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Sex disparities in gallstone disease: insights from the MAUCO prospective population-based cohort study

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ABSTRACT

Objective To investigate factors associated with the prevalence and incidence of gallstone disease (GSD) in women and men of the MAUCO population-based prospective cohort.

Design 8948 MAUCO participants (aged 38–74 years) underwent abdominal ultrasound at baseline (2015–2019); 4385 received follow-up ultrasound at years 2 or 4. Factors associated with prevalent GSD were assessed using Poisson multiple regression and with incident GSD using Cox regression models.

Results GSD prevalence was 40.4% in women (13.1% gallstones, 27.3% cholecystectomies) and 17.1% in men (8.9% gallstones, 8.2% cholecystectomies). In men, GSD prevalence rate ratio (PRR) by age in >64 years was 3.85 (95% CI 3.00 to 4.94), doubling that of women's PRR 1.78 (95% CI 1.57 to 2.01). In women, waist circumference and diabetes were stronger GSD factors; a higher number of children and worse metabolic and socioeconomic conditions were also highlighted. GSD men had higher cardiovascular disease and a family history of GSD and gallbladder cancer. 198 GSD cases developed during follow-up, with incidence increasing by 2% (95% CI 1.005% to 1.03%) per each centimetre above the ideal waist circumference, statistically significant only in women. In men, age was the strongest factor for incidence, followed by a family history of GSD and low high-density lipoprotein increased incidence risk.

Conclusions GSD burden was high in this population; a third of women had their gallbladder removed, which may pose them at risk of other health problems. Abdominal obesity was the only preventable GSD risk factor, highlighting the need for effective public health policies promoting obesity reduction.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chile has among the world's highest prevalence of gallstone disease (GSD) and mortality from gallbladder cancer. However, no prospective study has investigated its natural history in the country.

WHAT THIS STUDY ADDS

⇒ We found strong evidence that having older age, higher waist circumference, moderate-to-severe fatty liver and family history of GSD were associated with having GSD.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study confirms that the Chilean population has one of the highest GSD burdens worldwide, with a higher prevalence in women (40.4%) than in men (17.1%). To reduce GSD prevalence, public health programmes should focus on reducing obesity, the only modifiable risk factor identified in this study.

INTRODUCTION

Gallstone disease (GSD), including gallstones and cholecystectomy, affects 10–15% of adults in developed countries,[1] and its prevalence is increasing among younger individuals in line with the dramatic increase in obesity.[2–4] Gallstones are the leading risk factor for gallbladder cancer.[5–7] Chile has among the world's highest prevalence of GSD and mortality from gallbladder cancer.[8–10] Obesity, unhealthy diet, diabetes, non-alcoholic fatty liver disease and genetic variables are the main risk factors for GSD.[11 12] A recent meta-analysis of 436 636 elective cholecystectomies worldwide found an average prevalence of incidental gallbladder cancer of 0.6% (95% CI 0.5% to 0.8%).[13] Similarly, a study in Turkey reported incidental gallbladder cancer in 1%, ranging from 0.3% among individuals <60 years of age to 2.6% in those ≥60 years.[14] In Valdivia and Temuco, the high-risk areas for gallbladder cancer in Chile, researchers found a 4% incidental gallbladder cancer between 1990 and 2010 (personal communication Dr Enriqueta Bertrand and JCA), figures much higher than the 0.23% reported by Bragheto in 1999 in Santiago, the capital of the country

and a lower risk area.[15] Considering that studies in the field are limited and that the worldwide prevalence of GSD is evolving, this manuscript fills gaps in the literature and includes information relevant to other countries facing this new reality. The main aim of this study was to describe the prevalence and risk factors associated with GSD by sex in a population-based cohort of chronic diseases (the Maule Cohort (MAUCO)).[16 17] Moreover, to increase our understanding of the long-term effects of GSD, we are also investigating the association between risk factors and GSD incidence in the same cohort by sex. This study is the largest population-based cohort of ultrasound-detected GSD in Latin America.

METHODS

Study design, setting and selection of participants

The MAUCO target population includes residents of Molina County in Central Chile, which is representative of small counties of low socioeconomic levels. This population is covered by public health insurance and has a primary health hospital, two health centres and two rural health posts. Reference hospitals are between 20 and 56 km from Molina.

Through a household census, we invited all adults aged 38–74 years to enrol, excluding those unable to consent autonomously or who were terminally ill[16 17]; 72.3% accepted. Further methodological details are described elsewhere.[16 17] Between 2015 and 2019, individuals who accepted participation signed consent forms and completed a health and lifestyle survey (personal and family medical history, medication use, cardiovascular and digestive symptoms and neurocognitive state). They underwent anthropometric measurements, bioimpedance analysis and abdominal ultrasound at the study clinic as well as provided blood and saliva samples.[17] All participants received follow-up surveys 2 years later, and a subgroup of the cohort—those with abnormal baseline ultrasound and approximately 1:1 age-sex matched controls with normal baseline ultrasound—were invited for a new ultrasound exam (cohort control). The follow-up rate for ultrasound at 2 years was 96%.[17] Additionally, any participant who visited the MAUCO clinic was offered a follow-up ultrasound (opportunistic controls). Participants with abnormal health results at baseline or follow-up were referred to Molina Hospital. 8948 participants were finally included in the main baseline analyses (online supplemental figure 1). The distribution of missing variables at baseline by sex is available in online supplemental table 1.

Gallstone disease ascertainment

A medical technician (FH), trained and supervised by a radiologist (FC) at Pontificia Universidad Católica de Chile, performed and recorded all abdominal ultrasounds following the Rumack 2011 Guidelines.[18] Images were stored and reviewed by the radiologist as needed. GSD, the outcome variable, included cholecystectomy, gallstones or biliary sludge. Other abnormal gallbladder results included cholesterolosis, polyps, wall thickening, wall calcifications, scleroatrophic gallbladder, image suggestive of neoplasm, adenomyomatosis and adenomyosis (online supplemental table 2). Liver steatosis was classified as absent, mild, moderate or severe.[19]

Follow-up ultrasound examination

Among the 8609 MAUCO eligible for follow-up participants, 2353 participants with GSD, 1811 cohort controls and 597 MAUCO cohort opportunistic controls were invited for a follow-up ultrasound (online supplemental figure 1). The equipment and personnel were the same as at baseline.

Predictors of gallstone disease

Socio-demographic, lifestyle, familial and health-related factors were evaluated as prevalent and incident GSD risk factors. Socio-demographic factors included age at recruitment (38–44, 45–54, 55–64, 65–74 years), sex (women/men), self-reported schooling (<8, 8–12, >13 years), health insurance (levels A/B, C/D or private; where A corresponds to the lowest income level), and ancestry (self-identified Amerindian or Chilean Hispanic). Health factors included diet (Mediterranean Diet Score, defining healthy score ≥9),[20] food consumption patterns (eg, ≥1 fruit per day), alcohol intake (risky ≥20 g per week in women and ≥30 g in men), self-reported current or ever smoker (≥100-lifetime cigarettes), physical activity (low: <3, 30 min sessions per week), grip strength (low <27 kg in men and <16 kg in women), self-reported walking pace (slow, normal or brisk pace) and the number of teeth (assessed by a health technician and classified as ≥20 or <20). Women's hormonal factors included the number of children (count or dichotomised ≥3), hormonal contraception (ever) and hormonal replacement therapy (ever). Gallstones or gallbladder cancer in parents, children or full and half-siblings were also evaluated. Obesity was considered as a waist circumference ≥88 cm in women and ≥102 cm in men or body mass index (BMI)≥30 (weight (kg)/height (m^2)). Abnormal lipids were defined as low-density lipoprotein (LDL) cholesterol ≥160 mg/dL, high-density lipoprotein (HDL) cholesterol <50 mg/dL in women and <40 mg/dL in men and triglycerides ≥200 mg/dL. Altered bilirubin and liver enzymes were defined as total bilirubin >1.2 mg/dL, direct bilirubin >0.3 mg/dL, aspartate aminotransferase ≥48 IU/L, alanine aminotransferase ≥55 IU/L and alkaline phosphate ≥129 IU/L. Chronic diseases considered as risk factors for GSD included diabetes (self-reported, glycaemia ≥126 mg/dL or hypoglycaemic drug use), metabolic syndrome (≥3: abdominal obesity, high triglycerides, low HDL, high blood pressure and high fasting glucose), high blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥80 mm Hg or use of antihypertensive medication) and self-reported cardiovascular disease (myocardial infarction, heart failure, stroke or arrhythmia). Digestive symptoms

assessed were biliary colic (acute pain in epigastrium lasting >30 min without diarrhoea) and dyspepsia (Rome criteria III) in the past 12 months.

Statistical analyses

There was a very low percentage of missing data (online supplemental table 1), therefore, values were not imputed for these analyses.[21–23]

Prevalent gallstone disease

Factors associated with baseline GSD were tested with χ^2 tests or t-tests. GSD age prevalence curves by sex were tested with the Kolmogorov-Smirnov test. We tested the models with the Akaike information criteria for risk factors and confounder selection. We ran multiple logistic regression models separately by sex to obtain the prevalence of GSD by each baseline variable. Prevalence rate ratios (PRR) and 95% CI were calculated with age-adjusted robust Poisson multiple regressions.[24–26]

Incidental GSD and changes in gallbladder status

Participants at risk were those without gallstones or cholecystectomy at baseline (n=6256) who had a follow-up ultrasound by September 2022 (n=2284, 36.5%); gallstones or cholecystectomy detected in follow-up were considered incident GSD (online supplemental figure 1). We calculated the time from enrolment to the follow-up ultrasound to obtain person-time at risk between ultrasounds, estimating the incidence rate of GSD per 1000 person-years. We used Hazard Ratios (HRs) from age-sex adjusted multiple Cox regression to select variables predictive of GSD incidence. To investigate the risk factors associated with GSD incidence, we first produced directed acyclic graphs based on current knowledge about the disease (online supplemental figure 2).[27 28] Then, the following models were tested: (1) the overall and men model: adjusted by sex, schooling, family history of GBC, HDL cholesterol, diet, age and age x sex interaction; (2) the women model: as per the overall model but additionally adjusted for number of children. The proportional hazard assumption was checked using Schoenfeld residuals (the proportional hazard assumptions were all non-significant with a global p-value of 0.28). Finally, we assessed competing risk accounting for all-cause mortality using the Cox proportional hazard model for GSD incidence. The Fine-Gray subdistribution risk model was used to estimate the specific influence of obesity on the onset of GSD, adjusted for the aforementioned models and incorporating death as a competing event. The cumulative incidence curves for GSD and mortality, stratified by sex, were generated using the 'cmprsk' package in R. R V.4.5.1 and Stata V.15 (StataCorp LP) statistical software were used for the analyses.

RESULTS

Prevalent gallstone disease

Of the 8970 (69.6%) Molina residents who participated, 8948 (99.8%) had a valid ultrasound and were included in our prevalence analyses; 2692 (30%) had GSD and 6256 (69.9%) had normal gallbladder or other anomalies (table 1 and online supplemental figure 1). GSD was twice as frequent in women (40.4%) as in men (17.1%) (table 1). The proportion of GSD cases who had already received a cholecystectomy was 67.6% in women and 48% in men. Women tended to have larger stones, while men had a higher prevalence of other anomalies (2.3% vs 1.6% in women) (table 1 and online supplemental table 2). On the other hand, of the 1193 people who reported biliary colic at enrolment, 17.9% were gallstone

Table 1 Gallbladder ultrasound findings at baseline. MAUCO 2015–2019

Gallbladder status by ultrasound (n)	All n=8948	Women n=4918	Men n=4030	P value sex difference*
Normal gallbladder n=6082 (%)	68.0	57.9	80.3	<0.001
Gallstone disease GSD n=2692 (%)		40.4	17.1	<0.001
Cholecystectomy n=1674 (%)	18.7	27.3	8.2	<0.001
Gallstones n=1005 (%)	11.2	13.1	8.9	<0.001
# of stones, mean (SD)	4.7 (5.5)	4.6 (5.5)	4.8 (5.6)	0.710
Multiple gallstones (%)	57.8	57.9	57.7	0.884
Size of stones (%)				
<20 mm	66.3	63.6	70.9	<0.001
20–29 mm	24.5	26.0	21.8	<0.001
>29 mm	9.3	10.4	7.4	<0.001
Biliary sludge only n=13 (%)	0.14	0.04	0.27	0.204
Other anomalies† n=174 (%)	1.9	1.6	2.3	0.019

*P values for sex differences were calculated using χ^2 test the with Yates' continuity correction for categorical variables and the t-test for comparing means.
†Other anomalies include polyps, scleroatrophic gallbladder, wall thickening and cholesterolosis among others (online supplemental table 1).
GSD, gallstone disease.

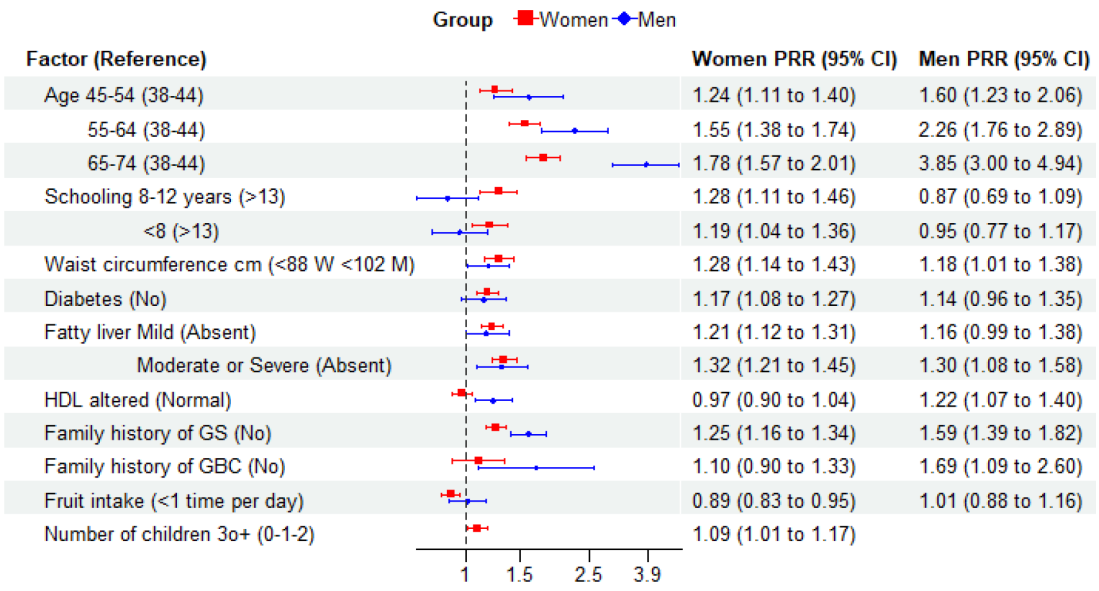

Figure 1 Factors associated with prevalent gallstone disease at baseline in 4838 women and 3936 men. MAUCO 2015–2019. Multiple robust Poisson regression model. GBC, gallbladder cancer; GS, gallstones; HDL, high-density lipoprotein; M, men; PRR, prevalence rate ratio; W, women.

carriers, 21.5% had a cholecystectomy and 60.5% were participants without GSD (online supplemental table 2).

Baseline characteristics of the included population by GSD status and sex are available in online supplemental table 3. Overall, GSD was associated with higher markers of obesity, metabolic syndrome and fatty liver. Men with GSD had higher cardiovascular disease and family history of gallbladder disease, while women with GSD had more children than women without GSD. Women were more likely to have digestive symptoms independent from their GSD status, while men with GSD were more symptomatic than those without GSD (online supplemental table 3). Other characteristics of cases, non-GSD ultrasound cohort controls and matched and opportunistic controls, can be found in online supplemental table 4.

Factors associated with prevalent GSD at baseline by sex are presented in figure 1 and online supplemental table 5. In multivariate models, age was the strongest factor associated with GSD in both sexes (figure 1). Compared with those aged 38–44, men aged ≥65 had a 3.85-times higher GSD PRR, while women ≥65 years had only a 1.78-times higher PRR. Additional strong factors that remained in the final model for both sexes included abdominal obesity, moderate-to-severe fatty liver and a family history of GSD, which was higher in men. Only in women diabetes was strongly associated with GSD independently of abdominal obesity, while having three or more children remained a relevant factor (PRR: 1.16 (95% CI 1.09 to 1.24). Other factors associated with GSD in women but not in men included low health coverage, few remaining teeth and direct bilirubin. In both sexes, GSD was associated with a family history of gallstones, being a stronger risk factor for men (PRR 1.60, 95% CI (1.40 to 1.82)) than for women (PRR 1.23 95% CI (1.15 to 1.34) (online supplemental table 5)).

Follow-up ultrasound findings

We invited all 2353 participants with GSD, 1811 non-GSD ultrasound-cohort controls and 595 non-GSD clinical controls for the follow-up ultrasound (online supplemental figure 1). The response rate was 89% in GSD cases, 93% in non-GSD ultrasound cohort controls and 100% in non-GSD opportunistic controls (online supplemental figure 1). During the follow-up, characteristics were similar between cases and non-GSD ultrasound cohort controls (online supplemental table 6). Findings did not change when excluding the opportunistic controls; thus, we presented the combined controls in the tables and text (online supplemental table 6).

Changes in GSD status during follow-up

Among 2239 participants with normal gallbladders at enrolment, 8.2% had GSD and 3.7% other anomalies at follow-up. Particularly notable, women had a 2.4-times higher risk of cholecystectomy (table 2A). Among 45 participants with other anomalies at baseline, 18.5% cleared their anomalies, 24.4% developed gallstones and 11.1% received a cholecystectomy; these results were similar by sex (table 2B). Among participants with gallstones since enrolment, 45.6% had a cholecystectomy, which was more likely in women (+69%, p<0.001); only nine participants (1.1%) cleared their gallstones (table 2C).

Associations between risk factors and GSD incidence

Over a median follow-up of 2.4 years (IQR: 2.04–2.84 years), 198 (139 women and 59 men) out of 2284 participants without GSD at baseline developed GSD.

The overall risk of GSD incidence increased by 2% (95% CI 1.005% to 1.03%) per each centimetre above the ideal waist circumference (online supplemental

Table 2 Changes in gallbladder status over 2.4 (IQR: 2.04–2.84) years of follow-up among MAUCO participants by sex 2015–2023

Gallbladder status at follow-up	All n=3074 (100%)			Women n=2017 (65.6%)			Men n=1059 (34.4%)			P value sex difference
	n	N*	Cum. Inc. (%)	n	N*	Cum. Inc. (%)	n	N	Cum. Inc. (%)	
P. normal gallbladder at baseline n=2239 (A)										
Gallstones (including biliary sludge)	136	2239	6.1	95	1468	6.5	41	771	5.3	0.08
Cholecystectomy	46	2239	2.05	35	1468	2.4	11	771	1.4	0.17
Other anomalies†	82	2239	3.7	58	1468	4.0	24	771	3.1	0.38
P. other anomalies at baseline n=45 (B)										
Gallstones (including biliary sludge)	11	45	24.4	6	27	22.2	5	18	27.8	0.93
Cholecystectomy	5	45	11.1	3	27	11.1	2	18	11.1	0.99
Clearance of other anomalies	10	45	18.5	6	27	22.2	4	18	22.2	0.99
P. gallstone at baseline n=790 (C)										
Cholecystectomy	360	790	45.6	273	522	52.3	87	268	32.5	<0.001
Clearance of gallstones	9	790	1.14	7	522	1.3	2	268	0.7	0.69

1308 cholecystectomies participants at enrolment were excluded from this analysis.
*N: people at risk.
†Other anomalies include polyps, scleroatrophic gallbladder, wall thickening and cholesterolosis. P value from χ^2 test with Yate's continuity correction.
Cum. Inc., cumulative incidence; P, participants with changes.

table 7). The association was similar in women but not in men (online supplemental table 7). Another strong predictor of GSD incidence was age (HR: 1.07 (95% CI 1.04 to 1.10)) (online supplemental table 8). When other predictors were investigated by sex, having a family history of gallbladder cancer (HR: 6.02 (95% CI 1.42 to 25.5)) and low HDL cholesterol (HR: 1.89 (95% CI 1.12 to 3.20)) were the strongest predictor in men but not in women (table 3).

Finally, the cumulative incidence of GSD and death by sex is shown in online supplemental figure 3. As it is observed, while the cumulative incidence of death was higher in men, women had a higher GSD cumulative incidence during the follow-up.

DISCUSSION

We report the occurrence of ultrasound-detected GSD in nearly 9000 women and men from the general population of an agricultural county in Central Chile. This study confirms that the Chilean population has one of the highest burdens of GSD (30%) reported worldwide, similar to the rates found among the Pima Indians in the USA in 1970 (48.6%).[8 29]

GSD prevalence

Older age, higher waist circumference, moderate-to-severe fatty liver and family history of GSD were all strongly associated with prevalent GSD. The prevalence of GSD was twice as high in women (40.4%) than in men (17.1%); one of the highest sex differentials reported, with GSD being 127% higher in women. Interestingly, Sun *et al* reported large heterogeneity in the sex ratio by BMI in China.[30] They found that among participants with normal BMI, the female-to-male (F:M) ratio of gallstone prevalence was 1.15, but among participants with BMI >25, the F:M ratio was 2.14.[30] The 2016 mean BMI in Chilean women (28.3) and men (28.0) was notably higher than the mean BMI reported in China (23.6 women and 24.3 men) or in Japan (21.8 women and 23.7 men).[31] In our study, obesity measured by BMI was very similar by sex; yet, abdominal obesity in women doubled that of men, being one of the main explanatory factors of the women's excess of GSD and potentially preventable risk factors of GSD incidence. A high prevalence of GSD has been reported in Chile from autopsy reports and cholecystography studies since 1960,[32] long before the epidemic of obesity currently affecting Chilean adults and children.[33] A high proportion of germline variants in the *ABCG8* and *TRAF3* genes, which are associated with GSD and gallbladder cancer, have been reported in the Chilean population, which could explain its GSD burden.[12 34]

Family history of GSD and gallbladder disease were stronger risk factors for GSD among men than women (60–70% vs 25%, respectively). This finding suggests that GSD in men has a stronger genetic component than for women, while metabolic and reproductive factors play a larger role in women. For men, GSD might be a problem of older ages, while most GSD in women have occurred during their reproductive life. Of note, the fastest GSD in

Table 3 Risk factors of GSD incidence by sex. MAUCO 2015–2023

	Total	New GBD	Person-years	Incidence 1000/p-y	Age-adjusted HR (95% CI)
A. Women	1495	139	7318.71	18.9	
Age					
<45 years	192	23	710.86	32.36	Ref
45–54 years	421	43	1233.30	34.87	1.25 (0.75 to 2.07)
55–64	537	43	1338.23	32.13	1.46 (0.87 to 2.44)
65+	345	30	877.13	34.20	1.73 (0.98 to 3.03)
Schooling					
+13 years	175	20	486.70	41.09	Ref
<4 years	159	14	432.28	32.87	0.83 (0.42 to 1.65)
4–8 years	549	53	1480.74	35.79	0.94 (0.56 to 1.59)
9–12 years	612	52	1759.80	29.55	0.77 (0.46 to 1.31)
Normal waist circumference	358	22	1030.48	21.35	Ref
Risk waist circumference*	1135	117	3122.45	37.47	1.84 (1.16 to 2.90)
HDL cholesterol					
≥50	580	49	1611.44	30.41	Ref
<50	914	90	2543.95	35.38	1.19 (0.84 to 1.69)
Diet Med Score					
<6	842	70	2318.06	30.20	Ref
≥6	530	49	1511.91	32.41	0.97 (0.67 to 1.40)
Children					
<3	796	73	2249.34	32.45	Ref
≥3	699	66	1910.19	34.55	1.11 (0.79 to 1.55)
Family history GBC					
No	1461	135	4060.51	33.25	Ref
Yes	34	4	99.02	40.40	1.21 (0.45 to 3.28)
B. Men	789	59	4087.56	14,4	
Age					
<45 years	95	3	398.12	7.54	Ref
45–54 years	211	15	679.69	22.07	3.18 (0.92 to 11.02)
55–64	293	17	901.12	18.87	2.85 (0.83 to 9.77)
65+*	190	24	555.86	43.18	8.94 (2.65 to 30.12)
Schooling					
+13 years	89	3	297.27	10.09	Ref
<4 years	84	4	261.20	15.31	1.02 (0.22 to 4.61)
4–8 years	315	30	980.43	30.6	2.25 (0.68 to 7.45)
9–12 years	301	22	995.89	22.09	1.99 (0.59 to 6.67)
Normal waist circumference	441	28	1454.1	19.26	Ref
Risk waist circumference	346	31	1076,49	28.80	1.37 (0.81 to 2.30)
HDL cholesterol					
≥40	478	27	1538.89	17.55	Ref
<40*	307	31	982.94	31.54	1.89 (1.12 to 3.20)
Diet Med Score					
<6	564	44	1849.47	23.79	Ref
≥6	158	12	495.7	24.21	1.09 (0.58 to 2.08)

Continued

Table 3 Continued

	Total	New GBD	Person-years	Incidence 1000/p-y	Age-adjusted HR (95% CI)
Family history GBC					
No	777	57	2509.05	22.72	Ref
Yes*	12	2	25.73	77.72	6.02 (1.42 to 25.5)

An HR from multiple Cox extended regression, all variables included in the model.
*p:<0.05
GBC, gallbladder cancer; GBD, gallbladder disease; Med, Mediterranean; p-y, person-years.

women occurred during the reproductive ages, reaching its peak at age 52–15 years earlier than in men, whose peak was at 65 years. This can be explained by the highest oestrogen levels in women during the reproductive years. Oestradiol has a lithogenic effect by stimulating oestrogen receptors in the liver, which disrupts bile acid metabolism, increasing bile saturation of cholesterol and inducing gallstone formation.[35] As previously described by other authors, we found that women with GSD had more children, implying longer exposure to high female hormone levels.[36–38] Finally, among GSD men, cardiovascular diseases appeared to be a strong risk factor. GSD and cardiovascular disease have been shown to share many common risk factors, while a recent prospective study highlighted that men with gallbladder diseases had a higher risk of a cardiovascular event.[39]

Cholecystectomy

At baseline, 68% of women with GSD and only 50% of men with GSD had undergone cholecystectomy. This could be explained by women having gallstones for a longer period than men, resulting in more opportunities for surgery. Women might have also been prioritised for cholecystectomy because gallbladder cancer was the number one cause of cancer death among Chilean women until 2010.[40 41] Also, women with gallstones had more digestive symptoms than men, which may cause more medical visits. There is mounting evidence of the long-term effects of cholecystectomy. The gallbladder extraction profoundly alters lipid metabolism and the enterohepatic circulation of bile. The permanent flux of diluted biliary acids to the intestines results in intestinal dysbacteriosis and a pro-inflammatory intestinal state.[42] Cholecystectomy also increases steatotic liver disease and elevates the risks of digestive and hepatobiliary cancers.[43 44]

In our study, 16% of participants who had already had cholecystectomy reported experiencing biliary colic at enrolment, suggesting that surgery did not solve the pain in these subjects. Only 17.9% of baseline biliary colic reported for all participants could be attributed to current gallstones. This lack of specificity of abdominal gallstone symptoms has also been reported by others,[45 46] suggesting that gallstone prevention is preferable to surgery.

GSD incidence

In our population, the cumulative incidence of GSD was 7.2%. While women had 57.7% higher GSD at baseline, the incidence of a new GSD was only 32.8% higher in women than in men. The latter indicates that most susceptible women acquired GSD before entering the cohort, while, for men, it is a disease of older age. Similarly, the only factor significantly associated with GSD incidence in women was waist circumference; in men, the strongest factor was older age, followed by a family history of gallbladder disease and low HDL.

The main preventable risk factor for GSD incidence was abdominal obesity, similar to other populations of diverse ethnic backgrounds.[35 47] We found that GSD was associated with metabolic disorders and diabetes, independent of abdominal obesity, which is consistent with cohort studies in the USA, Europe and Taiwan.[48] Aune and Vatten proposed that diabetic autonomic neuropathy affects gallbladder motility, favouring bile stasis, sludge and the stone cascade.[48] Recently, Cortés proposed that insulin resistance, independent of obesity, might play a causal role in GSD.[49]

GSD participants also had lower LDL levels than controls, similar to a previous study in the Chilean population that suggested that individuals predisposed to GSD displayed enhanced whole-body sterol clearance, a trait of ethnic groups at higher risk of GSD.[50] However, we did not see an association between LDL and GSD incidence, and the literature has been inconsistent in this regard.[51 52] The heterogeneity in the association of blood lipids with GSD across populations suggests confounding by gene-environment interaction.[53 54]

GSD and lifestyle factors

GSD participants had a lower prevalence of risky alcohol consumption. Several studies report a protective effect of alcohol against GSD,[55] including prospective studies[52 56 57] and a meta-analysis.[58] The mechanism for this association would be a direct effect of alcohol on lipid metabolism and gallbladder motility.[57] Adherence to the Mediterranean diet was low in our population, which has been reported to be protective against gallstones.[59] In Chileans, legumes were suggested as a risk factor for GSD by lowering plasma cholesterol and LDL but increasing

cholesterol saturation of the bile.[60] This hypothesis was confirmed in a study among men in the USA, suggesting that legumes partially interrupt the enterohepatic circulation of bile acids, increasing the hepatic secretion of cholesterol.[61] Chileans and American Indians consume high quantities of legumes, but prospective confirmatory studies are needed before considering a recommendation on this matter.

MAUCO is a unique Latin American cohort that enables analysis of GSD in a high-risk population with a wide range of exposures. The response rate was high, facilitated by a comprehensive surveillance system and low attrition.[17] However, this study also has limitations. First, MAUCO does not represent the overall Chilean population since the study was conducted in an agricultural county in Central Chile, which also limited the evaluation of potential geographical differences that may affect the GSD development. Second, we made many comparisons, and some of our findings may be explained by chance or confounding by unmeasured or uncontrolled factors. Also, the low number of cases in some variables could determine a lack of power to identify other risk or protective factors. Nonetheless, it is reassuring that our findings agree with studies conducted in different populations worldwide. Third, the short follow-up period of this cohort may explain the lack of associations between some risk factors and GSD incidence. Therefore, analyses must be conducted again in the upcoming years or in a similar population with longer follow-ups. Finally, as per any observational study, causality cannot be inferred.

In conclusion, GSD had a different presentation by sex, occurring much earlier in women associated with female hormones, higher cholecystectomy and less impact of genetics. In men, GSD occurred at older ages, mainly in men with cholecystectomy, showing a stronger genetic component and was associated with cardiovascular risks. Women and men shared the only preventable risk factor: abdominal obesity. Therefore, improving diet quality and physical activity to decrease obesity and the associated metabolic risk factors need to be further encouraged and stressed even after cholecystectomy to diminish the risks of liver steatosis.

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Sex disparities in gallstone disease: insights from the MAUCO Prospective Population-Based Cohort Study

SUPPLEMENTARY FILES

Supplementary Figure S1. Diagram of participants included in the study.
Supplementary Table S1. Distribution of missing variables at baseline characteristics of women and men by gallstone disease status (n=8774). MAUCO 2015-2019.
Supplementary Table S2. All gallbladder anomalies at baseline ultrasound. MAUCO 2015-2019.
Supplementary Figure S2. Directed acyclic graph of obesity effect on GBD.

Supplementary Table S3. Baseline characteristics of women and men by GSD status (n=8774). MAUCO 2015-2019.

Supplementary Table S4. Comparison of gallstone disease participants and matched and opportunistic non-GSD controls.
Supplementary Table S5. GSD-associated factors at baseline ultrasound by sex, age-adjusted. MAUCO 2015-2019.
Supplementary Table S6. Risk factors of GSD incidence by type of controls. MAUCO 2015-2023.
Supplementary Table S7. Waist Circumference and Incident GSD in MAUCO 2015-2023. Cox extended *MRM.
Supplementary Table S8. Predictors of incident GSD (n=198) among 2284 participants. MAUCO 2019-2023.
Supplementary Figure S3. Cumulative incidence (person-years) of GSD and Death from all-causes by follow-up years and sex.

Supplementary Figure S1. Diagram of participants included in the study.

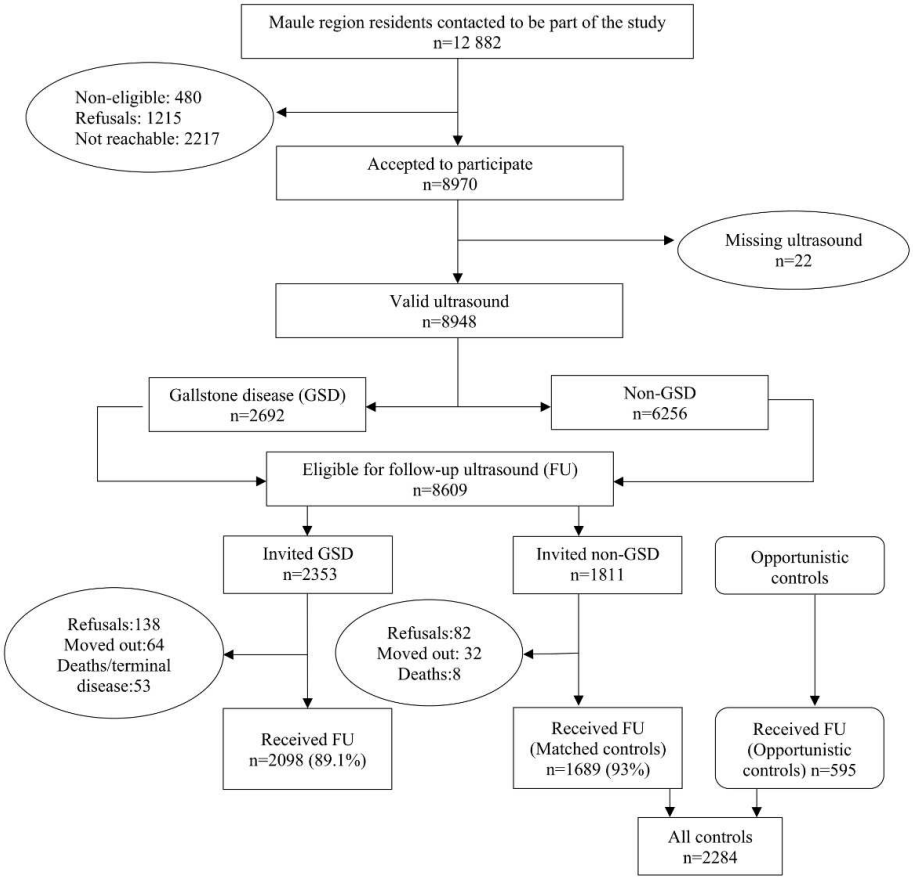


Table S1. Distribution of missing variables at baseline characteristics of women and men by gallstone disease status (n=8774). MAUCO 2015-2019.

Baseline characteristics	Women (n=4838)		Men (n=3936)	
	Non-GSD (n=2848) %	GSD (n=1990) %	Non-GSD (n=3234) %	GSD (n=702) %
Age	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Above 8 years of schooling	2 (0.07)	3 (0.15)	3 (0.09)	1 (0.14)
Low healthcare coverage [a]	17 (0.60)	10 (0.50)	17 (0.53)	4 (0.57)
Chilean-Hispanic ancestry	24 (0.84)	12 (0.60)	13 (0.40)	4 (0.57)
Less than 20 remaining teeth	13 (046)	9 (0.45)	18 (0.57)	5 (0.71)
Mediterranean Diet Score ≥9	167 (5.86)	128 (6.43)	149 (4.61)	35 (4.99)
High-fat red meat >2 t/w	76 (2.67)	66 (3.32)	50 (1.55)	13 (1.85)
Sugary drinks >2 t/d	48 (1.69)	50 (2.51)	30 (0.93)	9 (1.28)
Whole grains >3 t/w	141 (4.95)	104 (5.23)	119 (3.68)	27 (3.85)
Legumes >1 t/w	11 (0.38)	11 (0.55)	15 (0.46)	1 (0.14)
Fruit intake ≥1 t/d	3 (0.11)	5 (0.25)	6 (0.18)	0 (0.00)
Vegetable intake ≥1t/d	6 (0.21)	8 (0.40)	10 (0.31)	4 (0.57)
Risky alcohol intake	43 (1.51)	31 (1.56)	58 (1.79)	9 (1.28)
Ever smoker	20 (0.70)	17 (0.85)	26 (0.80)	4 (0.57)
Current smoker	36 (1.26)	37 (1.86)	43 (1.33)	11 (1.57)
Low physical activity [b]	24 (0.84)	31 (1.56)	31 (0.96)	11 (1.57)
Low grip strength [c]	33 (1.16)	26 (1.31)	29 (0.90)	10 (1.42)
Slow walking pace [d]	754 (26.47)	544 (27.33)	613 (18.95)	133 (18.95)
Children 3 and more	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Hormone contraception, ever	9 (0.32)	6 (0.30)	-	-
HRT, ever	37 (1.30)	21 (1.06)	-	-
Family history of gallstones	116 (4.07)	75 (3.77)	121 (3.74)	27 (3.85)
Family history of GBC	5 (0.18)	3 (0.15)	12 (0.37)	4 (0.57)
High body fat [e]	60 (2.11)	36 (1.81)	189 (5.84)	32 (4.56)
Abdominal obesity [f]	8 (0.28)	2 (0.10)	13 (0.40)	1 (0.14)
BMI ≥30 kg/m^2	7 (0.25)	1 (0.05)	3 (0.09)	0 (0.00)
LDL ≥160 mg/dL	34 (1.19)	34 (1.71)	113 (3.49)	30 (4.27)
Low HDL [g]	9 (0.32)	6 (0.30)	11 (0.34)	3 (0.43)
Triglycerides ≥200 mg/dL	10 (0.35)	6 (0.30)	10 (0.31)	3 (0.43)
Current use of statins	300 (10.53)	218 (10.95)	342 (10.57)	75 (10.68)
Bilirubin Total >1.2 mg/dL	12 (0.42)	6 (0.30)	12 (0.37)	4 (0.57)
Bilirubin Direct >0.3 mg/dL	11 (0.39)	5 (0.25)	15 (0.46)	5 (0.71)
AST ≥48 IU/L	11 (0.39)	5 (0.25)	11 (0.34)	3 (0.43)
ALT ≥55 IU/L	11 (0.39)	6 (0.30)	12 (0.37)	3 (0.43)
ALT/AST ≥1.33	11 (0.39)	6 (0.30)	12 (0.37)	3 (0.43)
ALP >129 IU/L	11 (0.39)	5 (0.25)	11 (0.34)	3 (0.43)
Fatty liver	5 (0.18)	2 (0.10)	6 (0.19)	1 (0.14)
Biliary colic	15 (0.53)	15 (0.75)	12 (0.37)	7 (0.99)

Dyspepsia	38 (1.33)	13 (0.65)	40 (1.24)	6 (0.85)
Diabetes	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
High blood pressure	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Cardiovascular disease	10 (0.35)	10 (0.50)	8 (0.25)	4 (0.57)
Metabolic Syndrome	15 (0.53)	9 (0.45)	24 (0.74)	5 (0.71)

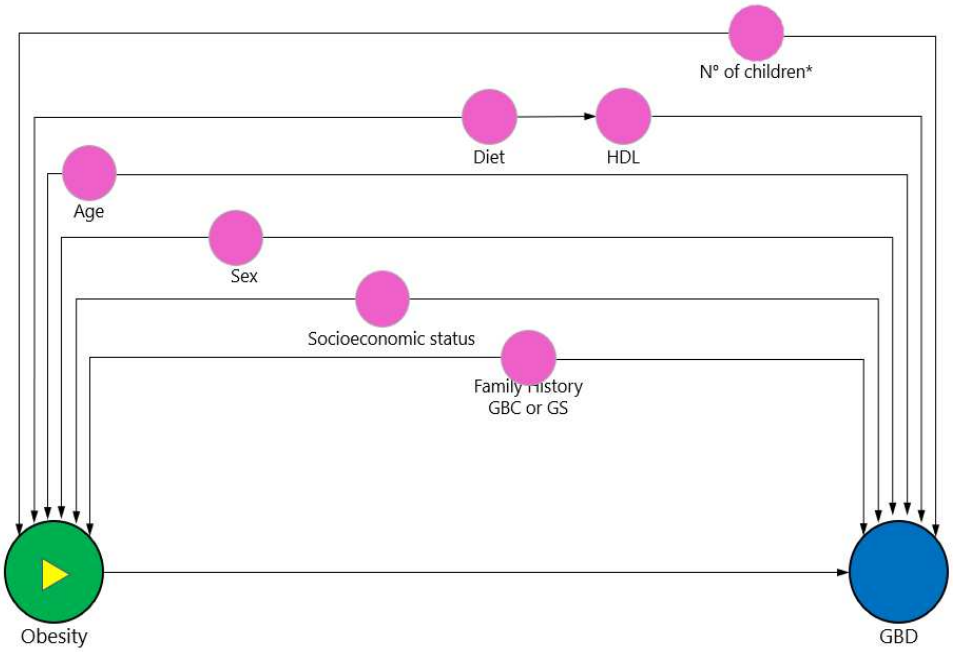
Other gallbladder anomalies (n=174) excluded. Abbreviations: GSD, gallstone disease; GBC, gallbladder cancer; HRT, hormone replacement therapy; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; t/w, times per week; t/d, times per day. [a] Public levels A or B. [b] < 3 times per week (30 minutes each time).[c] Low grip strength <16 kg in women, <27 kg in men. [d] Self-reported slow walking pace. [e] Waist circumference ≥88 cm in women and ≥102 cm in men. [f] <50 mg/dL in women and <40 mg/dL in men.

Table S2. All gallbladder anomalies and biliary colic at baseline ultrasound. MAUCO 2015-2019.

Baseline ultrasound findings	Frequency (%)			
	All n=8948	Women n=4918	Men n=4030	*p-value* [a]
A.Gallbladder anomalies at baseline ultrasound.				
Gallstone disease	2692 (30.0)	1990 (40.4)	702 (17.1)	<0.001
Other gallbladder anomalies	174 (1.9)	80 (1.6)	94 (2.3)	0.016
Polyps	134 (1.5)	59 (1.2)	75 (1.9)	0.010
Scleroatrophic gallbladder	57 (0.6)	32 (0.7)	25 (0.6)	0.858
Wall thickening	41 (0.6)	24 (0.7)	17 (0.5)	0.219
Cholesterolosis	40 (0.5)	16 (0.3)	24 (0.6)	0.057
Wall calcifications	21 (0.3)	12 (0.3)	9 (0.2)	0.461
Image suggestive of neoplasm	9 (0.1)	5 (0.1)	4 (0.1)	0.971
Adenomyomatosis	4 (0.0)	2 (0.0)	2 (0.0)	0.842
Adenomyosis	3 (0.0)	0 (0.0)	3 (0.0)	0.056
B.Biliary Colic at enrolment by gallbladder status at baseline.*				
Normal	722 (60.5)	495 (56.1)	227 (72.9)	<0.001
Gallstones	214 (17.9)	155 (17.6)	59 (19,0)	0.641
Cholecystectomy	257 (21.5)	232 (26.3)	25 (8.0)	<0.001

Gallstone disease: gallstones, biliary sludge, or cholecystectomy. [a] *p* values for sex differences from the Chi-square test with Yates' continuity correction. *Percentages were obtained from total of biliary colic; 1193 All, 882 Women and 311 Men.

Supplementary Figure S2. Directed acyclic graph of obesity effect on GBD



Abbreviature, SES: Socioeconomic status, GBD: Gallbladder disease ;*Only women

Table S3. Baseline characteristics of women and men by GSD status (n=8774). MAUCO 2015-2019.

Baseline characteristics	Women (n=4838)		Men (n=3936)	
	Non-GSD (n=2848) %	GSD (n=1990) %	Non-GSD (n=3234) %	GSD (n=702) %
Age, years 38-44	801 (28.1)	302 (15.2)	799 (24.7)	77 (11.0)
45-54	1042 (36.6)	623 (31.3)	1070 (33.1)	175 (24.9)
55-64	681 (23.9)	638 (32.1)	913 (28.2)	230 (32.8)
65-74	324 (11.4)	427 (21.5)	452 (14.0)	220 (31.3)
Above 8 years of schooling	1589 (55.8)	885 (44.5)	1671 (51.7)	351 (50.1)
Low healthcare coverage [a]	2117 (74.8)	1607 (81.2)	2331 (72.5)	511 (73.2)
Chilean-Hispanic ancestry	2773 (97.9)	1944 (98.1)	3137 (97.3)	682 (97.7)
Less than 20 remaining teeth	1140 (40.2)	1105 (55.8)	1352 (42.0)	358 (51.4)
Mediterranean Diet Score ≥9	66 (2.5)	32 (1.7)	28 (0.9)	6 (0.9)
High-fat red meat >2 t/w	262 (9.5)	141 (7.3)	371 (11.7)	67 (9.7)
Sugary drinks >2 t/d	267 (9.5)	164 (8.5)	639 (19.9)	116 (16.7)
Whole grains >3 t/w	547 (20.2)	326 (17.3)	267 (8.6)	63 (9.3)
Legumes >1 t/w	2374 (83.7)	1628 (82.3)	2714 (84.3)	590 (84.3)
Fruit intake ≥1 t/d	1640 (57.6)	1066 (53.7)	1419 (44.0)	329 (46.9)
Vegetable intake ≥1t/d	2117 (74.5)	1434 (72.4)	1950 (60.5)	418 (59.9)
Risky alcohol intake	321 (11.4)	152 (7.8)	1001 (31.5)	170 (24.5)
Ever smoker	1472 (52.1)	1008 (51.1)	2087 (65.1)	459 (65.8)
Current smoker	873 (31.0)	575 (29.4)	1124 (35.2)	194 (28.1)
Low physical activity [b]	2590 (91.7)	1831 (93.5)	2979 (93.0)	631 (91.3)
Low grip strength [c]	320 (11.4)	274 (14.0)	366 (11.4)	84 (12.1)
Slow walking pace [d]	532 (25.4)	440 (30.4)	464 (17.7)	152 (26.7)
Children 3 and more	1251 (43.9)	1071 (53.8)	-	-
Hormone contraception, ever	1690 (59.5)	1050 (52.9)	-	-
HRT, ever	351 (12.5)	288 (14.6)	-	-
Family history of gallstones	1407 (51.5)	1156 (60.4)	1132 (36.4)	333 (49.3)
Family history of GBC	58 (2.0)	57 (2.9)	20 (0.6)	12 (1.7)
Abdominal obesity [e]	2178 (76.7)	1716 (86.3)	1428 (44.3)	388 (55.3)
BMI ≥30 kg/m^2	1075 (37.8)	996 (50.1)	1166 (36.1)	299 (42.6)
LDL ≥160 mg/dL	324 (11.5)	189 (9.7)	314 (10.1)	44 (6.5)
Low HDL [f]	1651 (58.2)	1208 (60.9)	1251 (38.8)	322 (46.1)
Triglycerides ≥200 mg/dL	544 (19.2)	477 (24.0)	951 (29.5)	219 (31.3)
Current use of statins	215 (8.4)	216 (12.2)	140 (4.8)	46 (7.3)
Bilirubin Total >1.2 mg/dL	62 (2.2)	50 (2.5)	211 (6.5)	55 (7.9)
Bilirubin Direct >0.3 mg/dL	85 (3.0)	89 (4.5)	203 (6.3)	62 (8.9)
AST ≥48 IU/L	126 (4.4)	118 (5.9)	228 (7.1)	58 (8.3)
ALT ≥55 IU/L	193 (6.8)	185 (9.3)	499 (15.5)	106 (15.2)
ALT/AST ≥1.33	391 (13.8)	223 (11.2)	242 (7.5)	58 (8.3)
ALP >129 IU/L	276 (9.7)	287 (14.5)	208 (6.5)	65 (9.3)
Fatty liver Mild	799 (28.1)	691 (34.8)	905 (28.0)	222 (31.7)

Moderate	336 (11.8)	387 (19.5)	517 (16.0)	149 (21.3)
Severe	33 (1.2)	29 (1.5)	43 (1.3)	5 (0.7)
Biliary colic	495 (17.5)	387 (19.6)	227 (7.0)	84 (12.1)
Dyspepsia	920 (32.7)	671 (33.9)	534 (16.7)	134 (19.3)
Diabetes	320 (11.2)	409 (20.6)	439 (13.6)	147 (20.9)
Hypertension	1308 (45.9)	1167 (58.6)	1929 (59.6)	470 (67.0)
Cardiovascular disease	167 (5.9)	151 (7.6)	207 (6.4)	77 (11.0)
Metabolic Syndrome	1300 (45.9)	1153 (58.2)	1391 (43.3)	377 (54.1)

Other gallbladder anomalies (n=174) excluded. Abbreviations: GSD, gallstone disease; GBC, gallbladder cancer; HRT, hormone replacement therapy; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; t/w, times per week; t/d, times per day. [a] Public levels A or B. [b] < 3 times per week (30 minutes each time).[c] Low grip strength <16 kg in women, <27 kg in men. [d] Self-reported slow walking pace. [e] Waist circumference ≥88 cm in women and ≥102 cm in men. [f] <50 mg/dL in women and <40 mg/dL in men.

Table S4. Comparison of gallstone disease participants and matched and opportunistic non-GSD controls.

Baseline characteristics	GSD			Non-GSD				
				Matched control			Opportunistic control	
	Invited (n=2353)	Attended (n=2098)	*p*-value [a]	Invited (n=1811)	Attended (n=1689)	*p*-value [b]	(n=595)	*p*-value [c]
Sex, women	74.8	76.0	0.38	69.9	70.1	0.90	52.1	<0.001
Age, years 38-44	13.8	13.6		15.5	15.4		30.5	
45-54	29.6	30.7	0.71	31.3	31.9	0.92	42.5	<0.001
55-64	32.7	33.1		32.8	33.1		20.6	
65-74	23.9	22.6		20.5	19.6		6.4	
> 8 years of schooling	60.8	61.6	0.63	63.3	64.1	065	80.9	<0.001
Low healthcare coverage [d]	78.7	78.1	0.66	76.2	76.4	0.93	69.0	0.002
Chilean-Hispanic	98.0	98.0	0.99	97.8	97.7	0.99	97.8	0.97
< 20 remaining teeth	56.2	55.7	0.74	53.2	52.6	0.72	32.0	<0.001
Risky alcohol intake	3.9	3.6	0.85	3.3	3.5	0.91	3.1	0.98
Ever smoker	54.9	54.5	0.81	56.8	57.2	0.81	59.7	0.23
Family history of gallstones	56.7	57.6	0.57	47.3	47.9	0.72	48.7	0.59
Family history of GBC	2.3	2.5	0.83	2.0	2.1	0.86	1.7	0.76
High body fat [e]	88.2	88.2	0.99	81.3	81.1	0.93	75.9	0.006
Abdominal obesity [f]	78.3	78.9	0.70	69.3	69.4	0.99	64.0	0.02
BMI ≥30 kg/m^2	48.2	48.6	0.82	36.4	36.4	0.99	42.4	0.01
Low HDL [g]	58.5	58.9	0.81	54.1	53.9	0.94	50.8	0.16
Fatty liver Mild	35.9	36.9	0.89	31.9	32.0	0.99	27.0	<0.001
Moderate/Severe	20.4	20.2		14.1	14.1		21.1	
Biliary colic	18.4	18.2	0.88	15.4	15.7	0.82	12.6	0.11
Diabetes	19.3	18.2	0.36	14.1	13.6	0.75	7.4	<0.001
High blood pressure	65.6	65.2	0.82	60.5	59.7	0.65	53.8	0.004
Cardiovascular disease	8.7	8.0	0.48	8.2	7.9	0.75	4.7	0.005
Metabolic Syndrome	57.3	57.6	0.88	48.2	47.7	0.76	44.2	0.098
Incident GSD*				8.2	8.2	0.99	6.2	0.3

p-values calculated with Chi-square test with Yates' continuity correction. [a] GSD invited versus attended; [b] Matched controls invited versus attended; [c] Opportunistic versus matched controls. Abbreviations: GSD, gallstone disease; HDL, high-density lipoprotein; GBC, gallbladder cancer; US, ultrasound. [d] Participant with public health insurance level A or B. [e] Measured by bioimpedance analysis as >35% in women and>25% in men. [f] Waist circumference ≥88 cm in women and ≥102 cm in men. [g] <50 mg/dL in women and <40 mg/dL in men. * This is the only information estimated during the follow-up and that was used for creating the following Supplementary Tables.

Table S5. GSD-associated factors at baseline ultrasound by sex, age-adjusted. MAUCO 2015-2019.

Baseline factors (reference category)	Women with GSD			Men with GSD		
	Denominator N:4838	n (%) n:1990(41.4)	Age-adjust PRR (95% CI)	Denominator N: 3936	n (%) 702(17.8)	Age-adjust PRR (95% CI)
45-54 (38-44)	1665	623 (37.4)	1.37 (1.22-1.53)	1245	175 (14.1)	1.60 (1.24-2.06)
55-64	1319	638 (48.4)	1.77 (1.58-1.97)	1143	230 (20.1)	2.29 (1.80-2.92)
65-74	751	427 (56.9)	2.08 (1.85-2.32)	672	220 (32.7)	3.72 (2.93-4.73)
Schooling ≤8 years	2359	1102 (46.7)	1.17 (1.09-1.26)	1910	351 (18.3)	0.86 (0.75-0.98)
Low healthcare coverage [a]	3724	1607 (43.2)	1.16 (1.06-1.27)	2842	511 (21.9)	0.88 (0.76-1.02)
Less than 20 remaining teeth	2245	1105 (49.2)	1.18 (1.09-1.28)	1710	358 (20.9)	0.89 (0.77-1.04)
Mediterranean Diet Score ≥9	98	32 (32.7)	0.72 (0.55-0.96)	34	6 (17.6)	0.94 (0.46-1.92)
Fruit intake ≥1 time/day	2706	1066 (39.4)	0.87 (0.82-0.93)	1748	329 (18.8)	1.02 (0.89-1.16)
Ever smoker [b]	2480	1008 (40.6)	1.00 (0.94-1.07)	2546	459 (18.0)	1.07 (0.93-1.23)
Low grip strength [c]	594	274 (46.1)	1.05 (0.95-1.15)	450	84 (18.7)	0.83 (0.68-1.02)
Slow walking pace [d]	972	440 (45.3)	1.09 (0.99-1.18)	616	152 (24.6)	1.24 (1.05-1.47)
Three and more children	2322	1071 (46.1)	1.16 (1.09-1.24)	-	-	-
Family history of gallstones	2563	1156 (45.1)	1.23 (1.15-1.34)	1465	333 (22.7)	1.60 (1.40-1.82)
Family history of GBC	115	57 (49.6)	1.17 (0.97-1.40)	32	12 (37.5)	1.93 (1.27-2.95)
Abdominal obesity [f]	3894	1716 (44.1)	1.45 (1.31-1.61)	1816	388 (21.4)	1.36 (1.19-1.55)
Body Mass Index ≥30 kg/m^2	2071	996 (48.1)	1.32 (1.24-1.41)	1465	299 (20.4)	1.30 (1.14-1.48)
LDL ≥160 mg/dL	513	189 (36.8)	0.85 (0.76-0.96)	358	44 (12.3)	0.72 (0.55-0.96)
Low HDL [g]	2859	1208 (42.3)	1.09 (1.01-1.16)	1573	322 (20.5)	1.31 (1.15-1.50)
Triglycerides ≥200 mg/dL	1944	874 (45.0)	1.13 (1.06-1.21)	1170	219 (18.7)	1.18 (1.02-1.35)
Biliary colic	882	387 (43.8)	1.11 (1.03-1.21)	311	84 (27.0)	1.64 (1,35-1.99)
Dyspepsia	1591	671 (42.2)	1.04 (0.97-1.12)	668	134 (20.1)	1.22 (1.03-1.44)
Current use of statins	431	216 (50.1)	1.05 (0.95-1.17)	186	46 (24.7)	1.08 (0.84-1.39)
Fatty liver Mild (none)	1490	691 (46.4)	1.29 (1.19-1.39)	1127	222 (19.7)	1.28 (1.10-1.49)
Moderate	723	387 (53.5)	1.48 (1.36-1.61)	666	149 (22.4)	1.55 (1.30-1.83)
Severe	62	29 (46.8)	1.40 (1.08-1.81)	48	5 (10.4)	0.79 (0.35-1.80)
Total bilirubin >1.2 mg/dL	112	50 (44.6)	1.06 (0.87-1.28)	266	55 (20.7)	1.13 (0.89-1.43)
Direct bilirubin >0.3 mg/dL	174	89 (51.1)	1.18 (1.02-1.36)	265	62 (23.4)	1.18 (0.94-1.48)
AST ≥48 IU/L	244	118 (48.4)	1.18 (1.03-1.35)	286	58 (20.3)	1.28 (1.01-1.61)
ALT ≥55 IU/L	378	185 (48.9)	1.25 (1.12-1.39)	605	106 (17.5)	1.23 (1.02-1.48)
ALT/AST ≥1.33	1002	468 (46.7)	1.21 (1.12-1.31)	1494	251 (16.8)	1.16 (1.01-1.34)
Diabetes	729	409 (56.1)	1.30 (1.20-1.40)	558	140 (25.1)	1.26 (1.01-1.48)
Hypertension	2475	1167 (47.2)	1.15 (1.07-1.24)	2728	524 (19.2)	1.03 (0.88-1.21)
Cardiovascular disease*	318	151 (47.5)	1.03 (0.91-1.16)	284	77 (27.1)	1.30 (1.06-1.59)
Metabolic Syndrome	2453	1153 (47.0)	1.23 (1.15-1.32)	1768	376(21.3)	1.32 (1.16-1.51)

Poisson Multiple regression with robust variance age-adjusted. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CI, confidence intervals; GBC, gallbladder cancer; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PRR, prevalence rate ratios. [a] Public health insurance level A or B. [b] Self-reported ever smoked ≥100 cigarettes. [c] Low grip strength:<16 kg in women <27 kg in men. [d] Self-reported slow walking pace. [e] Bioimpedance >35% women, >25% men. [f] Waist circumference ≥88 cm women, ≥102 cm men. [g] <50 mg/dL women <40 mg/dL men.*: Self-reported heart attack, vascular accident, or heart failure

Table S6. Risk factors of GSD incidence by type of controls. MAUCO 2015-2023.

	Total	New GBD	Person-years	Incidence 1000/p-y	Age-sex adjusted HR(95% CI)
A. Model with all controls (n=2284)					
Age, <45 years	287	26	1108.98	23.45	Ref
45-54 years	632	58	1912.99	30.32	1.48 (0.92-2.35)
55-64	830	60	2239.35	26.79	1.60 (1.005-2.56)
65+	535	54	1433.0	37.68	2.66 (1.64-4.31)
Men	789	59	2534.78	23.28	Ref
Women	1495	139	4159.53	33.41	1.81 (1.33-2.47)
Schooling +13 years	264	23	783.97	29.34	Ref
<4 years	243	18	693.48	25.96	0.84 (0.46-1.58)
4-8 years	864	83	2461.17	33.72	1.17 (0.74-1.88)
9-12 years	913	74	2755.69	26.85	0.96 (0.60-1.54)
Normal waist circumference	799	50	2484.57	20.12	Ref
Risk waist circumference*	1481	148	4198.94	35.24	1.65 (1.18-2.31)
Diet Med Score < 6	1406	114	4167.53	27.35	Ref
≥6	688	61	2007.62	30.38	1.91 (0.80-1.49)
HDL cholesterol ≥50,	1058	76	3150.33	24.12	Ref
<50	1221	121	3526.89	34.31	1.55 (1.16-2.06)
Family hist. GBC, No	2233	191	6556.0	29.13	Ref
Yes	46	6	124.75	48.1	1.63 (0.72-3.68)
B. Model with age-sex matched controls (n=1589)					
Age, <45 years	157	17	423.0	40.19	Ref
45-54 years	419	41	1045.51	39.21	1.02 (0.58-1.80)
55-64	627	48	1528.70	31.40	0.85 (0.49-1.48)
65+	486	51	1225.87	41.60	1.14 (0.66-1.98)
Men	505	42	1315.23	31.93	Ref
Women	1184	115	2907.84	39.55	1.32 (0.99-2.03)
Schooling +13 years	167	18	414.34	43.44	Ref
<4 years	205	16	510.73	31.33	0.78 (0.39-1.53)
4-8 years	682	69	1710.82	40.33	0.98 (0.58-1.66)
9-12 years	635	54	1587.19	34.02	0.82 (0.48-1.40)
Normal waist circumpherence	571	38	1479.92	25.68	Ref
Risk waist circumpherence*	1114	119	2732.35	43.55	1.83 (1.25-2.68)
Diet Med Score < 6	1019	89	2551.94	34.88	Ref
≥6	501	47	1249.66	37.61	1.03 (0.72-1.48)
HDL cholesterol ≥50,	770	59	1942.24	30.38	Ref
<50	916	97	2270.94	42.71	1.46 (1.05-2.01)
Family hist. GBC, No	1650	150	4135.93	36.27	Ref
Yes	36	6	80.86	74.20	2.74 (1.21-6.24)
C. Model with opportunistic controls (n=595)					

Age, <45 years	130	9	685.98	13.12	Ref
45-54 years	213	17	867.48	19.60	1.38 (0.61-3.11)
55-64	203	12	710.65	16.89	1.56 (0.64-3.81)
65+	49	3	207.13	14.48	2.98 (0.75-11.78)
Men	284	17	1219.56	13.94	Ref
Women	311	24	1251.69	19.17	1.62 (0.82-3.20)
Schooling +13 years	97	5	369.63	13.53	Ref
<4 years	38	2	182.76	10.94	0.56 (0.11-2.95)
4-8 years	182	14	750.35	18.66	0.96 (0.33-2.73)
9-12 years	278	20	1168.51	17.12	0.98 (0.36-2.67)
Normal waist circumpherence	228	12	1004.65	11.94	Ref
Risk waist circumpherence*	367	29	1466.59	19.77	1.36 (0.65-2.83)
Diet Med Score < 6	387	25	1615.59	15.47	Ref
≥6	187	14	757.96	18.47	1.28 (0.66-2.49)
HDL cholesterol ≥50	288	17	1208.09	14.07	Ref
<50	305	24	1255.95	19.10	1.57 (0.83-2.98)
Family hist. GBC, No	583	41	2420.07	16.94	Ref
Yes	10	0	43.89	0.0	-

Opportunistic controls: participants who attended the MAUCO clinic for other reasons and were offered an ultrasound examination. Abbreviations: GBD, gallblader disease; p-y, person years; HR, hazard ratio; CI, confidence intervals; GBC, gallbladder cancer. a Hazard ratio from Multiple Cox extended regression.

Supplementary Table S7. Waist Circumference and Incident GSD in MAUCO 2015-2023. Cox extended *MRM.

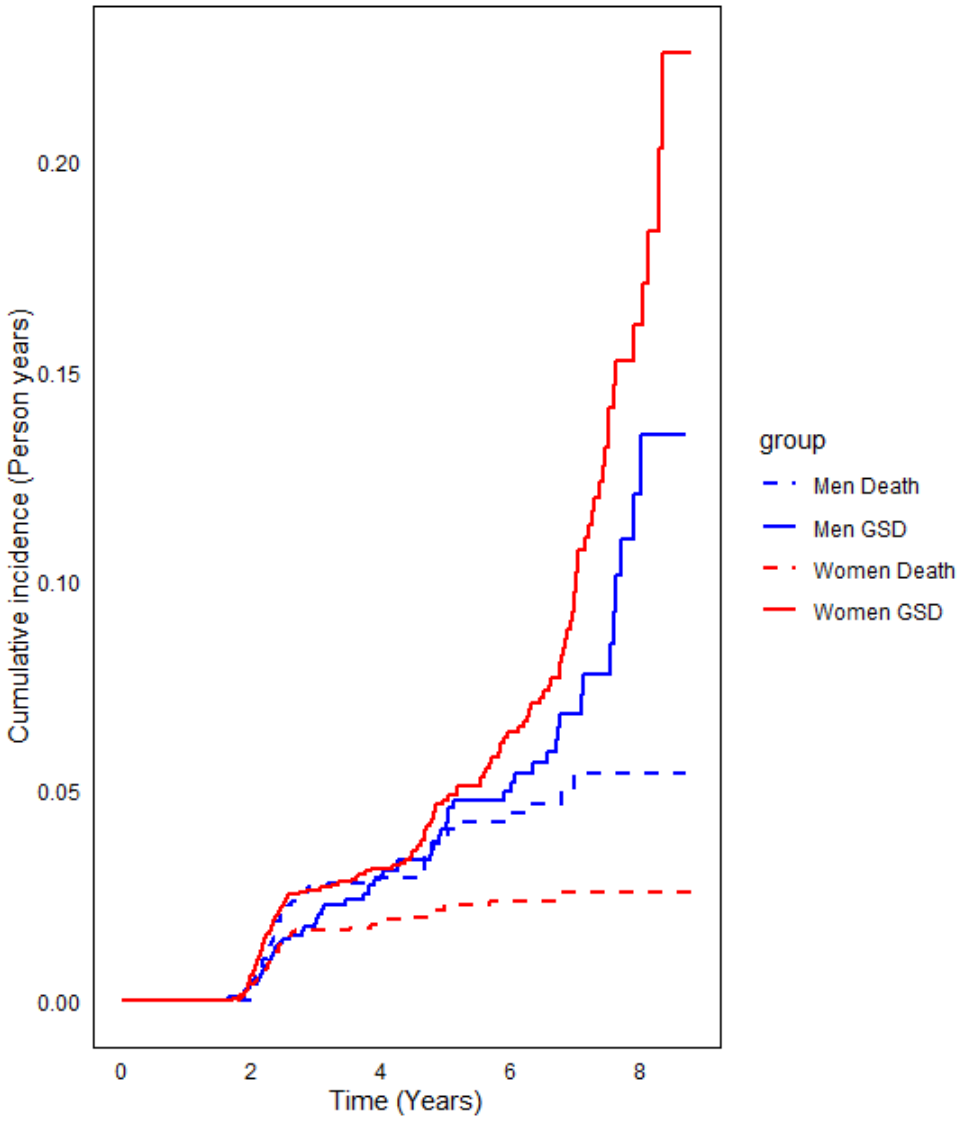
	Hazard Ratio (95% CI) Per cm above ideal[1]
Overall	
Waist circumference, cm	**1.02 (1.005-1.03)**
Women	
Waist circumference, cm	**1.02 (1.006-1.04)**
Men	
Waist circumference, cm	1.01 (0.99-1.04)

*MRM: Multiple regression model; [1]: 80 cm for women and 94 cm for men.
Overall was adjusted by: Sex, Schooling, Family history of GBC, HDL cholesterol. Diet, Age as well as Age*Sex interaction.
Women was adjusted as per overall model but additionally for n° of children.
Men was adjusted as per overall.

Table S8. Predictors of incident GSD (n=198) among 2284 participants. MAUCO 2019-2023.

	HR (95% CI)
Waist circumference	1.02 (1.005-1.03)
Age	1.07 (1.04-1.10)
Women (Men)	1.15 (0.79-1.67)
Schooling <4 years (≥13)	0.69 (0.33-1.43)
4-8 (≥13)	0.94 (0.56-1.57)
9-12	0.88 (0.53-1.46)
Diet med score	0.98 (0.89-1.68)
Family history GBC	1.86 (0.82-4.22)
HDL cholesterol <50 W <40m	1.22 (0.88-1.68)
Sex*Age	0.95 (0.92-0.98)

Abbreviations: GBD, gallbladder disease; p-y, person years; HR, hazard ratio; CI, confidence intervals; GBC, gallbladder cancer. a Hazard ratio from Multiple Cox extended Regression, all variables included in the model.



Supplementary Figure S3. Cumulative incidence (person-years) of GSD and Death from all-causes by follow-up years and sex.