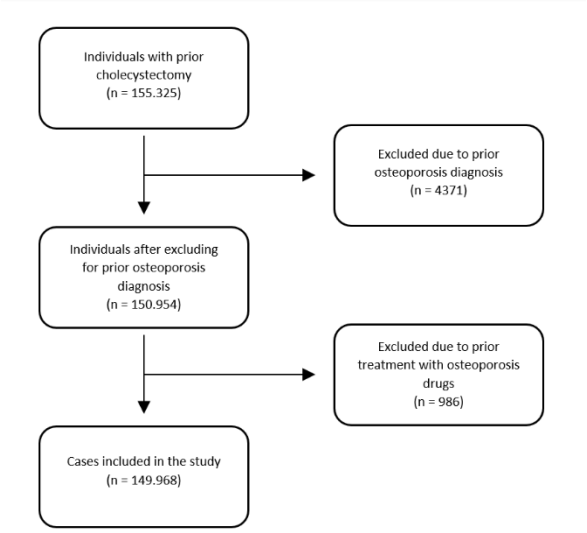
2.2 Data sources

In Denmark, every person with a permanent residence is assigned a unique personal identification number. This personal identification number makes it possible to link data from different registers. Information on gender, date of birth, place of residence, emigration and immigration is recorded in The Danish Civil Registration System (12). The Danish National Patient Registry contains information on visits to hospital outpatient clinics and emergency departments, hospital admissions and discharges, surgical procedures and medical diagnoses (11). Information on dispensed prescriptions is recorded at the individual level in The Danish National Prescription Registry since 1994 (13). Together these registers supply the information necessary for the present study.

2.3 Study population

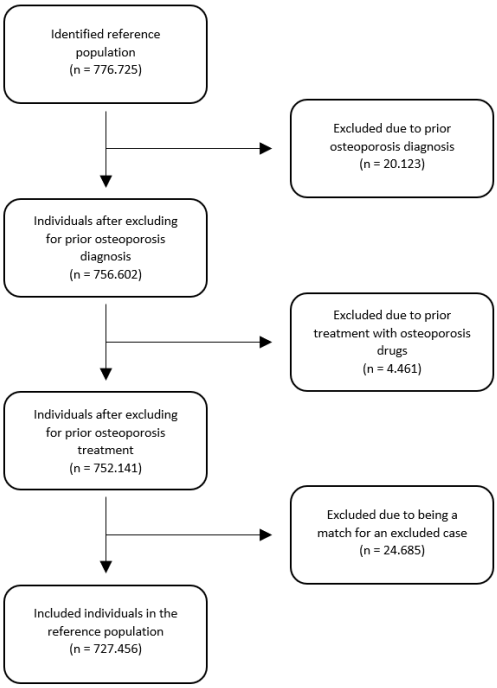
We identified 155.325 individuals with prior cholecystectomy using the Danish National Patient Registry that were registered with a Nordic Surgical Code for cholecystectomy including both open and laparoscopic procedures (KJKA2 and KJKA21). We excluded 4371 individuals from the identified cohort with a prior diagnosis of osteoporosis on the date of surgery (defined as ICD-10 diagnoses of M80* or M81* in the Danish National Patient Registry). In addition, 986 were excluded since they had used specific osteoporosis treatments defined as at least one dispensed prescription for drugs with ATC codes M05BA (bisphosphonates), M05BX04 (denosumab), M05BX03 (strontium ranelate), H05AA02 (teriparatide), G03XC01 (raloxifene) or M05BX06 (romosozumab) in the Danish National Prescription Registry on the date of surgery. The total number of cases included in the study population was 149.968 (Figure 1a)

Figure 1a Identification of cases included in the cholecystectomy cohort



For every case with prior cholecystectomy, we identified 5 sex- and age-matched controls. We excluded 20.123 individuals due to prior osteoporosis diagnosis on the index date. In addition, 4.461 individuals were excluded due to the use of specific osteoporosis treatments before the index date. 24.684 individuals were excluded due to being a reference individual to an excluded case. We included 727.456 individuals in the reference population. These reference individuals were randomly selected from the general population (Figure 1b)

Figure 1b Identification of control subjects included in the reference population



2.4 Data variables and outcomes

We defined that a fracture was sustained on the date that a fracture ICD-10 diagnosis (Table 1) was entered into the Danish National Patient Registry. We also defined that a patient was diagnosed with osteoporosis on the date an ICD-10 diagnosis of osteoporosis was entered into the Danish National Patient Registry or on the date that a relevant anti-osteoporotic drug was registered as dispensed in the Danish National Prescription Registry. A person who had a registration of a dispensed prescription of prednisolone (ATC H02AB06) was defined as a prior prednisolone user. We calculated the Charlson comorbidity index (CCI) on the index date for all participants using the methods described by Christensen et al (14).

Table 1: ICD-10 codes for fractures used in the study

Fracture site	ICD-10 code
Humerus	S422, S422A, S422B, S422C, S423, S423A, S424, S424A, S424B, S424C, S427, S427B, S428, S429
Forearm	S52, S520, S520A, S520B, S520C, S520D, S521, S521A, S521B, S522, S523, S524, S525, S525A, S525B, S525C, S526, S527, S528, S528A, S528B, S528C, S529
Hand	S62
Clavicular	S420, S427A
Scapula	S421, S427
Hip	S720, S721, S722, S723
Pelvis	S32 (excluding S320)
Femur	S724, S727, S728, S729
Lower leg and ankle	S82
Foot	S92
Head	S02
Sternum	S222
Ribs	S223, S224, S225
Cervical spine	S12
Thoracic spine	S220, S221
Lumbar spine	S320
Sacrum	S321
Multiple body regions	T02

The Danish National Patient Registry is used for governance. Therefore, multiple registrations may occur for the same fracture event (*ie.* emergency department visits, orthopaedic surgery ward stays, and outpatient clinic visits may all be registered with different dates but relate to the same fracture occurrence). To overcome this, we instituted a 180 days wash-out period before counting a fracture as a new fracture if it occurred at the same skeletal site as a previous fracture with the same ICD-10 diagnosis.

2.5 Statistical methods

Data are presented descriptively as n(%N), mean (±SD), median [inter quartile range] and incidence rates as n (95%CI) per 10.000 person-years. Kaplan-Meier estimate curves were fitted to show the osteoporosis-free survival from the index date. We present hazard ratio (HR) and incidence rate ratios (IRR) between the

cholecystectomy cohort and the reference population with 95% confidence intervals. Between-group comparisons were done using the chi-squared test for categorical variables.

The association between cholecystectomy (yes/no) and fracture rates adjusted for age at surgery was evaluated using a Poisson regression model with the total number of fractures as the outcome variable, and logarithmic transformation of follow-up time within each age strata as the offset. Age groups were ≤30 years of age, 31-50 years of age, 51-65 years of age, 66-80 years of age and 80+ years of age. We used relatively large age bands to define groups of individuals with similar baseline fracture risks while at the same time having sufficient statistical power to evaluate differences in fracture rates between the two cohorts. Since we expected an interaction between age at surgery and the risk of fractures, we added an interaction term to our final model. We used the likelihood ratio test to identify statistically significant interactions, accepting a p-value below 0.05 as statistically significant. The independent variables in the final model were surgery, age group and the interaction term. We defined the IRR to be statistically significant if the 95% CI did not include 1.00.

In addition, we defined a composite endpoint for osteoporosis consisting of the diagnoses of 1) hip or spine fracture, 2) osteoporosis based on ICD diagnosis or 3) osteoporosis treatment. We calculated the HR for osteoporosis by comparing the two groups by fitting a Cox proportional hazards model and running the model for each age group. We evaluated the model assumptions by plotting a log-log plot of survival and visually assessing if the assumption of proportional hazards held. Proportionality was accepted if the group graphs did not cross. HRs were considered statistically significant if the 95% CI did not include 1.00.

All statistical analyses were done remotely, using the Statistics Denmark VPN remote desktop. To ensure participant anonymity only summary data will be shown. All analyses were done using Stata 17 (StataCorp, Texas, USA).

3 Results

As detailed above, we included 149.968 individuals in the cholecystectomy cohort and 727.456 in the reference population. In the cholecystectomy cohort, the median follow-up time from surgery was 9.2 years, while the total person-years at risk was 1.447.451 (Table 2). In the reference population, the median follow-up time was 9.3 years, while the total person-years at risk was 7.117.119. The median age at the time of surgery in the cholecystectomy group was 50 years (IQR 37-62). Approximately two-thirds of the cholecystectomy cohort and reference population were females. In all age groups ~~T~~the use of prednisolone

was more prevalent in the cholecystectomy group (23.6%) compared to the reference population (18.1%, p<0.001)

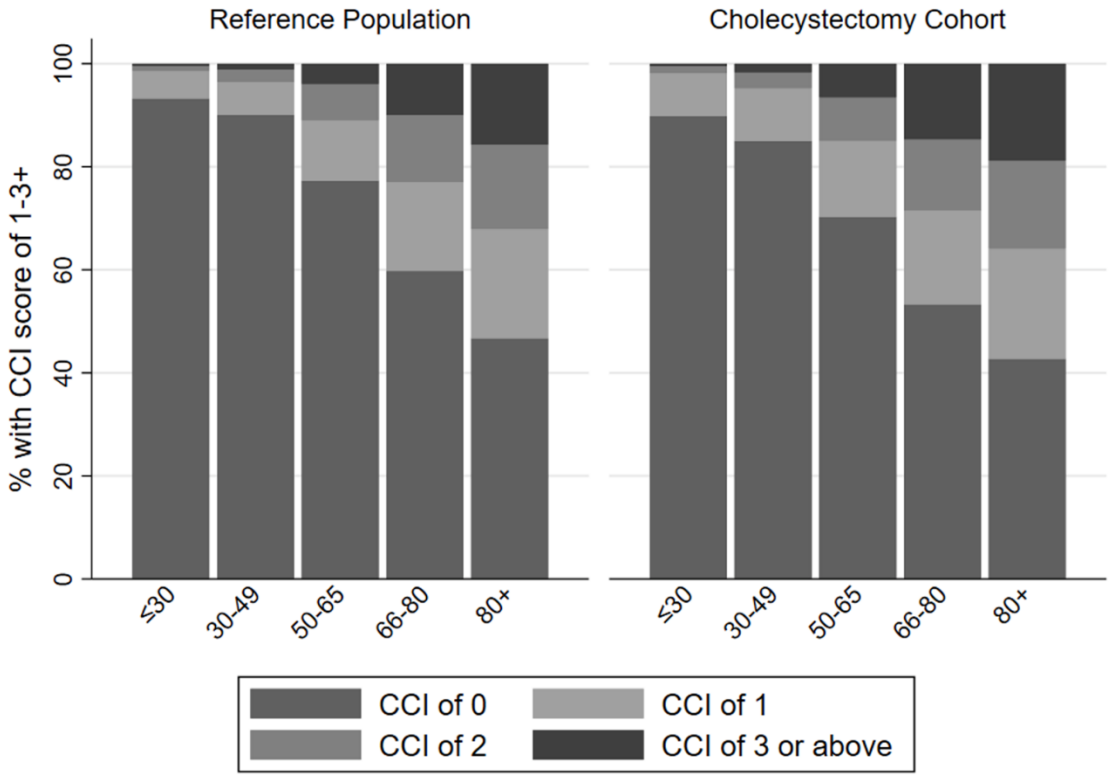
Table 2: Baseline characteristics of the cholecystectomy cohort and reference population

	Cholecystectomy cohort	Reference population	
Mean Age (SD)*	50.1 (15.9)	49.6 (15.7)	NA
Median follow-up time (years [IQR])	9.2 [4.2-15.1]	9.3 [4.3-15.3]	
Total person-years at risk (years)	1.447.451	7.117.119	
Females (n(%N))	106.654 (71.1%)	515.777 (70.9%)	p=0.09
Males (n(%N))	43.314 (28.9 %)	211.679 (29.1 %)	p=0.21
Prednisolone user (n(%N))	35.401 (23.6%)	131.654 (18.1%)	p<0.001

IQR inter quartile range. *at index date. NA= not applicable

Figure 2 shows the CCI score for the reference population and the cholecystectomy cohort. The patients in the cholecystectomy cohort had a significantly higher CCI score than the reference population (p-value < 0.001). In the cholecystectomy group, 74.9 % had a CCI score of 0, compared to 80.9 % in the reference population. The number of patients with a CCI ≥ 3 was 5.6 % in the cholecystectomy group and 3.6 % in the reference population.

Figure 2 Charlson Comorbidity Index (CCI) score in the reference population and cholecystectomy cohort



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175

176 *3.1 Fractures rates*

177 As shown in Table 3, the total number of fractures was slightly higher in the cholecystectomy cohort
178 compared to the reference population. In the cholecystectomy cohort, the total number of fractures was
179 32.717 compared to 151.712 in the reference population (IRR 1.06 (1.05-1.07)). In the age group ≤ 30 years
180 the IRR when comparing the cholecystectomy cohort to the reference population was 1.38 (1.32-1.43),
181 while the IRR were 1.10 (1.08-1.12) in the age group 31-50 years. In the remaining age groups, the number
182 of fractures was not significantly different between the groups.

183

184 **Table 3: Number of fractures in the cholecystectomy cohort and reference population**

Age group	Cholecystectomy cohort			Reference population			IRR	95% CI
	Number of fractures	Person-years at risk	IR per 10.000 person-years	Number of fractures	Person-years at risk	IR per 10.000 person-years		
≤30	2.914	192.977	151.0	10.396	947.510	109.8	1.38	1.32-1.43
31-50	10.160	619.273	164.1	45.918	3.078.539	149.2	1.10	1.08-1.12

51-65	10.678	438.209	243.7	52.864	2.169.722	243.6	1.00	0.98-1.02
66-80	7.694	205.033	375.3	36.982	978.188	378.1	0.99	0.97-1.02
80+	1.271	18.517	686.4	5.552	78.389	708.3	0.97	0.91-1.03
All	32.717	1.474.009	222.0	151.712	7.252.348	209.2	1.06	1.05-1.07

3.2 Osteoporosis risk

Based on an ICD-10 diagnosis the prevalence of osteoporosis was 3,5 % in both the cholecystectomy cohort and reference population (HR 1.00, 0.97-1.03) (Table 4). In the age group ≤ 30 years of age, the number of patients with an ICD-10 diagnosis of osteoporosis was significantly higher in the cholecystectomy group, where 0.3 % of the patients had the diagnosis compared to 0.2 % in the reference population yielding an HR of 1.40 (1.04-1.87).

Table 4: Number of patients with osteoporosis based on ICD diagnosis in the cholecystectomy cohort and reference population

Age group	Cholecystectomy cohort	Reference population	HR	95%CI
≤30	58 (0.3%)	204 (0.2%)	1.40	1.04-1.87
31-50	828 (1.4%)	4.214 (1.5%)	0.98	0.91-1.05
51-65	2.193 (5.0%)	11.345 (5.3%)	0.95	0.91-1.00
66-80	1.898 (7.3%)	9.009 (7.5%)	1.00	0.95-1.05
80+	229 (7.1%)	867 (6.4%)	1.09	0.94-1.26
All	5.206 (3.5%)	25.639 (3.5%)	1.00	0.97-1.03

In the cholecystectomy cohort, 6.~~6~~4 % had reached the composite endpoint defined as either a hip or spine fracture, osteoporosis based on an ICD-10 diagnosis or the use of osteoporosis treatment, while the similar number was 6.~~6~~4 % in the reference population (HR of 0.98 (0.96-1.00) (Table 5).

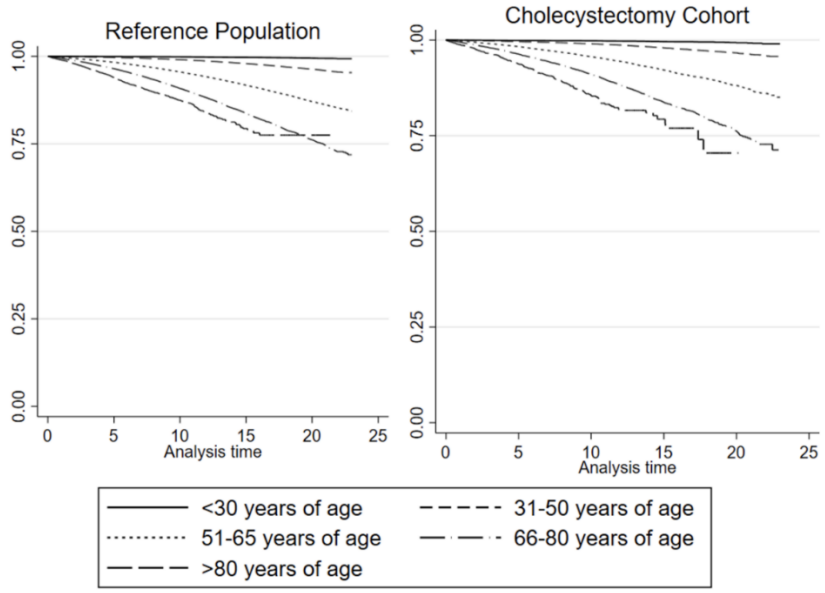
In the cholecystectomy cohort 0.8 % of the patients ≤30 years of age at surgery had reached the composite endpoint during follow-up, compared to 0.6 % in the reference population, resulting in an HR of 1.42 (1.19-1.71) (Table 5 and Figure 3). When comparing the other age groups the number of patients fulfilling the composite endpoint did not differ significantly between the cholecystectomy cohort and the reference

population, except for the age group 51-65 years where the number of patients who reached the composite endpoint was significantly lower in the cholecystectomy cohort compared to the reference population (HR of 0.92 (0.85-0.95)). Figure 3 shows the osteoporosis-free survival in different age groups based on the composite endpoint for osteoporosis in the cholecystectomy cohort and the reference population.

Table 5: Number of individuals in the cholecystectomy cohort and reference population who fulfilled the composite endpoint for osteoporosis defined as a hip or spine fracture, an ICD diagnosis of osteoporosis or had received osteoporosis treatment.

Age group	Cholecystectomy cohort	Reference population	HR	95%CI
≤30	150 (0.8%)	517 (0,6 %)	1.42	1.19-1.71
31-50	1.502 (2.6%)	7.669 (2.7%)	0.97	0.92-1.03
51-65	3.754 (8.5%)	19.999 (9.1%)	0.92	0.85-0.95
66-80	3.649 (14.0%)	17.628 (14.7%)	0.98	0.95-1.02
80+	556 (17.2%)	2.381 (18.2%)	0.92	0.84-1.01
ALL	9.611 (6.4%)	48.294 (6.6%)	0.98	0.96-1.00

Figure 3 Osteoporosis-free survival based on the composite endpoint for osteoporosis



4 Discussion

This nationwide, register-based, population-based study evaluated the risk of osteoporosis and fractures in patients with prior cholecystectomy. Overall, prior cholecystectomy was not associated with an increased risk of osteoporosis based on a composite endpoint of spine or hip fracture, ICD diagnosis of osteoporosis or osteoporosis treatments. However, we did observe a minor increase in the risk of ICD-diagnosed osteoporosis in the youngest age group and a minor increase in the risk of fractures in the cholecystectomy cohort that was caused by an increased incidence of fractures in the younger age groups.

The incidence rate ratio for fractures when comparing the cholecystectomy cohort and the reference population decreased with increasing age and was similar in age groups above 50 years of age. In the age groups ≤30 years of age and 31-50 years of age, those with prior cholecystectomy had an increased risk of fractures and this was most evident in the age group ≤30 years of age. In terms of ICD-diagnosed osteoporosis across age groups, the proportion was similar in the cholecystectomy cohort and the reference population. As for fracture incidence, however, in those below 30 years, the proportion with an ICD diagnosis of osteoporosis was higher in the cholecystectomy cohort. Likewise, using a composite endpoint for osteoporosis an increased proportion was observed in those aged below 30 years while similar risks were found in all remaining age groups. In accordance with our findings, an epidemiological study from South Korea in individuals above 40 years of age found that those with prior cholecystectomy had a small increase in fracture risk which was more pronounced in younger compared to older individuals (10)

The reason for the observed increase in osteoporosis and fracture risk after cholecystectomy in the younger age groups as opposed to similar risks in the older age groups is unknown. In theory, the increase in fracture risk and osteoporosis after cholecystectomy could relate to reduced gastrointestinal absorption of vitamin D. As alluded to above, two small, cross-sectional studies from Turkey reported lower 25-hydroxy vitamin D in middle-aged patients with prior cholecystectomy compared to controls (7, 8). Vitamin D is a fat-soluble vitamin and its absorption from the intestine is bile-dependent. After cholecystectomy, bile quality and secretion from the liver are altered which may compromise vitamin D absorption (8, 15, 16). Vitamin D plays important roles in bone metabolism and calcium homeostasis (6) and vitamin D insufficiency causes reduced calcium absorption from the gut and reduced calcium reabsorption in the kidney leading to hypocalcemia, hyperparathyroidism and increased bone resorption (6). In Denmark, located on the 55 – 57th northern latitude, vitamin D precursor synthesis occur in the skin from April to October and vitamin D and parathyroid hormone show a seasonal variation (17). Sufficient vitamin D intake and absorption are therefore necessary to abate these seasonal variations where a large part of the population has vitamin D insufficiency in winter and early spring. Vitamin D supplements are over-the-counter medication in Denmark and information on the use of vitamin D is therefore not available in the

health registries. In general, older compared to younger individuals might be more reliant on the absorption of vitamin D for adequate vitamin D status since with increasing age, solar exposure may be limited because of changes in lifestyle factors such as clothing and less time spent outdoors. In light of this, it was somewhat surprising that the increased risk of fracture after cholecystectomy was exclusively found in younger age groups and do not favour lower vitamin D absorption as a dominant etiological link between cholecystectomy and increased fracture risk.

The increased risk of fracture and osteoporosis after cholecystectomy in the younger age groups might also relate to reduced gastrointestinal absorption of vitamin K. Vitamin K is pivotal for the synthesis of osteocalcin which is secreted by osteoblasts (18) and is important for bone mineralization and regulation of bone remodelling (19) and vitamin K insufficiency is associated with an increased risk of fractures (4, 5, 20). Like vitamin D, vitamin K is fat-soluble and absorption is bile-dependent (21). Thus changes in bile flow and concentration after cholecystectomy could in theory lead to lower vitamin K levels and increased fracture risk (4-6, 21).

The age-related discrepancy in osteoporosis and fracture risk after cholecystectomy may relate to differences in fracture epidemiology and a dissimilar number of competing risk factors across the lifespan. Thus, most risk factors for fractures and osteoporosis such as postmenopausal status, predisposing comorbidities and falls increase with increasing age. In the young, few risk factors are generally present and cholecystectomy may therefore increase fracture and osteoporosis risk, albeit slightly, but to a level that becomes detectable in a large cohort study as the one performed here. On the contrary, in the old, numerous risk factors for fractures and osteoporosis may be present and any small risk increase caused by pathophysiological alterations related to cholecystectomy may not change fracture risk significantly.

As described above, cholecystectomy was associated with an increased risk of fractures and osteoporosis in the younger age groups. In the other age groups, the incidence of fractures and osteoporosis were the same in the cholecystectomy cohort and reference population, except in the age group 51-65 years. In this group, the incidence of the composite endpoint for osteoporosis was significantly *lower* in the cholecystectomy cohort. This may relate to the fact that some of the risk factors that increase the risk of gallstone, and thereby the risk of having cholecystectomy performed, decreases the risk of osteoporosis. First, estrogen is an important risk factor for gallstones (22) while it generally protects against bone loss (23). Estrogen increases the amount of cholesterol secreted into bile from the liver and diminishes bile salt secretion, thereby promoting the development of gallstones (22). Likewise, postmenopausal estrogen therapy increases the risk of gallstone disease (22, 24-26). Dissimilar estrogen levels or age at menopause might underlie the observed ~~higher~~ lower osteoporosis risk in the age group 51-65 in the cholecystectomy

cohort compared to the reference population. Second, obesity increases the risk of gallstones (2) while decreasing the risk of osteoporosis (27) and any difference between the groups in body weight may underlie the observed difference in risk. There are however also some confounders that would increase the risk of osteoporosis in the cholecystectomy cohort. A larger proportion of individuals in the cholecystectomy cohort had received treatment with prednisolone compared to the reference population. Glucocorticoids have detrimental effects on bone and this may have led to an increase in the incidence of osteoporosis and fractures (28). We did not observe any significant imbalance in risk factors for osteoporosis (such as chronic obstructive pulmonary disease, diabetes, colitis or coeliac disease, rheumatoid arthritis, thyrotoxicosis, vitamin D insufficiency or breast cancer that could explain the increase in fracture risk in the younger age groups nor the *lower* risk of osteoporosis assessed using the composite endpoint in the cholecystectomy cohort in those aged 51-65 years (data not shown). ~~The cholecystectomy cohort also had significantly more comorbidities. This is likely because risk factors for gallstone, and thereby also cholecystectomy, include obesity, a diet low in fibre and a sedentary lifestyle that would increase the risk of a number of those diseases that are assessed in the CCI~~ (29).

Our study has some limitations. First, we were not able to control for some confounders that could influence both the risk of fractures and the risk of cholecystectomy such as weight, diet, exercise and estrogen levels. In addition, supplements with calcium and vitamin D are over-the counter medications and cannot be accounted for in the registries. These factors may have affected the results in opposite directions; some of these confounders would increase the risk of osteoporosis in the cholecystectomy cohort while others would decrease the risk. Second, with the available information in the registries, we were only able to follow the participants for a median time of approximately nine years. Thus, individuals that had cholecystectomy performed at an early age would suffer from any pathophysiological effect for the remainder of their lives leading to greater cumulative risk. Whether this leads to changes in the risk of fractures later in life is unknown. Third, in young adults assessment of BMD performed before the attainment of peak bone mass, which typically occur in the 3rd decade of life, may reveal low BMD that subsequently could lead to an ICD-10 diagnosis of osteoporosis although this risk would be similar in both the cholecystectomy cohort and controls. In addition, vitamin D insufficiency can cause osteomalacia that would also present as low BMD. Since we did not have data on Vitamin D status we cannot exclude that the higher incidence of osteoporosis in the younger age groups in the cholecystectomy cohort may relate to vitamin D insufficiency with varying degrees of osteomalacia rather than genuine osteoporosis. To that end, vitamin D insufficiency might cause muscle weakness that could influence the risk of falls and thus fracture risk.

Our study also has some important strengths. We used data from the Danish National Patient Registry which generally has high data quality in terms of validity and completeness (11). Hospitals and outpatient clinics must report data to the registers, and therefore the register contains data on all contacts to Danish hospitals. The registry made us able to identify a large cohort of patients with prior cholecystectomy and provided valid information about fractures, osteoporosis diagnosis, comorbidities and drug use. By using the registry, we avoided problems with recall bias that might occur when using interviews or self-reported data. In addition, we were able to identify a large age- and sex-matched reference population for comparison.

In conclusion, in a nationwide, cohort study we found that individuals with prior cholecystectomy had a similar risk of osteoporosis compared to controls of the same age and sex. In younger subgroups, however, we did find that the risk of osteoporosis and fractures was slightly increased. Absolute risks were very small and cholecystectomy only led to small increases in risk. The pathophysiological mechanism underlying the increase in fracture and osteoporosis risks observed in the young is unknown and could be further explored. From a clinical perspective, however, the concern that cholecystectomy leads to any major calcium-metabolic disturbance is limited.

5 Ethical approval

Analysis was conducted via VPN exclusively on de-identified microdata hosted by Statistics Denmark with no access to subject names or other personal identifiers. To ensure participant anonymity only summary data will be presented. The investigators were blinded to identify the patients and the reference population. Statistics Denmark (project id 707791) approved the study. Ethical approval is not required for this type of study in Denmark.

6 References

1. Stinton LM, Myers RP, Shaffer EA. Epidemiology of gallstones. Gastroenterol Clin North Am. 2010;39(2):157-69, vii.
2. Shaffer EA. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? Curr Gastroenterol Rep. 2005;7(2):132-40.
3. Turumin JL, Shanturov VA, Turumina HE. The role of the gallbladder in humans. Rev Gastroenterol Mex. 2013;78(3):177-87.
4. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000;71(5):1201-8.
5. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr. 1999;69(1):74-9.
6. Barnes MS, Robson PJ, Bonham MP, Strain JJ, Wallace JM. Effect of vitamin D supplementation on vitamin D status and bone turnover markers in young adults. Eur J Clin Nutr. 2006;60(6):727-33.
7. Polat HB, Beyazal MS. The effect of cholecystectomy on 25-hydroxyvitamin D levels and bone mineral density in postmenopausal women. Arch Osteoporos. 2018;13(1):61.
8. Ekiz T, Yeğen SF, Katar MK, Genç Ö, Genç S. 25-Hydroxyvitamin D levels and bone mineral density evaluation in patients with cholecystectomy: a case-control study. Arch Osteoporos. 2018;13(1):14.
9. Marcinowska-Suchowierska EB, Tałałaj MJ, Włodarcyzk AW, Bielecki K, Zawadzki JJ, Brzozowski R. Calcium/phosphate/vitamin D homeostasis and bone mass in patients after gastrectomy, vagotomy, and cholecystectomy. World J Surg. 1995;19(4):597-601; discussion -2.
10. Lee EJ, Shin CM, Lee DH, Han K, Park SH, Kim YJ, et al. The Association Between Cholecystectomy and the Risk for Fracture: A Nationwide Population-Based Cohort Study in Korea. Front Endocrinol (Lausanne). 2021;12:657488.
11. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol. 2015;7:449-90.
12. Pedersen CB. The Danish Civil Registration System. Scand J Public Health. 2011;39(7 Suppl):22-5.
13. Kildemoes HW, Sørensen HT, Hallas J. The Danish National Prescription Registry. Scand J Public Health. 2011;39(7 Suppl):38-41.
14. Christensen S, Johansen MB, Christiansen CF, Jensen R, Lemeshow S. Comparison of Charlson comorbidity index with SAPS and APACHE scores for prediction of mortality following intensive care. Clin Epidemiol. 2011;3:203-11.
15. Handzlik-Orlik G, Holecki M, Wilczyński K, Duława J. Osteoporosis in liver disease: pathogenesis and management. Ther Adv Endocrinol Metab. 2016;7(3):128-35.
16. Stoker GE, Buchowski JM, Stoker ME. Prior cholecystectomy as a predictor of preoperative vitamin D deficiency in adults undergoing spine surgery. Arch Surg. 2012;147(6):577-8.
17. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol (Oxf). 2005;62(3):265-81.
18. Tsugawa N, Shiraki M. Vitamin K Nutrition and Bone Health. Nutrients. 2020;12(7).
19. Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. J Cell Physiol. 2013;228(6):1149-53.
20. Hao G, Zhang B, Gu M, Chen C, Zhang Q, Zhang G, et al. Vitamin K intake and the risk of fractures: A meta-analysis. Medicine (Baltimore). 2017;96(17):e6725.
21. Fusaro M, Mereu MC, Aghi A, Iervasi G, Gallieni M. Vitamin K and bone. Clin Cases Miner Bone Metab. 2017;14(2):200-6.
22. Simonsen MH, Erichsen R, Frøslev T, Rungby J, Sørensen HT. Postmenopausal estrogen therapy and risk of gallstone disease: a population-based case-control study. Drug Saf. 2013;36(12):1189-97.
23. Kanis JA. Estrogens, the menopause, and osteoporosis. Bone. 1996;19(5 Suppl):185s-90s.

24. Grodstein F, Colditz GA, Stampfer MJ**.** Postmenopausal hormone use and cholecystectomy in a large prospective study. Obstet Gynecol. 1994;83(1):5-11.
25. Dhiman RK, Chawla YK**.** Is there a link between oestrogen therapy and gallbladder disease? Expert Opin Drug Saf. 2006;5(1):117-29.
26. Cirillo DJ, Wallace RB, Rodabough RJ, Greenland P, LaCroix AZ, Limacher MC, et al. Effect of estrogen therapy on gallbladder disease. Jama. 2005;293(3):330-9.
27. Qiao D, Li Y, Liu X, Zhang X, Qian X, Zhang H, et al. Association of obesity with bone mineral density and osteoporosis in adults: a systematic review and meta-analysis. Public Health. 2020;180:22-8.
28. Chotiyarnwong P, McCloskey EV**.** Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. Nat Rev Endocrinol. 2020;16(8):437-47.
29. Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F**.** Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr. 2004;23(3):187-96.